A Sara, Michela, Fabrizio, Laura ma soprattutto a Stefano:
senza di loro questo lavoro non esisterebbe.
E alla mia famiglia,
senza la quale non sarei nulla!

Abstract

Perfluoroalkyl substances (PFASs) are emerging pollutants with so peculiar physico-chemical characteristics that these compounds found wide applications in several industrial processes and daily products. Their characteristics and the wide use make them widespread in all environmental compartments, very persistent and partially bioaccumulable.

A past survey at the basin closures of the main European rivers highlighted that river Po was the most contaminated (200 ng/L) by perfluorooctanoic acid (PFOA), the currently most diffused perfluoroalkyl acid (PFAA). This evidence led to a funded project which aimed to evaluate the PFAA distribution in main Italian river basins and to assess the correlated environmental and sanitary risks.

An UHPLC-MS/MS multi-residue method based on an on-line SPE procedure was developed for the simultaneous determination of 12 PFAAs to support the sampling program in river basins that covered about 40% of the Italian surface area and about 45% of the Italian population.

In Italy the most important sources of PFAA are fluorochemical plants, followed by discharges from tannery and textile industrial districts, while the contribution of urban areas is limited. Considering the most populated and industrialized Italian area, the river Lambro basin, Lombardy, detailed mass balance of the emission sources have been carried out.

The environmental risks for the aquatic ecosystem were evaluated in river Bormida (Piedmont) downstream the discharge of a fluoropolymer plant which is the most significant source of PFOA in Italy. An effect-based approach was applied to the study of macrobenthic community, evaluating the ecological quality, the community structure and the genetic variance.

Indice

Introduzione

Gli inquinanti emergenti

Gli ultimi cinquant'anni sono stati caratterizzati da un'esponenziale crescita demografica a livello mondiale accompagnata ma anche agevolata da un ingente sviluppo industriale che, tra l'altro, ha significato la produzione e la conseguente immissione in ambiente di numerose molecole di sintesi. Centinaia di questi composti che sono riversati quotidianamente in ambiente provengono da fonti di inquinamento diffuso (i.e. utilizzo di prodotti in agricoltura ed emissioni atmosferiche industriali) o da sorgenti puntuali (i.e. scarichi di impianti di depurazione urbani o industriali). Tuttavia solo una minima parte di queste molecole è stata studiata per conoscerne la distribuzione in ambiente e gli effetti tossicologici a livello ambientale. Di conseguenza una frazione ancora inferiore della totalità delle molecole immesse in ambiente è soggetta a regolamentazione. Le molecole più studiate sono quelle considerate persistenti, in particolare gli organoclorurati che rientrano tra quelli regolamentati dalla Convenzione di Stoccolma e sono definiti Persistent Organic Pollutants (POP). Solo recentemente, tuttavia, la comunità scientifica ha iniziato a considerare un nuovo gruppo di contaminanti, meno persistenti dei POP, che vengono definiti "emergenti" (Emerging Pollutants o Emerging Substances, ES) in quanto sono costantemente presenti anche in minime concentrazioni nelle matrici ambientali ma non sono correntemente inclusi nei programmi di monitoraggio delle agenzie di controllo. Molti di questi composti, sebbene siano meno persistenti, tuttavia mantengono concentrazioni costanti nell'ambiente poiché sono costantemente emesse da attività umane legate alla vita quotidiana: benché il destino, gli effetti tossicologici ed ecotossicologici degli inquinanti emergenti non siano pienamente conosciuti, è possibile prevedere che anche bassissime concentrazioni di alcuni di queste molecole biologicamente attive possano indurre effetti negli ecosistemi acquatici, ad esempio interferendo con il sistema endocrino e riproduttivo ma anche alterando il comportamento animale e danneggiando specifici tessuti (Jaspers et al., 2013; Gago-Ferrero et al., 2012; O'Toole et al., 2006).

Alcuni esempi di inquinanti emergenti sono i prodotti per la cura e l'igiene della persona, inclusi fragranze, schermi solari e detergenti, i farmaci, le droghe d'abuso, gli additivi alimentari, le nanoparticelle e i composti perfluorurati.

I composti perfluorurati (PFAS)

I composti perfluorurati , o sostanze perfluoroalchiliche (PFAS), sono composti organici costituiti da una catena alchilica di lunghezza variabile (in genere da 4 a 14 atomi di carbonio) parzialmente o totalmente fluorurata e da un gruppo funzionale idrofilico.

La presenza di numerosi legami carbonio-fluoro conferisce particolari caratteristiche fisico-chimiche a queste sostanze, incluse la repellenza all'acqua e ai grassi, la stabilità termica e la tensioattività che le rendono molto utili in un ampio campo di applicazioni industriali e nella produzione di prodotti di largo consumo (Kissa, 2001).

A partire dagli anni '50 i PFAS hanno trovato applicazione in svariati campi, come emulsionanti e tensioattivi nella pulizia di tappeti, pelli e tessuti, nella formulazione di insetticidi, di rivestimenti protettivi e di vernici, e sono stati impiegati anche nelle schiume antincendio. Sono presenti anche nei prodotti per stampanti, nelle

pellicole fotografiche e nei materiali per la microelettronica e in prodotti di uso quotidiano come shampoo e dentifrici e vengono utilizzati anche nei contenitori per cibo come ad esempio i cartoni delle pizze d'asporto o i contenitori da "fast-food". Sono impiegati nella produzione del politetrafluoroetilene (PTFE) (commercializzato con vari marchi di cui il più famoso è Teflon®) alla base anche della produzione di tessuti tecnici e ad alte prestazioni (i.e. il Gore-Tex®) ma impiegato anche in applicazioni di diverse tipo come guarnizioni e isolanti (Kannan et al., 2002; Minoia et al., 2008).

Come conseguenza dell'estensiva produzione e uso dei PFAS e a causa delle loro caratteristiche fisico-chimiche, questi composti sono stati spesso rilevati in concentrazioni significative in campioni ambientali e in organismi viventi, incluso esseri umani (Kannan, 2011).

PFAS: Proprietà chimico-fisiche

Gli acidi perfluoroalchilici (PFAA, PerFluoroAlkyl Acids) sono i composti che sono stati più frequentemente ritrovati in ambiente; sono ionici, e quindi di elevata solubilità in acqua, e possono trovarsi in forma più o meno dissociata a seconda del valore di pKa. Ne fanno parte gli acidi perfluorocarbossilici (PFCA, PerFluoroalkyl Carboxylic Acids), che hanno un gruppo –COOH terminale e tra i quali il più studiato è l'acido perfluoroottanoico (PFOA), e gli acidi perfluorosolfonati (PFSA, PerFluoroalkyl Sulfonic Acids) dei quali il più noto è l'acido perfluoroottansolfonico (PFOS) e che contengono il gruppo –SO_3^-. Gli acidi perfluorocarbossilici sono usati come coadiuvanti nei processi per la produzione di fluoropolimeri (come il PTFE) e come tensioattivi; inoltre i PFCA sono i prodotti terminali di degradazione di altri PFAS, come i fluorotelomeri degli alcoli (Ellis et al., 2004) e altri fluorotelomeri, utilizzati nella preparazione di trattamenti superficiali antigrasso e impermeabilizzanti. E' stato stabilito che circa l'80% degli acidi perfluorocarbossilici rilevati in ambiente derivino da emissioni dirette, cioè emissioni di cui si possono stabilire i punti di origine, che sono principalmente gli impianti di produzione (Prevedouros et al., 2006).

I composti perfluorurati ionici sono stabili oltre i 150 °C, non infiammabili, non degradabili da acidi e basi forti o da agenti ossidanti, non soggetti alla fotolisi e alla degradazione microbica (Lau et al., 2007). Le loro caratteristiche chimiche e chimico-fisiche sono le seguenti: possiedono un gruppo alchilico perfluorurato lipofobico, una testa polare (che può trovarsi in forma totalmente o parzialmente dissociata nei diversi comparti ambientali e biologici), una bassa tensione di vapore (che diminuisce con l'aumentare della lunghezza della catena e che ostacola la loro volatilizzazione da sistemi acquatici e terrestri) ed una buona solubilità (che come la tensione di vapore, diminuisce all'aumentare degli atomi di carbonio); ed infine, si comportano come tensioattivi. Queste proprietà sono state studiate sperimentalmente solo su alcuni isomeri lineari di acidi perfluorocarbossilici e perfluorosolfonici, tra i quali l'acido perfluoroottanoico (PFOA) e l'acido perfluoroottansolfonico (PFOS), ma mancano ancora dati per gli altri congeneri ritrovabili nelle miscele industriali (Rayne e Forest, 2009).

PFAS: Produzione

In natura è molto rara l'esistenza di composti fluorurati a causa dell'energia richiesta per formare il legame C-F (>450 kJ/mol), quelli prodotti dagli esseri viventi contengono spesso un solo atomo di fluoro e riescono ad essere degradati (Giesy e Kannan, 2002); la quasi totalità delle sostanze fluorurate ritrovate in ambiente sono quindi di origine antropica.

Dal punto di vista industriale le tecniche di produzione sono principalmente due: la fluorurazione diretta tramite fluorurazione elettrochimica (ECF) e la telomerizzazione.

Il processo di fluorurazione diretta inizia da una materia prima organica, come ad esempio l'1-ottansolfonil fluoruro ($C_8H_{17}SO_2F$) immersa in una soluzione di acido fluoridrico in cui viene fatta passare della corrente elettrica che determina la sostituzione degli atomi di idrogeno con atomi di fluoro e che, in questo caso, forma il corrispettivo perfluoroottansolfonil fluoruro ($C_8F_{17}SO_2F$), precursore del PFOS. Durante il processo può verificarsi la frammentazione e il riarrangiamento dello scheletro carbonioso portando alla formazione di composti fluorurati di diversa lunghezza e una miscela di isomeri lineari, ramifi cati e ciclici. La mancanza di purezza degli isomeri è indice di questo processo produttivo (Lau et al., 2007).

Nonostante il rendimento modesto e la formazione di numerosi prodotti collaterali, la fluorurazione elettrochimica è stata usata fin dagli anni '50 dalla 3M, grazie al basso costo sia dei reagenti utilizzati sia della corrente elettrica. Tale processo è tutt'oggi utilizzato da diverse aziende (Moody et al., 2000, Lau et al., 2004). Le sostanze prodotte sono principalmente precursori degli acidi perfluorosolfonati e del PFOA. (Buck et al., 2011).

La telomerizzazione consiste in un processo di condensazione tra un composto detto telogeno (AB) e un monomero detto tassogeno (M), si ottengono così dei telomeri, ovvero composti omologhi di struttura A-(M) n-B. L'indice (n) è il grado di telomerizzazione ed è un numero intero dato per ogni specie telomerica (Negri et al., 2008).

Nel caso dei fluorotelomeri, un telogeno come il pentafluoroetil ioduro ($F(CF_2)_2I$) polimerizza con tetrafluoroetilene per formare intermedi con una catena carboniosa della lunghezza desiderata (tipicamente fino a 12 atomi di carbonio), successivamente i telomeri formati reagiscono con etilene mediante l'aggiunta di radicali liberi formando fluorotelomeri degli alcoli (FTOH) che possono essere convertiti nei prodotti finali da commercializzare (Lau et al., 2004).

PFAS: Degradazione

I polimeri organici fluorurati sono molto stabili e caratterizzati da un'emivita che va dai cinque ai cinquecento anni. I composti perfluorurati sono quelli più difficili da degradare, poiché sono resistenti a processi di riduzione, ossidazione e irradiazione con raggi UV, anche se i composti più complessi possono essere degradati a PFOS e PFOA (Negri et al., 2008). Recenti studi sulla decomposizione termica di alcuni sali di PFAS però suggeriscono che questi composti possono essere più labili, in determinate condizioni, di quanto comunemente creduto (Rayne e Forest, 2009).

Gli acidi perfuoroalchilici e le altre molecole perfluorurate presenti negli scarichi non sono in genere rimossi negli attuali processi di depurazione e alcuni studi hanno dimostrato che gli impianti di trattamento delle acque reflue sono tra le fonti più consistenti di contaminazione degli ecosistemi acquatici (Sinclair e Kannan, 2006; Ahrens, 2011). Le tecniche di sonicazione sembrano dare risultati incoraggianti, ma anche l'integrazione di tecniche di filtrazione e assorbimento nel trattamento delle acque reflue contribuirà a ridurre il rilascio ambientale di questi composti. Nuove ricerche potrebbero sfruttare il carattere proteinofilo degli acidi perfluorurati per costruire filtri più efficaci (Kannan, 2011).

Altri PFAS, come i fluorotelomeri degli alcoli (FTOH), si degradano più facilmente; gli FTOH, per esempio, hanno un tempo di persistenza di 20 giorni in atmosfera e poi vengono degradati con reazioni di ossidazioni con i radicali ossidrilici presenti nella troposfera (in assenza di ossidi di azoto) (Ellis et al., 2004). Anche le perfluorosolfonammidi subiscono lo stesso destino, ma il loro tempo di vita è stato stimato tra i 20 e i 50 giorni (Martin et al., 2006).

PFAS: Sorgenti e distribuzione in ambiente

I PFAS sono stati rilevati nella maggior parte dei comparti ambientali in tutto il mondo, tra i quali le acque di superficie, l'aria, i fanghi di depurazione, i suoli, i sedimenti e le calotte polari (Lau et al., 2007).

Le sorgenti dei PFAS nell'ambiente possono essere generalmente raggruppate in sorgenti puntuali e sorgenti diffuse. Per quanto riguarda i corpi idrici, ad esempio, le sorgenti puntuali sono: gli impianti di trattamento delle acque, per i quali è stato stimato che il flusso in uscita ha la stessa concentrazione del flusso in entrata o addirittura più elevata (Sinclair e Kannan, 2006), gli impianti di produzione (vedi il modello di Davis et al., 2007) e i percolati da discarica, che rappresentano però solo l'1% rispetto agli scarichi degli impianti di depurazione (Busch et al., 2010).

Le sorgenti diffuse comprendono le deposizioni atmosferiche, poiché nelle idrometeore può avvenire la degradazione di precursori più volatili quali i fluorotelomeri, il deflusso (soprattutto da suoli agricoli), l'uso delle schiume antincendio e la contaminazione delle acque sotterranee per infiltrazione. In generale, la predominanza delle sorgenti puntiformi o diffuse dipende dall'area considerata (Ahrens, 2011).

Una volta immessi nell'ambiente i PFAS dimostrano un'elevata persistenza, e anche se non hanno caratteristiche comuni ai classici POP, come questi ultimi hanno contaminato il globo in modo ubiquitario, anche gli ambienti remoti come l'Artico (Armitage et al., 2009). Sono possibili differenti percorsi di diffusione in ambiente che dipendono dalle caratteristiche chimico-fisiche di questi composti. Secondo Ahrens (2011) i perfluorurati
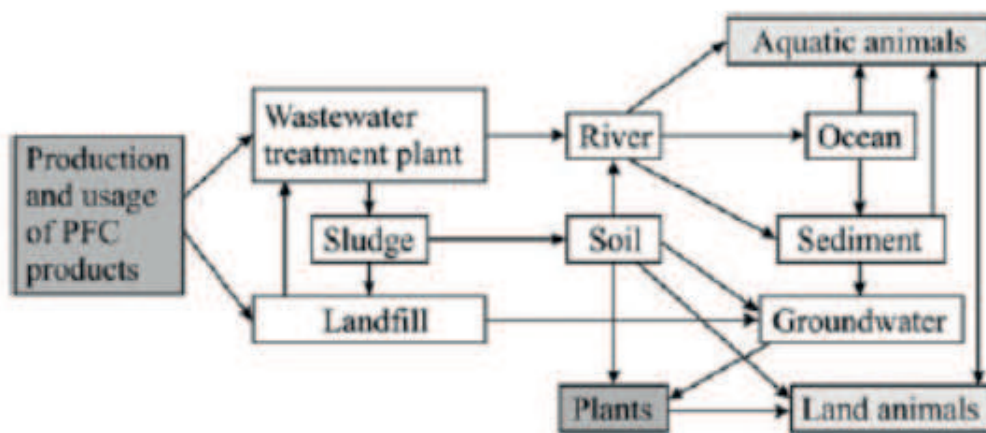
Figura 1 Distribuzione dei PFAS ionici in ambiente acquatico (Ahrens, 2011)

ionici sono anioni molto solubili e, perciò, il comparto acquoso è la loro principale destinazione. Giunti nei fiumi, si ripartiscono tra il sedimento, gli organismi viventi e l'acqua stessa in base alla lunghezza della catena alchilica e del gruppo funzionale. Raggiunto il mare i PFAS possono essere ulteriormente trasportati verso l'oceano aperto (Figura 1). Le zone costiere e i gli estuari dei fiumi sono considerati quindi sorgenti, mentre lo strato superficiale degli oceani è ritenuto il serbatoio più importante per le sostanze perfluoroalchiliche. La loro distribuzione spaziale pelagica dipende dalla distanza dalle fonti e dall'influenza delle correnti oceaniche. Il trasporto è molto lento, nell'ordine di anni (Ahrens, 2011). Concentrazioni minime di PFOA e PFOS sono state osservate nel Pacifico Centrale e Meridionale, nell'Oceano Indiano e nell'Oceano Antartico, mentre i mari che circondano le zone maggiormente industrializzate e popolate, come Oceano Atlantico e Oceano Pacifico Occidentale, presentano le maggiori concentrazioni pelagiche (Yamashita et al., 2005).

L'aerosol marino, secondo Prevedouros et al. (2006), è un ulteriore mezzo di trasporto per i composti ionici. Il micro-strato superficiale del mare, infatti, arricchendo l'atmosfera con le sostanze perfluorurate, è da
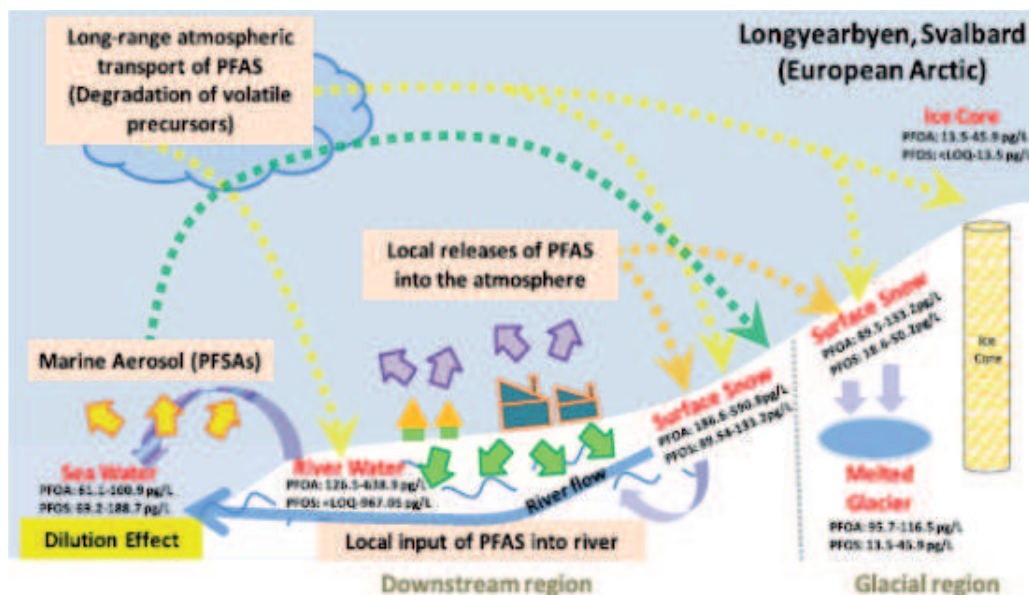
Figura 2 Schema del trasporto atmosferico dei PFAS per la contaminazione di aree remote (Artico europeo) (Kwok et al., 2013)

considerarsi un possibile contributo al trasporto a lunga distanza ed alla distribuzione globale dei PFAS. Questa tipologia di trasporto può essere rilevante soprattutto per i PFCA, i quali tendono ad accumularsi negli strati superficiali dell'oceano. Ulteriori ricerche sono necessarie per valutare l'effettivo contributo dell'aerosol all'inquinamento globale (Prevedouros et al., 2006).

I composti neutri e quindi più volatili, come i fluorotelomeri, subiscono un trasporto atmosferico a più lungo raggio, vengono ossidati e poi rimossi dall'atmosfera tramite deposizione umida. Sono ritenuti i responsabili della contaminazione di aree remote come la regione artica da parte di acidi perfluorurati (Figura 2). La condizione è di avere un tempo di vita sufficientemente lungo per raggiungere queste zone. Kwok et al. (2013) hanno analizzato i composti trovati nelle carote di ghiaccio raccolte a Svalbard e non trovando correlazione tra la concentrazione dell'anione solfato (che ha sicura provenienza oceanica) e quella dei PFAS, confermano così che la sorgente della contaminazione è atmosferica e può essere dovuto sia a trasporti a lungo raggio che ad emissioni locali. I suoi risultati confermano quelli ottenuti da Young (2007) nell'Artico Canadese.

I PFAS sono ubiquitari nel comparto acquoso, in quanto sono stati determinati nella neve, nel ghiaccio, nelle precipitazioni, nelle acque potabili, nelle acque superficiali, negli scarichi dei depuratori e nelle acque sotterranee con concentrazioni che variano da pochi pg a µg per litro per ogni composto (Ahrens, 2011).

Uno studio condotto su carote di ghiaccio, prelevate nell'Artico Canadese, rileva livelli di PFOS tra 2,6 e 86 pg/L, di PFOA tra 12 e 147 pg/L e tracce di altri acidi perfluorocarbossilici (Young et al., 2007).

Il PFOS è stato individuato in acque potabili, in Giappone, con concentrazioni inferiori a 4 ng/L, tranne in un caso dove raggiunge i 50 ng/L. L'origine della contaminazione è probabilmente da ricercarsi nel Fiume Tama, che è stato contaminato da PFOS (Harada et al., 2003). Loos et al. (2007) hanno trovato acidi perfluorurati in acque potabili nei paesi situati vicino al Lago Maggiore con concentrazioni tra 1 e 9 ng/L. Il risultato è stato confermato da Ullah et al. (2011), che ha indagato successivamente l'acqua di rubinetto di altre sei città europee,

Tabella 1 Concentrazioni di PFAS (espresse in ng/L) in acque dolci superficiali

COMPARTO	COMPOSTI			REFERENCE
	PFOS	PFOA	ALTRI PFAS	
Fiume USA	17–144	< LOQ – 598		Hansen et al., 2002
Lago Shikotsu, Hokkaido, Giappone	2,5			Taniyasu et al., 2003
Baia di Tokyo, Giappone	26			
Baia di Osaka, Giappone	8,7			
Baia di Hiroshima, Giappone	4			
Fiume Raisin, USA	3,5	14,7		Kannan et al., 2005
Fiume St. Clair, USA	2,6	4,4		
Fiume Ai, Giappone	2,9-37,1	16 – 63		Morikawa et al., 2006
Fiume Elba, Germania		7,6	2,7 (PFHpA)	McLachlan et al., 2007
Fiume Po, Pontelagoscuro, Italia		200		
Fiume Danubio, Europa		16,4	0,95 (PFHpA) 3 (PFHxA)	
Fiume Senna, Francia		8,9	3,7 (PFHpA)	
Fiume Tamigi, Gran Bretagna		23	4,1 (PFHpA)	
Fiume Reno, Germania		12	1,8- 3,3 (PFHpA)	
Fiume Loira, Francia		3,4	0, 90 (PFHpA) 3,4 (PFHxA)	
Fiume Tanaro, Alessandria, Italia		1270	18 (PFHpA) 6 (PFNA)	Loos et al., 2008

tra le quali Amsterdam (dove il PFAA più abbondante è risultato essere l'acido perfluorobutansolfonico, PFBS), Antwerp, in Belgio e Stoccolma. In queste ultime due città la concentrazione dei composti è compresa tra 0,07 e 8,81 ng/L. In Germania, nella regione del Ruhr, sono stati rilevati elevati livelli di PFAS nell'acqua potabile (598 ng/L come somma delle concentrazioni); il problema è stato causato da acque provenienti da suoli contaminati che hanno raggiunto la falda (Skutlarek et al., 2006).

PFCA sono stati determinati in acque sotterranee nelle vicinanze di una base militare in Michigan con concentrazioni incluse tra 3 e 120 µg/L. La contaminazione era dovuta alle schiume antincendio usate nelle esercitazioni, ma il fatto che i campioni siano stati raccolti dopo cinque anni e più di inattività del sito, da un'idea della persistenza di questi composti (Moody et al., 2003).

Le concentrazioni di acque di fiumi e laghi sono state oggetto di numerose pubblicazioni (Tabella 1). In generale si può affermare che i composti dominanti sono PFOS e PFOA, anche se in alcuni dati recenti (Moller et al., 2010) troviamo che a prevalere sono i composti a corta catena, come PFBA e PFBS (Ahrens, 2011). Gli effluenti finali degli impianti di depurazione sono stati identificati come sorgenti di PFAS per diversi fiumi, tra i quali il Reno (Moller et al., 2010).

Oltre che nelle matrici abiotiche, i PFAS sono stati misurati anche in organismi viventi. I primi rilevamenti si sono verificati all'inizio del 2000, con l'identificazione del PFOS in animali marini raccolti in varie parti del mondo, tra cui Artide ed Antartide (Giesy e Kannan, 2001).

Inizialmente il numero degli studi di biomonitoraggio è stato esiguo, soprattutto per la mancanza di standard certificati e strumenti adeguati, ma negli ultimi anni è andato aumentando. Ora il numero dei composti misurati è moltiplicato, i siti di campionamento coprono molte regioni del globo (Europa, Nord America, Asia, Artide ma anche Sud America, Antartide e Russia) e le analisi riguardano diversi taxa di animali, dai molluschi ai mammiferi (Houde et al., 2011).

Il progetto di tesi

Durante il progetto europeo FP6 PERFORCE (http://www.science.uva.nl/PERFORCE/index.htm?http%3A// www.science.uva.nl/PERFORCE/perforce.htm) venne condotta una campagna di misura dei composti perfluoroalchilici nelle acque e nei sedimenti di alcuni fiumi europei (McLachlan et al., 2007). In questa ricerca la concentrazione più elevata di PFOA fra tutti i fiumi europei fu rilevata nel fiume Po, alla chiusura di bacino, nel sito di Pontelagoscuro vicino a Ferrara (200 ng/L). La concentrazione di PFOA misurata nel Po era di un ordine di grandezza superiore a quella degli altri fiumi europei; questo dato suggeriva, perciò, che il Po fosse il fiume che in misura maggiore contribuiva al carico totale di PFOA riversato a mare dai principali fiumi europei. Concentrazioni così elevate sono in genere messe in relazione con la presenza di sorgenti puntiformi industriali lungo il corso del fiume (Prevedouros et al., 2006; Davis et al., 2007).

Successive indagini sul bacino del fiume Po hanno confermato i valori di concentrazione di acidi PFCA e di PFOS nell'acqua del Po e dei suoi principali tributari misurati nel progetto PERFORCE (Loos et al., 2008). Tutti i tributari, ad eccezione del fiume Tanaro hanno mostrato livelli di PFOA (1-20 ng/L) compatibili con una contaminazione diffusa. I livelli di PFOA nel fiume Tanaro, pari a 1270 ng/L, hanno suggerito la presenza di una fonte puntuale di PFOA nel bacino Bormida/Tanaro e il Tanaro è stato identificato come la maggior sorgente di PFOA del bacino del fiume Po.

A seguito dell'allarme generato da questi dati pubblicati a livello europeo, il ministero dell'Ambiente e della Tutela del Territorio e del Mare (MATT) ha stipulato con l'Istituto di Ricerca Sulle Acque del Consiglio Nazionale delle Ricerche (IRSA-CNR) una convenzione per la "Realizzazione di uno studio di valutazione del rischio ambientale e sanitario associato alla contaminazione da sostanze perfluoroalchiliche nel bacino del fiume Po e nei principali fiumi italiani" al fine di generare un quadro realistico sulla situazione italiana.

Durante i due anni di convenzione sono state svolte diverse attività, sia a livello di monitoraggio per l'identificazione di hot-spot e possibili sorgenti, sia per studiare l'impatto che i composti perfluorurati possono avere a livello delle comunità residenti in siti contaminati per determinare le possibili conseguenze ecologiche (http://www.irsa.cnr.it/ShPage.php?lang=it&pag=PFAS).

Il presente progetto di dottorato si è svolto nell'ambito di questo progetto finanziato ed ha avuto i seguenti obiettivi principali:

- Sviluppare un metodo analitico HPLC-MS con metodica di estrazione automatizzata per la determinazione multicomponente di acidi perfluoroalchilici

- Determinare la distribuzione di composti perfluorurati e le aree maggiormente impattate nel territorio italiano

- Stimare le emissioni di PFAA delle principali sorgenti urbane ed industriali

- Valutare l'effetto ecotossicologico su popolazioni e comunità bentoniche residenti in un sito contaminato da una sorgente puntuale industriale di PFAS

Schema della tesi

Capitolo 1: Sviluppo del metodo analitico

Per la determinazione dei composti perfluorurati in campioni acquosi è stato sviluppato un metodo in cromatografia liquida ad alta pressione e rivelazione in spettrometria di massa a triplo quadrupolo (UHPLC-MS/MS), con sorgente a ionizzazione negativa (ESI -electrospray ionisation), accoppiata a pre-concentrazione on-line del campione. Il metodo multiresiduale sviluppato ha permesso di identificare simultaneamente

durante un'unica analisi 12 composti perfluorurati (9 acidi perfluorocarbossilici e 3 acidi perfluorosolfonici, Tabella 2) e di limitare il pre-trattamento dei campioni ad un'eventuale centrifugazione per eliminare particelle che possano intasare il sistema d'iniezione e all'acidificazione del campione. La ridotta manipolazione del campione permette di evitare la contaminazione da composti perfluorurati per rilascio di polimeri fluorurati largamente diffusi nei materiali da laboratorio.

Lo sviluppo di tale metodo ha permesso inoltre di ridurre il volume di campione necessario a soli 5 mL, mantenendo limiti di rivelabilità, compresi tra 0,2 a 5,0 ng/L a seconda delle molecole, sufficienti per l'indagine ambientale. Ridurre i volumi di acqua campionata ha permesso di semplificare la fase di campionamento e trasporto dei campioni, rendendo possibile campagne di monitoraggio più vaste e con un numero maggiore di siti di campionamento. L'automazione del metodo ha permesso infine di processare in tempi relativamente brevi e con notevole risparmio di solventi per estrazione e cromatografia numerosi campioni raccolti nelle campagne di monitoraggio eseguite sul territorio italiano. Il tempo totale di analisi, comprensivo di estrazione e preconcentrazione del campione e ricondizionamento della colonna analitica, è solamente di 20 minuti. I principali vantaggi di questo metodo sono l'elevata produttività analitica, la limitata manipolazione del campione e la scarsa possibilità di errori grazie all'automazione del sistema.

La validazione del metodo è stata eseguita su diversi tipi di campioni di acqua ed ha dimostrato l'affidabilità del metodo applicato anche su campioni acquosi con matrici molto diverse come acque potabili, fluviali, lacustri, salmastre, di scarichi da impianti di depurazione industriali e urbani.

Lo sviluppo del metodo e la sua validazione è descritta nella pubblicazione "Michela Mazzoni, Marianna Rusconi, Sara Valsecchi, Claudia P. B. Martins and Stefano Polesello, An on-line solid phase extraction-liquid chromatography-tandem mass spectrometry method for the determination of perfluoroalkylated acids in drinking and surface waters", sottomesso per la pubblicazione al Journal of Analytical Methods in Chemistry, riportato al Capitolo 1.

Tabella 2: Elenco degli acidi perfluoroalchilcarbossilici e perfluoroalchilsolfonici analizzabili con il presente metodo

Composto	Sigla	Formula	N° Atomi Carbonio	Peso molecolare	N° CAS
Acido perfluorobutanoico	PFBA	$C_4HF_7O_2$	4	214	375-22-4
Acido perfluoropentanoico	PFPeA	$C_5HF_9O_2$	5	264	2706-90-3
Acido perfluoroesanoico	PFHxA	$C_6HF_{11}O_2$	6	314	307-24-4
Acido perfluoroeptanoico	PFHpA	$C_7HF_{13}O_2$	7	364	375-85-9
Acido perfluorottanoico	PFOA	$C_8HF_{15}O_2$	8	414	335-67-1
Acido perfluorononaonico	PFNA	$C_9HF_{17}O_2$	9	464	375-91-1
Acido perfluorodecanoico	PFDA	$C_{10}HF_{19}O_2$	10	514	335-76-2
Acido perfluoroundecanoico	PFUnDA	$C_{11}HF_{21}O_2$	11	564	2058-94-8
Acido perfluorododecanoico	PFDoDA	$C_{12}HF_{23}O_2$	12	614	307-55-1
Acido perfluorobutansolfonico	PFBS	$C_4HF_9O_3S$	4	300	375-73-5
Acido perfluoroesansolfonico	PFHxS	$C_6HF_{13}O_3S$	6	400	355-46-4
Acido perfluorottansolfonico	PFOS	$C_8HF_{17}O_3S$	8	500	1763-23-1

Lo sviluppo del metodo si è basato su un'ampia rassegna della letteratura scientifica che ha portato alla stesura delle seguenti pubblicazioni, non inserite nella presente tesi:

Valsecchi S., Rusconi M., Polesello S., 2013. Determination of perfluorinated compounds in aquatic organisms: a review. Analytical and Bioanalytical Chemistry, 405, 143-157

Rusconi M., Polesello S., Valsecchi S., 2014. Chapter 12. Liquid Chromatography-Mass Spectrometry for the Analysis of Perfluorinated Compounds in Water Samples in "Fast Liquid Chromatography-Mass Spectrometry Methods for Food and Environmental Analysis" (O. Núñez, H. Gallart-Ayala, C. Martins and P. Lucci eds), Imperial College Press, in press.

Capitolo 2: Indagine sui principali bacini idrici italiani

L'indagine svolta sul territorio italiano si è occupata di determinare la concentrazione dei PFAS nei principali bacini fluviali della penisola (bacini dei fiumi Po, Adige, Tevere, Arno e Brenta), coprendo un'area di circa il 40% sulla quale incide il 45% della popolazione italiana. Sono stati campionati 35 fiumi, appartenenti ai bacini sopra elencati, in stazioni di chiusura di bacino, mentre nel caso di Po, Arno e Tevere sono state condotte campagne di monitoraggio lungo l'asta del fiume per evidenziare le possibili sorgenti lungo il fiume. Laddove disponibile la portata istantanea o media della stazione di monitoraggio, sono stati calcolati i flussi di PFAS provenienti dai bacini o dai sottobacini. I dati di monitoraggio raccolti durante il progetto sono stati archiviati in un database relazionale georeferenziato, che sarà disponibile a breve sul portale europeo IP-Chem gestito dal Joint Research Centre di Ispra (VA) della Commissione Europea (http://ipchem.jrc.ec.europa.eu/). Negli stessi bacini fluviali sono stati anche campionate acque di falda o acque potabili, in generale di origine sotterranea tranne alcune acque di origine superficiale (come nel caso di Ferrara). In Tabella 2 è presentato un sommario statistico dei dati di monitoraggio nei bacini esaminati, nei diversi anni di campionamento.

L'analisi dei dati ha messo in luce che nei bacini interessati principalmente da territorio agricolo o da aree urbane, la concentrazione di PFAS nelle acque superficiali è spesso vicino al limite di rivelabilità o ai livelli di fondo, mentre le situazioni in cui le concentrazioni sono più elevate si riferiscono a fiumi nei cui bacini sono presenti insediamenti industriali che recapitano più o meno direttamente i propri reflui nel fiume.

I fiumi che apportano il maggiore flusso di PFAS a mare sono Po e Brenta , nei bacini dei quali sono presenti due stabilimenti industriali che producono rispettivamente polimeri fluorurati e intermedi fluorurati per la sintesi di polimeri; le loro emissioni raggiungono il 57% del carico totale di PFAS stimato. Entrambi questi fiumi sfociano nel mar Adriatico contribuendo con un carico di PFOS di circa 0.3 t/anno che può avere un impatto sull'ecosistema marino. Tra le altre attività produttive emergono come sorgenti di PFBS e di PFOA accoppiati ai PFCA a corta catena le industrie tessili e conciarie. L'impatto dovuto a questo tipo di attività è stato studiato nel bacino del fiume Arno, lungo il cui corso sono situati il distretto conciario di S. Croce sull'Arno e il distretto tessile di Prato che da solo produce emissioni equivalenti al carico domestico stimato dell'intera popolazione presente in tutti i bacini studiati.

I risultati sono riportati e discussi nella seguente pubblicazione che costituisce il Capitolo 2:

Valsecchi S., Rusconi M., Mazzoni M., Viviano G., Pagnotta G., Pagnotta R., Zaghi C., Serrini G., Polesello S., 2014. Occurrence and sources of perfluoroalkyl acids in Italian river basins. Chemosphere, http://dx.doi.org/10.1016/j.chemosphere.2014.07.044, Available online 6 August 2014.

Tabella 2 Sommario dei dati di PFAA nei fiumi, acque potabili e sotterranee italiane (LOD= Limite di rilevabilità)

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFBS	PFHxS	PFOS
FIUMI												
2010/2011												
102 campioni da 17 fiumi												
Mediana (ng/L)	<LOD	2	4	2	42	1	<LOD	<LOD	<LOD	3	<LOD	<LOD
90°percentile (ng/L)	15	30	70	40	895	7	4	1	0	16	<LOD	16
Frequenza di rilevamento (%)	10	52	79	72	87	62	35	12	10	44	0	43
2012/2013												
138 campioni da 35 fiumi												
Mediana (ng/L)	<LOD	<LOD	4	2	22	<LOD	<LOD	<LOD	<LOD	3	<LOD	6
90°percentile (ng/L)	110	72	82	48	974	13	11	4	3	75	<LOD	38
Frequenza di rilevamento (%)	43	43	70	59	87	39	49	34	20	61	6	70
ACQUE POTABILI												
2008/2011 (N= 34)												
Mediana (ng/L)	<LOD	<LOD	3	1	8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
90°percentile (ng/L)	<LOD	18	13	5	25	1	1	<LOD	<LOD	4	<LOD	14
Frequenza di rilevamento (%)	0	47	68	53	65	15	21	6	9	24	3	41
2012/2013 (N= 181)												
Mediana (ng/L)	<LOD	<LOD	<LOD	<LOD	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
90°percentile (ng/L)	55	14	20	15	171	1	1	0	2	50	31	86
Frequenza di rilevamento (%)	41	23	43	30	51	10	13	5	14	28	20	44
ACQUE SOTTERRANEE												
2012/2013 (N= 68)												
Mediana (ng/L)	<LOD	<LOD	2	<LOD	8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
90°percentile (ng/L)	24	6	26	63	1936	<LOD	<LOD	<LOD	<LOD	2	5	16
Frequenza di rilevamento (%)	28	25	62	40	71	4	4	4	4	28	12	44

Capitolo 3: Stima delle emissioni di PFAS

La ricerca delle sorgenti sul territorio ha evidenziato l'area del bacino del Lambro come sorgente significativa di PFAS senza però presentare un composto prevalente. Il bacino del Lambro presenta infatti diversi tipi di impatto antropico (urbano, agricolo e industriale) ed è un'area che oltre ad essere altamente urbanizzata, in quanto include l'area metropolitana di Milano, è anche la regione più industrializzata del Paese. Lo studio di quest'area ha reso possibile stimare separatamente il carico industriale da quello urbano poiché, oltre ai corsi d'acqua, sono stati campionati sei impianti di depurazione, tre dei quali collettano unicamente scarichi urbani di Milano, mentre gli atri tre invece collettano scarichi industriali nei distretti brianzoli del tessile e del mobile. Conoscendo la dimensione degli impianti di depurazione e le portate giornaliere dei reflui in ingresso e uscita è stato possibile calcolare il carico giornaliero o fattore di emissione pro capite dei PFAS analizzati: il carico in uscita dagli impianti depurativi che collettano scarichi industriali è risultato anche cinquanta volte superiore al carico in uscita dai depuratori urbani. Il bilancio di massa delle emissioni nel bacino del Lambro mostra una costante crescita nelle emissioni da nord a sud e rileva le differenze nella composizione degli omologhi da est a ovest, infatti l'area ovest del bacino è sorgente di PFBS che probabilmente è emesso dai distretti industriali del tessile e del mobile. Nel complesso il bacino del Lambro emerge come una delle principali sorgenti di PFAS per il fiume Po per quanto riguarda i composti perfluoroalchilsolfonici (PFBS e PFOS) che sono diffusi in molte lavorazioni dove è richiesta una impermeabilizzazione delle superfici, ma anche in altre attività come le cromature galvaniche e la produzione di schiume antiincendio.

I risultati sono riportati e discussi nella seguente pubblicazione che costituisce il Capitolo 3:

Castiglioni S., Valsecchi S., Polesello S., Rusconi M., Melis M., Palmiotto M., Manenti A., Davoli E., Zuccato E., 2014. Sources and fate of perfluorinated compounds in the aqueous environment and in drinking water of highly urbanized and industrialized area in Italy. Journal of Hazardous Materials, 282 (2015) 51–60. http://dx.doi.org/10.1016/j.jhazmat.2014.06.007

Capitolo 4: Effetti ecotossicologici su una comunità residente in un fiume contaminato da una sorgente industriale

Per valutare se la costante presenza di PFAS può avere degli effetti a lungo termine sulle popolazioni residenti in ambiente fluviale contaminato, è stato scelto come caso studio il fiume Bormida, corpo recettore dello scarico diretto dell'industria di polimeri fluorurati che è la principale sorgente di PFOA per il fiume Po.

Benché la pressione industriale sul fiume Bormida è stata molto alta fin dal secolo scorso (si ricordi il caso dell'Acna di Cengio), negli ultimi anni, tuttavia, dai monitoraggi periodici di ARPA Piemonte, il fiume ha mostrato di aver recuperato la qualità sia chimica che ecologica tanto da venire classificato nella classe di qualità buona. Nei monitoraggi dei PFAS eseguiti negli ultimi anni (Valsecchi et al., 2014), la concentrazione di PFOA a valle dello scarico del polo chimico è sempre risultato rilevante, variando tra 250 ng/L e 6480 ng/L, in funzione della portata. Questi dati suggeriscono che le popolazioni bentoniche residenti nel fiume a valle dello scarico per anni siano state esposte ad elevate concentrazioni di PFOA e altri composti fluorurati. L'obiettivo che ci siamo posti è stato dunque quello di valutare se la sorgente inquinante ha un impatto sulla struttura della comunità macrobenthonica residente a valle dello scarico e se si rilevano effetti permanenti sulla popolazione legati ad una possibile pressione selettiva generata del composto. L'approccio utilizzato per affrontare entrambi i quesiti è stato un confronto "monte-valle". Per rispondere alla prima domanda è stata

valutata la struttura della comunità nei due siti campionati. Le analisi eseguite hanno evidenziato una leggera differenza nelle due comunità che comunque non incide sulla qualità ecologica dei siti, valutata secondo i criteri del campionamento standard di monitoraggio.

L'analisi genetica è stata eseguita su individui di Hydropsyche modesta, taxon presente sia a monte che a valle dello scarico. Attraverso la tecnica molecolare Amplified Fragment Lenght Polymorphism (AFLP) sono stati genotipizzati venti individui della popolazione di monte e venti di quella di valle ed è stata valutata la varianza genetica tra le due popolazioni, evidenziando una limitata percentuale di varianza compatibile con una pressione che agisce non ubiquitariamente sul genoma ma solo su un numero limitato di loci. L'analisi di outlier loci non ha però determinato la presenza di una pressione selettiva. La divergenza genetica potrebbe quindi essere legata più a fattori ambientali che alla presenza del contaminante stesso.

Non è stato possibile però confermare questi dati con una ulteriore campagna di misura in quanto a valle dello scarico dell'industria di fluoropolimeri imponenti lavori di regimazione dell'alveo hanno modificato drasticamente l'area oggetto della prima indagine.

I risultati sono riportati e discussi nella seguente pubblicazione in preparazione che costituisce il Capitolo 4:

> Rusconi M., Bettinetti R., Marziali L., Mazzoni M., Polesello S., Stefani F., Valsecchi S. Impact of a fluoropolymer plant on a river ecosystem: evaluation using a combined chemical, ecological and genetic approach. *In preparation*

An on-line solid phase extraction-liquid chromatography-tandem mass spectrometry method for the determination of perfluoroalkylated acids in drinking and surface waters

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The authors declare that there is no conflict of interests regarding the publication of this paper

Abstract

An UHPLC-MS/MS multi-residue method based on an on-line SPE procedure was developed for the simultaneous determination of 9 perfluorinated carboxylates (from 4 to 12 carbon atoms) and 3 perfluorinated sulphonates (from 4 to 8 carbon atoms).

The transition from a chromatographic elution gradient separation, commonly used by an analysis performed by direct injection, to an on-line SPE elution gradient, including chromatographic separation, is proposed. Manual sample preparation was reduced to sample centrifugation and acidification, thus eliminating several procedural errors and significantly reducing time consuming and costs. Ionization suppression between target perfluorinated analytes and their co-eluting SIL-IS were detected for homologues with number of carbon atoms less than 9 but quantitation was not affected. Total matrix effect corrected by SIL-IS, inclusive of extraction efficacy and of ionization efficiency, ranged between -34 and + 39 %. The percentage of recoveries, between 76 and 134%, calculated in different matrices (tap water and rivers impacted by different pollutions) were generally satisfactory. LODs and LOQs of this on-line SPE method, which also incorporate recovery losses, ranged from 0.2 to 5.0 ng/L and from 1 to 20 ng/L respectively.

Validated on-line SPE-LC/MS/MS method has been applied in a wide survey for the determination of perfluoalkylacids in Italian surface and groundwaters.

Keywords

Perfluorinated compounds, water, on-line SPE, LC/ESI-MS/MS, drinking water, surface freshwater

1.   Introduction

In the last decade concern about perfluoroalkyl substances (PFAS) rapidly raised in the scientific community because of their worldwide distribution in the different environmental compartments [1-4]. This class of chemicals have been used into a wide range of industrial and consumer products for the past six decades mainly to repel dirt, water and oil [5-6]. PFAS includes thousands of chemicals but the environmental studies have been concentrated mainly on two classes of perfluoalkylacids (PFAA), i.e. perfluorosulfonic acids (PFSA), which include perfluorooctanesulfonic acid (PFOS), and perfluorocarboxylic acids (PFCA), which include perfluorooctanoic acid (PFOA). PFSA and PFCA are low molecular weight surfactants in which all carbons are bonded to fluorine atoms, consisting of homologous series of molecules that differ in carbon chain length. PFOS and PFOA have been demonstrated to be persistent in the environment and bioaccumulative in the trophic chain. The accumulation in the aquatic trophic chain poses concern about the risks for the end consumers, including humans. After a risk assessment study, the European Commission very recently introduced PFOS in the list of priority hazardous substances which must be monitored in the EU water bodies, setting an Environmental Quality Standard (EQS) of 0.65 ng/l for freshwater [7], while US Environmental protection Agency (EPA) proposed a Provisional Health Advisories of 400 ng/l and 200 ng/l respectively for PFOA and PFOS in drinking waters [8].

The introduction of regulatory restrictions in the use of PFOS and PFOA [9-10] induced the major PFAS producers to find other substitutes for these compounds especially among the congeners with different chain lengths. These series of homologues, usually from 4 to 14 for PFCA and from 6 to 10 for PFSA show vary different physico-chemical behaviours which put a serious challenge for the simultaneous determination of PFAA in water samples.

Solubility strongly decreases by increasing the chain length, e.g. from 100 g/L for PFHpA to 0.1 g/L for PFUnDA [11-12], while acidity decreases as the chain length increases (pKas vary from 0.1 to 3.8 in the range PFBA – PFDoDA) [13 and references therein]. The increase of number of CF2 moieties also leads to a significant increase in lipophilicity expressed as pKow: e.g. from PFHxA to PFDoDA pKows increase from 3.68 to 9.21 [13 and references therein]. Generally for a certain chain length sulfonates are more lipophilic than carboxylates. All these physico-chemical variables drastically influence recoveries with classical extraction techniques such as Liquid-Liquid Extraction (LLE) or Solid Phase Extraction (SPE). According to the review of Jahnke and Berger [14] LLE worked best for PFAA with carbon chain lengths > 7 whereas SPE was best suited for PFAA with < 10 carbon atoms. Major drawbacks of the SPE approach in PFAA analysis are the sample contamination and possible losses of the surface-active PFAA to container walls and other materials (tubing, connections) besides the problems inherent in SPE such as breakthrough and clogging of the column [14]. Moreover, it requires the use of large sample volumes followed by solvent evaporation, which translates into the extension of the analysis time to hours or even days.

Since many years the capability of SPE of being automatized has been exploited [15-16] and on-line SPE coupled to LC-MS has been also applied to the analysis of PFAA in water samples [17-21]. The online SPE made possible the development of faster methods by reducing the analysis time and thus increasing the analytical

productivity [16]. The challenge of the on-line SPE methods is still to optimize pre-concentration and elution procedures to achieve a satisfactory accuracy in a single run for a class of compounds with different physico-chemical variables such as PFAA. In addition, the coupling of on-line SPE with UHPLC is not easy due to the high back pressure generated from the combination of a high flow rate with low particle size column (<2μm). Furthermore, it is common to have release of PFAA (mainly PFOA) from the UHPLC hardware since polytetrafluoroethylene is often used in the manufacturing of tubing, fittings and seals in UHPLC system. An extra column can be placed before the injector to delay the elution of PFAA from the pump and distinguish between PFAA from sample and from the configuration/material used. This means that the system may contain up to 3 columns connected in-line (trap, pre-concentration and chromatographic column).

The aim of our work is to explore the possibility of optimizing an on-line SPE/UHPLC-MS/MS method for PFCA and PFSA ranging respectively from 4 to 12 and from 4 to 8 carbon atoms, understanding the factors that more influence the method accuracy. The optimized and validated method has then applied to a large survey of Italian surface and drinking waters whose preliminary results are presented in the present paper.

2.   Experimental

2.1.   *Chemicals and reagents*

Perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), tetrabutylammonium perfluorobutane sulfonate (PFBS), potassium perfluorohexane sulfonate (PFHxS) and tetrabutylammonium perfluorooctane sulfonate (PFOS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Separate stock solutions of the analytes were prepared in methanol at a concentration of 1.0 mg/mL of the anionic compound. A multicomponent standard solution containing the 12 analytes at 10 μg/mL was prepared diluting the stock solutions. Aqueous standard solutions (2-200 ng/L) containing all analytes were freshly prepared by serial dilution of the methanol standard mixed solution in clean tap water. Stable isotope-labelled PFCA and PFSA internal standard compounds (SIL-IS) were purchased from Wellington Laboratories (Guelph, ON, Canada) in 2 μg/mL solution mixtures. SIL-IS were 13C4-PFBA , 13C2-PFHxA, 13C4-PFOA, 13C5-PFNA, 13C2-PFDA, 13C2-PFUnDA, 13C2-PFDoDA, 18O2-PFHxS and 13C4-PFOS. The solution of SIL-IS was diluted to a concentration of 40 ng/mL with methanol. All standard solutions were stored at + 4°C. All reagents were analytical reagent grade. Supelco-Supelclean EnviCarb®, LC-MS Chromasolv® methanol, LC-MS Chromasolv® acetonitrile, ammonium acetate (99%), concentrated formic acid were purchased from Sigma–Aldrich. Water (<18 MΩcm resistivity) was produced by a Millipore Direct-QUV water purification system (Millipore, Bedford, MA, USA).

2.2.   *Samples*

Samples were collected from Italian water bodies between 2010 and 2013. The surveyed river basins were rivers Po, Adige and Brenta in Northern Italy and rivers Arno and Tevere in Central Italy. In the river Po basin, samples were collected also from the main tributaries Bormida, Tanaro, Ticino, Lambro, Adda, Oglio and Mincio (Figure 1). River waters were collected by means of a bucket at the centre of the river bed. Samples from lake (lake Como) and transitional environments (lagoons of Venice and Goro in the Po Delta) were directly collected in the sampling vials 1 meter below the water surface. Groundwaters were also collected between
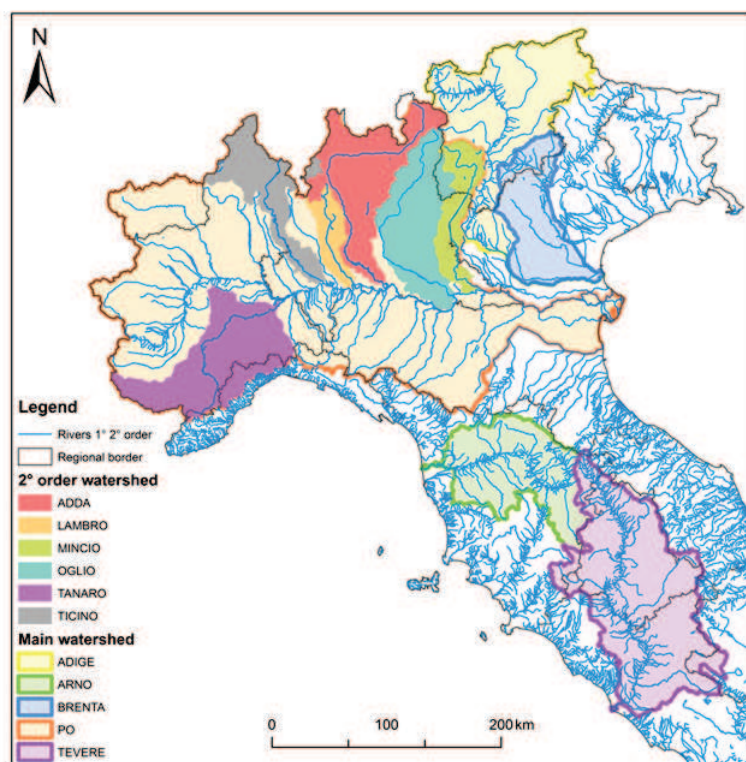
Figure 1. Watersheds of the first and second order rivers which were sampled.

2010 and 2012 from wells or piezometers. The samples were collected in polypropylene (PP) centrifuge tubes and kept in a refrigerator at 4 °C until analysis. Analyses were carried out within the next 5 days. Contamination or adsorption of target compounds onto tube surfaces were assessed by analysing selected samples (3 poorly contaminated drinking water samples, 3 poorly contaminated river samples and 3 highly contaminated river samples) just after collection and after 1 month, without significant differences in the resulting concentrations. In order to prevent the clogging of any parts of the injector or column all aqueous samples were centrifuged. Before injection, in the final analytical procedure, standards and

samples were acidified to pH 3 by adding, directly into the glass vials of the autosampler, 50 µl of concentrated formic acid to 10 mL of sample.

For native spiking experiments, known volumes of standard mixed solution containing the 12 analytes were added to aqueous sample to give a target concentration of 100 ng/L. For SIL-IS spiking experiments 25 µL of the diluted SIL-IS solution were added to native-spiked aqueous samples to obtain concentrations of 100 ng/L both of native and of stable isotope labelled perfluorinated compounds.

2.3. On-line SPE and analytical separation

The on-line SPE has been carried out by a Thermo EQuan system which consists of two Thermo Scientific Accela
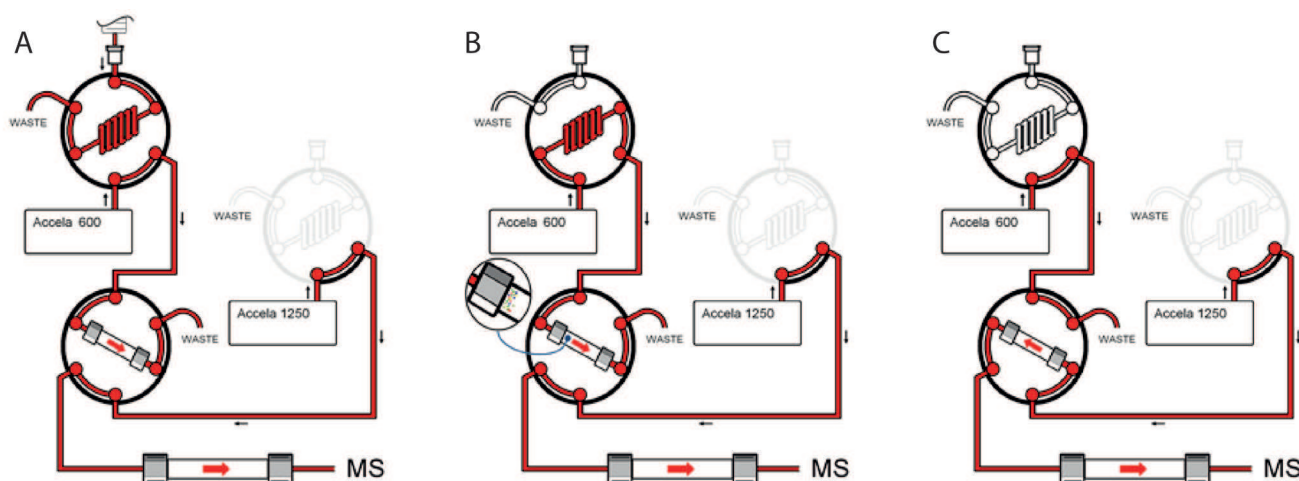
Figure 2. Schematic representation of the on-line SPE system used. A) Loading of the sample into the high volume loop. B) Transfer of the sample from the injection loop to the preconcentration column. C) Transfer of the analytes retained in the preconcentration column to the chromatographic column.

LC pumps (600 and 1200) with a pre-concentration column (Thermo Hypersil GOLD aQ 12 μm, 20 × 2.1 mm), an analytical column (Thermo Hypersil GOLD PFP 1.9 μm, 50 × 2.1 mm), a CTC PAL autosampler equipped with three 6-way VICI valves (Figure 2). Samples were injected into a high volume loop (figure 2A) and then transferred onto the pre-concentration column by the loading pump (Thermo Scientific Accela 600) using 2 mM ammonium acetate (NH4OAc) with 5% methanol (MeOH) eluent at 1200 μL/min (Figure 2B). When the enrichment step was completed (260 s), a 6-way valve on the auto-sampler switched over and the elution pump (Accela 1200) flowed the elution gradient, composed by two eluents [(A) 2mM NH4OAc -5% MeOH and (B) methanol] at 300 μL/min, through the pre-concentration column to the analytical column (Figure 2 C). The loading and the elution gradients are illustrated in Table 1. In order to delay the interfering background peaks of perfluorinated compounds, which are present in solvents or are released from the analytical system, a trap column (Thermo Hypersil GOLD 1.9 μm, 50 × 2.1 mm) has been placed between the analytical pump and the injection valve.

2.4.   Mass spectrometry

A triple quadrupole mass spectrometer (Thermo Scientific TSQ Quantum Access MAX) equipped with a heated-electrospray ionization (HESI-II) probe was used. The source-dependent parameters were as follows: spray voltage (3000 V); sheath gas pressure (25 psi); auxiliary gas pressure (10 arbitrary units); skimmer offset (0 V), ion transfer tube temperature (270 °C), vaporizer temperature (40 °C), high purity argon (>99.98%) was used as the collision gas (1.5 mTorr). The mass spectrometer operated at a resolution of 0.7 Da in negative selected reaction monitoring (SRM) mode. Table 2 lists the MS/MS transitions, tube lens offset and collision energies applied for the different target analytes and isotope labelled standards. The Xcalibur. 2.1 (Thermo Scientific) was used for instrument control, data acquisition and processing.

3.   Results and discussion

3.1.   Method development

In a SPE procedure, several experimental variables, such as sample volume and sample pH, should be optimized in order to achieve the maximum extraction efficiency of the target analytes. If a multi-residual analysis of a series of analytes with a broad polarity range is carried out, it may be difficult to achieve reliable results for all target compounds. Ideally by the time the extraction column is switched into the analytical flow path, the trapped analytes should be eluted and re-focused onto the analytical column by the analytical elution gradient. However in multi-residual analysis, the gradient elution for reversed-phase separations usually starts with high percentage of aqueous in the mobile phase, and the slow elution from the SPE pre-concentration column results in peak broadening, which may cause a decrease in sensitivity [22-23]. For these reasons, before carrying out the method validation, the evaluation of the effect of the elution gradient, sample volume as well as the effect of matrix modification of the aqueous sample was performed in order to achieve the best recovery and sensitivity for the on-line SPE/UHPLC-MS/MS analysis of the perfluorinated carboxylic and sulfonic acids.

3.1.1.   Optimisation of the elution gradient

Optimisation of the analytical separation was carried out by direct injection of standard solutions with a 25μL loop. The elution gradient started with only the 5% of solvent mobile phase B (methanol) in order to analyse also the shorter chain PFCA (PFBA and PFPeA). An initially fast gradient with mobile phase B raising from 5 to 70 % within 2 min followed by a slower gradient with mobile phase B raising up to 100% within 5 min allowed

the separations of all perfluorocarboxylic acids and the elution of the longer chain homologues (PFUnDA and PFDoDA) in 5 min. Then 100 % of methanol was used for 3.5 min followed by 95 % of mobile phase A reached within 1 min and by a conditioning step of 4 min for the following analysis.

In the on-line SPE method, during the sample loading step, analytes are trapped by the stationary phase of the pre-concentration column. Then elution of analytes is achieved in back-flush mode by putting in-line the pre-concentration column with the eluting mobile phase.

The first approach was to transfer the separation gradient elution program, optimised by direct injection of analytes, to the on-line procedure. In this case (Table 1: "unchanged gradient") the gradient program started soon after the end of the loading of the sample into the pre-concentration column (4.34 min). Throughout the so called loading time, an isocratic flow with the initial gradient conditions (5% methanol) is used in the chromatographic column. By using these settings, peak broadening and distortion were observed for the shorter chain and more polar homologues (PFBA and PFPeA), because these compounds, which show lower affinity for the pre-concentration column stationary phase, were poorly focused on the pre-concentration column.

Table 1. Elution gradients used by the loading pump and the elution pump. Elution pump flowed at 300 µl/min. Loading time was 260 s. Sample volume was 5 mL.

Time (min)	Elution pump (unchanged gradient) A%	B%	Elution pump (plug gradient) A%	B%	Elution pump (early gradient) A%	B%	Loading pump Flow (µL/min)	A%	B%
0	95	5	95	5	95	5	1200	100	0
3.00					95	5			
3.99			95	5					
4.00			20	80					
4.34	95	5							
4.50			20	80			1200	100	0
4.51			65	35					
4.75			55	45					
5.00			30	70	30	70			
6.00			30	70					
6.34	30	70							
6.50							200	10	90
10.00					0	100			
11.00			0	100					
11.34	0	100							
11.50							200	10	90
13.50					0	100			
14.50			0	100	95	5	1200	100	0
14.84	0	100							
15.50			95	5	95	5			
15.84	95	5							
16.50	95	5	95	5			1200	100	0

To overcome this problem the "solvent plug injection technique" [22] was implemented. This technique provides elution bands of only a few seconds width with high percentage solvent in a way to obtain a rapid transfer of the analytes from the pre-concentration column to the analytical column as well as to keep them focused. Gode et al. [22] generated short plugs of high-elution strength solvent by means of an external loop and an additional LC-pump. We achieved the same high-elution strength solvent plugs by using the separation UHPLC pump which is characterised by very low dead volumes. A high percentage solvent step (80 % B) was inserted in the eluting gradient at the switching time, in order to provide narrow high-elution strength eluent band containing all the eluted analytes (Table 1: "plug gradient"). The subsequent elution gradient was modified in order to obtain a chromatographic separation similar to the one used for direct injection of the sample (Table 1).

The effects of the different elution gradient are reported in figure 3A which shows the height ratios between the analyte peaks detected with the plug gradient and the analyte peaks obtained with the unchanged gradient. Both early- and late- eluting perfluorocarboxylic acids benefited from "plug gradient" by improving the shape of their peaks whereas no significant effects were detected for PFSA. The peak heights improved up to 3.7 times for the less retained PFCA. Nevertheless the optimization of the "solvent plug injection technique" is laborious and high time-consuming because involves several runs to find out the best high-elution strength solvent and
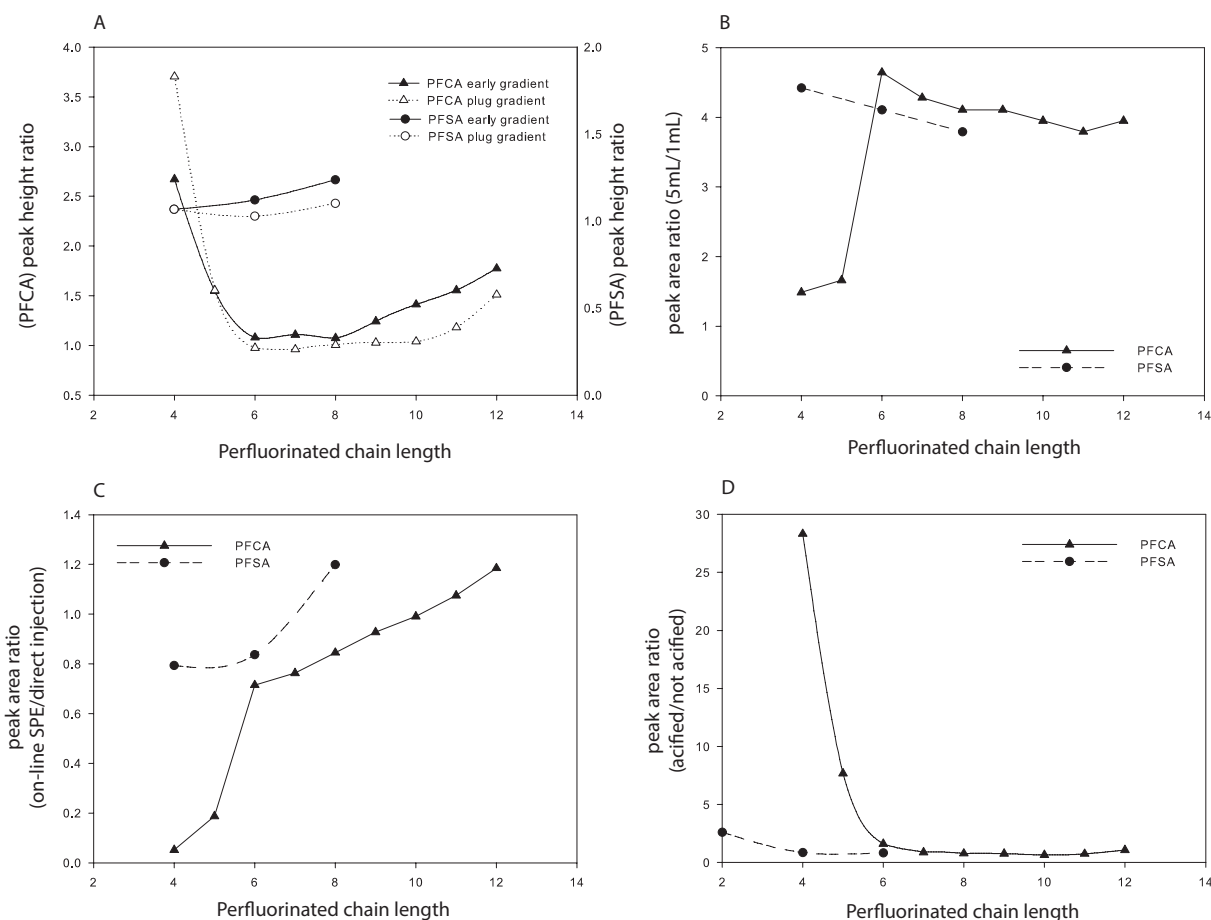
Figure 3. Development of the on-line SPE method: (A) Effect of different elution gradients, "plug gradient" or "early gradient" compared with "unchanged gradient", on the analyte peak height; injection of 5 mL of acidified aqueous standard at 200 ng/L. (B) Effect of the sample volume on the peak area; injection in "early gradient" mode of aqueous standard at 200 ng/L. (C) Extraction efficiency of the 5 mL on-line SPE injection volume; on-line SPE injection in "early gradient" mode of 5 mL of aqueous standard at 200 ng/L and direct injection of 25 µL of aqueous standard at 40 µg/L. (D) Effect of acidification of the sample on the peak area; injection in "early gradient" mode of aqueous standard at 200 ng/L.

Table 2. LC/MS/MS parameters for all target analytes and internal standards

Compound	Abbreviation	Precursor ion (*m/z*)	Product ion		
			(*m/z*)	Collision Energy	Tube Lens Offset
Target analytes					
Perfluorobutanoic acid	PFBA	212.9	168.9	11	80
perfluoropentanoic acid	PFPeA	262.9	69.0	39	85
			218.9	11	85
perfluorohexanoic acid	PFHxA	312.9	119.1	22	86
			268.9	11	86
perfluoroheptanoic acid	PFHpA	362.9	169.0	18	91
			318.9	12	91
perfluorooctanoic acid	PFOA	412.9	169.0	19	96
			368.9	13	96
perfluorononanoic acid	PFNA	462.9	218.9	18	104
			418.9	13	104
perfluorodecanoic acid	PFDA	512.9	268.9	18	97
			468.9	13	97
perfluoroundecanoic acid	PFUnDA	562.9	268.8	20	96
			518.8	14	96
perfluorododecanoic acid	PFDoDA	612.9	318.8	20	107
			568.9	14	107
Perfluorobutane sulfonate	PFBS	298.9	80.2	44	85
			99.1	32	85
Perfluorohexane sulfonate	PFHxS	398.9	80.1	38	91
			99.0	34	91
Perfluorooctane sulfonate	PFOS	498.9	80.3	45	104
			99.1	45	104
Internal standards					
Perfluoro-n-[$^{13}C_4$] butanoic acid	$^{13}C_4$-PFBA	216.9	171.9	11	80
Perfluoro-n-[$^{13}C_2$] hexanoic acid	$^{13}C_2$-PFHxA	314.9	269.9	11	86
Perfluoro-n-[$^{13}C_4$] octanoic acid	$^{13}C_4$-PFOA	416.9	371.9	13	96
Perfluoro-n-[$^{13}C_5$] nonannoic acid	$^{13}C_5$-PFNA	467.9	422.9	13	104
Perfluoro-n-[$^{13}C_2$] decanoic acid	$^{13}C_2$-PFDA	514.9	469.9	13	97
Perfluoro-n-[$^{13}C_2$] undecanoic acid	13C$_2$-PFUnDA	564.9	519.8	14	96
Perfluoro-n-[$^{13}C_2$] dodecanoic acid	$^{13}C_2$-PFDoDA	614.9	569.9	14	107
Perfluoro-n-hexane [$^{18}O_2$] sulfonate	$^{18}O_2$-PFHxS	402.9	103.0	34	91
Perfluoro-n- octane [$^{13}C_4$] sulfonate	$^{13}C_4$-PFOS	502.9	99.1	45	104

elution band width as well as the subsequent elution gradient in order to avoid loss of retention along with severe peak distortion and artefact formation [22].

Finally we tested a further and simpler approach: we anticipated the starting of the gradient program in separation column when the loading on pre-concentration column is still on going, and in this way, when the mobile phase is switched on the pre-concentration column, the mobile phase composition, having an higher solvent percentage, is able to transfer quickly analytes on the separation column with a focusing effect (Table 1: "early gradient"). The optimum mobile phase composition at the switching time is determined by calculating the composition when the first peak (PFBA) elutes in direct injection analysis. Improvements in the PFAA peak heights, compared with "unchanged gradient", were analogous to those of the "injection plug" and still better for the longer chain PFCA (Figure 3A). Because of its simplicity the "early gradient" was chosen for the rest of the optimization procedure.

3.1.2. Sample volume

Sample volume effect was evaluated comparing the peak areas obtained by injecting 1 and 5 ml of aqueous standard (200 ng/L). Proportionality was satisfactory (Figure 3B), with an average response ratio of 4.1, for PFSA and PFCA from 6 to 12 carbon atoms, despite a slight peak broadening. Only PFBA and PFPeA did not show proportional improvement in the response when the loop volume was increased, suggesting that the shorter homologues in the perfluorocarboxylic acid series have smaller breakthrough volumes on the concentration column.

The extraction efficiencies of 5 mL on-line SPE injection volume were determined comparing the peak areas obtained analysing aqueous standards by the on-line SPE method and those obtained from 25 µL direct injection at the same injected mass (100 pg). As shown in figure 3c ratios of peak areas close to 1 (0.75-1.2) were obtained for all PFSA homologues and the PFCA with carbon chain length greater than 6, indicating an efficient extraction for these compounds. Differently the response of the more soluble PFCA (PFBA and PFPeA) determined by on-line SPE method was much less the response obtained by direct injection (0.1-0.2) suggesting poor affinity for the pre-concentration column stationary phase.

3.1.3. Chemical modification of the sample before injection

Since all target analytes are acid compounds, samples were acidified to pH 3 by adding 50 µl of concentrated formic acid in order to improve their retention on the endcapped C18 phase of the pre-concentration column. Acidification significantly improved the peak areas of the less retained and more soluble homologues (PFBA, PFPeA, PFHxA and PFBS) whereas no effects were pointed out for the homologues with a longer carbon chain (Figure 3D). This is due to the fact that the acidification suppresses the ionization of the PFCA, and thereby increases their affinity for the reverse stationary phase of the pre-concentration column.

The effect on the LC-MS/MS response for the PFAA was also examined as a function of the % of solvent (acetonitrile and methanol) in the water samples. Addition of organic solvent (10%) caused a decrease in the area response for shorter and longer-chain perfluorocarboxylic homologues (peak area ratio ranging from 0.65 to 0.75). However, this effect was not observed when injecting 25 µL of the aqueous standards directly onto the analytical column without pre-concentration step: in this case peak areas and symmetry increased with perfluorinated carbon chain in standard prepared in water and methanol. The response decrease with solvent addition in pre-concentration may be attributed to a loss of retention on the pre-concentration column, when

compounds partially dissolved in solvent, are transferred to the pre-concentration column.

3.2.   Method validation

The optimised method (early gradient), with 5 ml injection volume and acidified samples, was subjected to the validation procedure.

3.2.1.   Matrix effect and mutual suppression effect

LC-ESI-MS/MS is the most suitable analytical technique for the analysis of the target compounds though it may suffer of matrix effects on the ionization efficiency [24 and reference therein, 25]. Matrix effects on the ionization in ESI-MS/MS determination are usually evaluated by comparing the signal responses of target compounds in matrix extracts, spiked immediately before instrumental analysis, with those in standard solutions. But when the extraction step is on-line connected with the HPLC separation the effect of sample matrix on extraction efficacy cannot be evaluated separately from the matrix effect on efficiency of ionization. Total matrix effects in the on-line SPE analysis of target compounds were determined analysing native-spiked aqueous samples at 100 ng/L level. In order to explore different matrices drinking water produced from ground water (TW1 and TW2) and from lake water (TW3), and water samples collected in low polluted rivers (RW1 and RW3), in a river impacted by industrial discharges (RW2) and in an organic polluted river (RW4) were chosen. Figure 4A shows the difference, in percentage, of the concentration measured with respect to the addition. Signal response suppression was more common than enhancement. Total matrix effect ranged between -70 and + 35 with more than 80% of the results between ± 30 %. Suppression of the signal response of the target compounds were found in some samples, both drinking and river water, for short as well as long carbon chain perfluorinated homologues (Figure 4). We tested the matrix removal by dispersive SPE clean-up with activated graphitized carbon (ENVI-Carb), which has been successfully employed to purify methanol and acetonitrile extracts for PFAA analysis [26], but very low recoveries of the target analytes in aqueous samples (results not showed) were achieved. We explored, thus, the utilisation of SIL-IS analogues to correct matrix effects on ionization efficiency as well as to compensate for variation in injection, sample extraction and instrumental parameters. For some target compounds the peak area of the co-eluting SIL-IS decreased with increasing native analyte concentration. Figure 5a shows the PFOA and PFHxS results obtained in the analysis of calibration standards spiked with SIL-IS at 100 ng/L level. This effect was also observed with a 25 μL injection directly onto the analytical column indicating that this is not due to a competition on the active site in the pre-concentration column but it is a suppression effect in the electrospray. Previous reports have noted mutual ionization suppression between target analytes and their co-eluting SIL-IS [27-28] due to the competition among ions for the limited number of excess charge sites on the generated droplet during electrospray ionization [29-30]. Liang et al. [28] investigated this phenomenon for nine drugs: generally, the extent of suppression in each drug-SIL-IS pair was concentration-dependent and was correlated with the hydrophobicity of the compounds. Maximum suppression was calculated for each perfluorinated SIL-IS at 200 ng/L native concentration (Figure 5B). The suppression of PFCA decreases with the increase of the number of carbon chain length but the suppression of PFSA is lower for the shorter-chain homologues. Quantitation, however, was not affected by the variation in the peak area of the internal standard. Mutual suppression effects of target analytes and SIL-IS are equal and this ensures good linearity of peak area ratio vs. analyte concentrations [27]. Results of Figure 4A were recalculated using SIL-IS linear calibrations (Figure 4B). The scattering of the results was significantly reduced: total matrix effect ranged between -34 and + 39 %. SIL-IS successfully corrected the effects on the
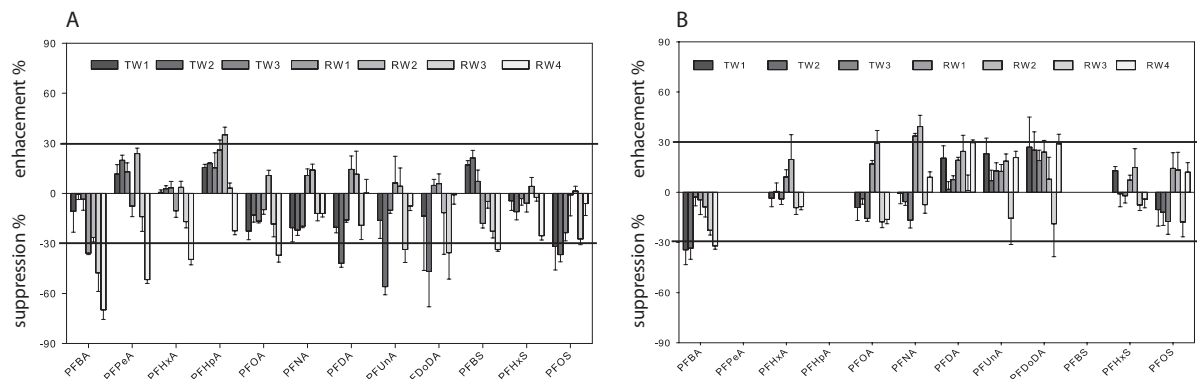
Figure 4. Not corrected (A) and corrected by SIL-IS (B) total matrix effects (%). TW1 and TW2 are drinking water samples produced from ground water; TW3 is a drinking water sample produced from lake water. RW1 and RW3 are water samples collected in low polluted rivers; RW2 and RW4 are water samples collected in a river impacted by industrial discharges and in an organic polluted river respectively. Spiked concentration of native and co-eluting SIL-IS at 100 ng/L level. n.d. not determined.
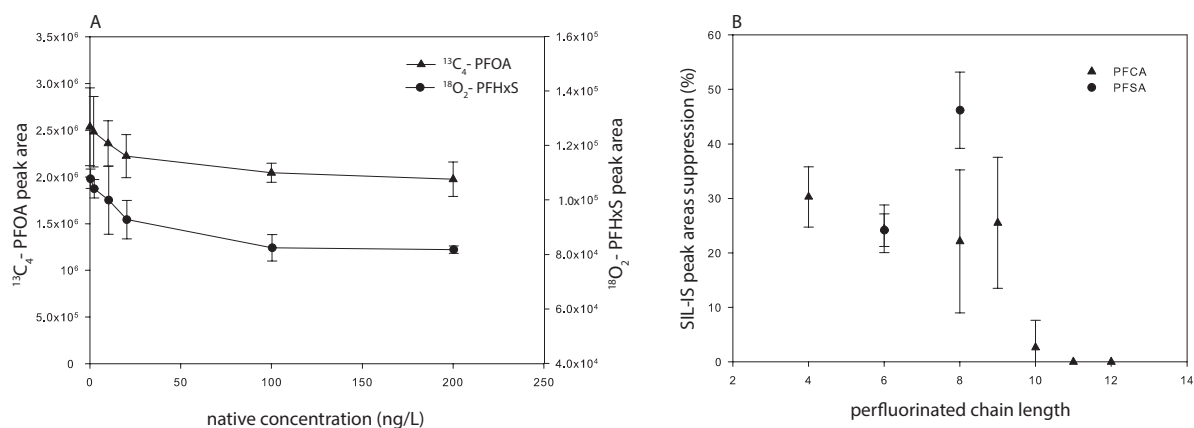
Figure 5. Effect of native concentration on co-eluting SIL-IS peak area. (A) Injection in "early gradient" mode of 5 mL of acidified aqueous standards spiked with 100 ng/L of SIL-IS (B) Extent of suppression of SIL-IS peak area at 100 ng/L was calculated as: (SIL-IS peak area of 0 ng/L native standard solution minus SIL-IS peak area of 200 ng/L native standard solution)/ SIL-IS peak area of 0 ng/L native standard solution X 100.

Table 3. Validation parameters (injection in "early gradient" mode of 5 mL of acidified standards and samples)

	Linearity[a]		Precision[b]		Trueness (%)[c]		Sensitivity (ng/L)[d]	
	(ng/L)	R[2]	Intra-day	Inter-day	TW	RW	LOD	LOQ
PFBA	2-200	0.955	7-27	10-33	76	83	5.0	20
PFPeA*	2-200	0.962	10-32	10-28	115	88	2.0	4
PFHxA	2-200	0.969	2-8	8-33	98	103	0.2	1
PFHpA*	2-200	0.970	2-9	6-14	116	111	0.2	5
PFOA	2-200	0.963	1-13	8-12	91	103	0.5	3
PFNA	2-200	0.960	4-12	10-16	92	119	0.5	1
PFDA	2-200	0.985	10-20	16-39	110	119	0.5	1
PFUnDA	1-100	0.972	10-31	24-53	119	109	0.5	1
PFDoDA	1-100	0.971	10-35	30-65	134	112	1.0	2
PFBS*	2-200	0.990	6-28	11-44	115	80	1.0	10
PFHxS	2-200	0.970	4-13	10-25	103	103	5.0	20
PFOS	2-200	0.989	8-49	14-48	87	105	2.5	10

* values not corrected by SIL-IS; [a] linearity was calculated with the analysis of five levels of aqueous standard solutions;
[b] precision is calculated by injecting standards in the 1-100 ng/L range for PFUnDA and PFDoDA and 2-200 ng/L range for the other target compounds (intra-day: n=3; Inter-day: n=12);
[c] trueness is expressed as recoveries determined by analysing native-spiked tap waters (TW) and river waters (RW) at 100 ng/L level;
[d] Sensitivity was estimated as three-fold (LOD) and ten-fold (LOQ) the standard deviation of the aqueous standard solution at the lowest injected concentration.

ionization as well as any eventual losses during the on-line extraction step.

3.2.2. *Linearity and sensitivity*

The linearity of the instrumental response was assessed by injecting five levels aqueous standards with native analyte concentration spanning two orders of magnitude (1-100 ng/L range for PFUnDA and PFDoDA and 2-200 ng/L range for the other compounds) spiked with SIL-IS at 100 ng/L level. Working calibration curves presented good linearity range and coefficients of determination (R2) higher than 0.95 for all target compounds (Table 3). Limit of detection (LOD) and limit of quantification (LOQ) were estimated as three-fold and ten-fold, respectively, the standard deviation of the aqueous standard solution at the lowest injected concentration (1 and 2 ng/L). LODs and LOQs ranged from 0.2 to 5.0 ng/L and from 1 to 20 ng/L respectively (Table 3). It is important to emphasize that these values refer to the whole analytical procedure, incorporating mutual ionization suppression and recovery losses, while the detection limits estimated from solvent calibration curves usually underestimate the true values.

Table 4. Summary of PFAS concentrations in Italian surface and groundwaters

Samples	Monitoring period	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFBS	PFHxS	PFOS
Rivers in Northern Italy	2010-2013												
(n° samples= 222)	min (ng/L)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	median(ng/L)	<LOD	<LOD	4	2	28	<LOD	<LOD	<LOD	<LOD	3	<LOD	3
	max (ng/L)	411	974	892	946	6.480	174	99	58	19	4.328	36	218
	% positive sample	28	48	75	64	88	46	43	25	16	65	4	55
Rivers in Central Italy	2011-2013												
(n° samples = 26)	min (ng/L)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	median(ng/L)	16	<LOD	3	2	14	2	<LOD	<LOD	<LOD	<LOD	<LOD	3
	max (ng/L)	79	15	40	29	222	30	51	11	4	335	<LOD	34
	% positive sample	73	31	58	69	73	69	35	12	8	42	0	62
Transitional waters	2011-2013												
(n° samples = 14)	min (ng/L)	<LOD	<LOD	1	<LOD	1	<LOD	<LOD	<LOD	<LOD	1	<LOD	<LOD
	median(ng/L)	<LOD	2	2	<LOD	5	<LOD	1	1	2	5	<LOD	4
	max (ng/L)	31	8	4	1	19	<LOD	1	1	3	10	<LOD	9
	% positive sample	14	64	100	7	100	0	64	79	93	100	0	57
WWTP effluents	2011-2013												
(n° samples = 18)	min (ng/L)	<LOD	<LOD	<LOD	1	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	median(ng/L)	<LOD	<LOD	38	22	78	16	14	4	<LOD	<LOD	<LOD	<LOD
	max (ng/L)	<LOD	582.881	811.431	171.231	712.877	1.080	1.414	1.128	4	4.833.734	<LOD	18.688
	% positive sample	0	44	72	100	100	94	94	78	39	17	0	28
Groundwaters	2010-2012												
(n° samples =77)	min (ng/L)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	median(ng/L)	<LOD	<LOD	0.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	max (ng/L)	20	22	37	31	53	13	16	9	13	29	45	121
	% positive sample	67	29	48	40	47	17	16	12	16	38	22	45

3.2.3. Accuracy

Method precision has been evaluated by 3 replicate injections of standards in the 1-100 ng/l range for PFUnDA and PFDoDA and 2-200 ng/l range for the other compounds on 4 not consecutive days (Table 3). Repeatabilities (intra-day precision) and reproducibilities (inter-day precision) were under 20% for carboxylic acid homologues from 7 to 9 carbon atoms while PFBS, PFHxS, PFOS, PFBA, PFPeA, PFHxA, PFDA, PFUnDA and PFDoDA showed RSD from 30 to 65% at the lower limit of the explored range which was very close to their detection limits. Trueness is expressed as recoveries determined by analysing spiked drinking and river waters since no certified reference materials were available for PFAA in these matrices. The recovery values were between 76 and 134 % for PFCA and between 87 and 115 % for PFSA.

4.   Applications to real samples

The validated on-line SPE/LC-MS/MS methodology has been applied in a wide survey of the PFAA concentrations in Italian surface and drinking waters with the aim to get a reliable picture of PFAA contamination and sources in Italian waters. The low volumes needed for the on-line extraction allow us to minimise the duration of the sampling campaigns, avoid the need for large refrigeration system during the travel and thus minimise the risk of sample degradation and contamination during the transport to the laboratory.

Data summarized in Table 4 showed that the method can be applied to the monitoring of different typologies of waters, including complexes matrices such as saline waters of coastal lagoons and discharges from industrial and urban wastewater treatment plants (WWTP).

Results of monitoring of Italian surface waters have been recently discussed by the Authors [31].

Monitoring data of rivers in Northern Italy, which includes rivers in the basins of river Po, Adige and Brenta which discharges in the Adriatic Sea, shows that the highest concentrations were measured for PFOA and PFBS, while the highest detection frequencies were found for PFOA, PFHxA and PFBS. The highest concentrations were measured downstream the discharges of perfluorinated compounds and polymers factories, but the detection frequency suggests that the same compounds are diffused in many rivers of the explored territories. Rivers in Central Italy (belonging to the basins of rivers Tevere and Arno) were less impacted and PFOA and PFBS were the only substances measured at concentrations above 100 ng/L.

The highest detection frequency of PFAA in groundwaters used for drinking water abstraction were measured for PFHxA, PFOA and PFOS (Table 4) and reflects a diffuse and historical pollution coming both from urban and industrial pressure on the territory. In fact the highest concentrations were measured for PFOS (up to 121 ng/L) notwithstanding it was subjected to restrictions in use [9]. Nevertheless it should be underlined that all measured concentrations were significantly lower than the Provisional Health Advisories of 400 ng/l e 200 ng/l proposed by US EPA respectively for PFOA and PFOS in drinking waters [8], showing a very limited risk for the final consumer.

The validated on-line SPE-UHPLC-LC/MS/MS method has been successfully used also for determining PFAA in difficult matrices. We were able to determine low concentrations of PFAA in transitional waters, characterised by a range of salinity from 23 to 31 o/oo, collected in the lagoons of Venice and in the Po Delta. We also analysed effluents from industrial and urban WWTPs and, after the appropriate dilution, concentrations up to 4.8 mg/L of PFBS and 0.7-0.8 mg/L for PFOA and PFHxA were measured. Samples were injected in the system after centrifugation and dilution and matrix effects were minimized by using internal labelled standards.

5.   Conclusions

The use of on-line SPE coupled with UHPLC using sub 2μm particle size columns made possible the development of faster methodology, by reducing the analysis time and thus increasing the sample throughput. However, the reducing of the total analysis time originated from the development of ultra fast separation and the reduced sample treatment may introduce new analytical challenges during method development: the increasing of ionization matrix effect and the loss of chromatographic efficiency may be observed.

By understanding the factors that impact negatively an on-line SPE methodology, a rapid analytical method for the simultaneous determination of compounds with different polarities and other physicochemical properties such as PFCA and PFSA was developed. Optimization of elution gradient and acidification of the aqueous samples allowed good analytical parameters for all compounds, including perfluorocarboxylates such as PFBA and PFPeA. Ionization suppression between target perfluorinated analytes and their co-eluting SIL-IS was reported for the first time for perfluorinated compounds. Generally, the extent of suppression in each target compound-SIL-IS pair was correlated with the carbon chain length. Validated on-line SPE-LC-MS/MS method has been applied in a wide survey of the concentrations of PFAA in Italian surface and drinking waters. Manual sample preparation was reduced to sample centrifugation and acidification, thus eliminating several procedural errors and significantly reducing time consuming and costs.

References

[1] J.P. Giesy, K. Kannan, Environ. Sci. Technol. 35 (2001) 1339.

[2] J.P. Giesy, K. Kannan , Environ. Sci. Technol. 36 (2002) 146A.

[3] K. Kannan, Environ. Chem. 8 (2011) 333.

[4] A.B. Lindstrom, M.J. Strynar, E.L. Libelo, Environ. Sci. Technol. 45 (2011) 7954.

[5] K. Prevedouros, I.T. Cousins, R.C. Buck, S.H. Korzeniowski, Environ. Sci. Technol. 40 (2006) 32.

[6] R.C. Buck, J. Franklin, U. Berger, J.M. Conder, I.T. Cousins, P. de Voogt, A.A. Jensen, K. Kannan, S.A. Mabury, S.P. van Leeuwen, Integrated Environ. Assess. Manag. 7 (2011) 513.

[7] European Commission, Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, Official Journal of the European Union 24.8.2013 L 226/1.

[8] US Environmental Protection Agency (2009) Provisional Health Advisories for Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS). http://water.epa.gov/action/advisories/drinking/upload/2009_01_15_criteria_drinking_pha-PFOA_PFOS.pdf

[9] European Commission (2006) Directive 2006/122/EC of the European Parliament and of the Council of 12 December 2006 amending for the 30th time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on

the marketing and use of certain dangerous substances and preparations (perfluorooctane sulfonates) Official Journal of the European Union 27.12.2006 L 372/32

[10] US Environmental Protection Agency (2006) PFOA Stewardship Program. http://www.epa.gov/opptintr/pfoa/pubs/stewardship/pfoastewardshipbasics.html

[11] A.A. Jensen, P.B. Poulsen, R. Bossi, (2008) Survey and Environmental/Health Assessment of Fluorinated Substances in Impregnated Consumer Products and Impregnating Agents, n. 99, Danish Environmental Protection Agency.

[12] US Environmental Protection Agency (2003) Environmental and Health Assessment of Perfluoro Sulfonic Acid and its Salts, AR:226-602

[13] G.Ding, W.J.G.M. Peijnenburg, Crit. Rev. Env. Sci. Technol. 43 (2013) 598.

[14] A. Jahnke, U. Berger, J. Chromatogr. A 1216 (2009) 410.

[15] M. Petrovic, M. Farré, M. Lopez de Alda, S. Perez, C. Postigo, M. Köck, J. Radjenovic, M. Gros, D. Barcelo, J. Chromatogr. A 1217 (2010) 4004

[16] O. Nunez, H. Gallart-Ayala, C.P.B. Martins, P. Lucci , J. Chromatogr. A 1228 (2012) 298.

[17] M. Takino, S. Daishima, T. Nakahara, Rapid. Comm. Mass. Spectrom. 17 (2003) 383.

[18] S.R. Wilson, H. Malerød, A. Holm, P. Molander, E. Lundanes, T. Greibrokk, J. Chromatogr. Sci. 45 (2007) 146.

[19] F. Gosetti, U. Chiuminato, D. Zampieri, E. Mazzucco, E. Robotti, G. Calabrese, M.C. Gennaro, E. Mareng,. J. Chromatogr. A 1217 (2010) 7864.

[20] R. Enevoldsen, R.K. Juhler, Anal. Bioanal. Chem. 398 (2010) 1161.

[21] M. Llorca, M. Farré, Y. Pico, J. Müller, T.P. Knepper, D. Barcelò, Sci. Total. Environ. 431 (2012) 139.

[22] D. Gode, M.M. Markus, F. Steiner, C.G. Huber, D.A. Volmer, Anal. Bioana.l Chem. 404 (2012) 433.

[23] D.R. Rodier, J.W. Birks, Chromatographia 39 (1994) 45.

[24] S.A. Tittlemier, E. Braekevelt, Anal. Bioanal. Chem. 399 (2011) 221.

[25] J.W. Martin, K. Kannan, U. Berger, P. de Voogt, J. Field, J. Franklin, J.P. Giesy, T. Harner, D.C.G. Muir, B. Scott, M. Kaiser, U. Järnberg, K.C. Jones, S.A. Mabury, H. Schroeder, M. Simcik, C. Sottani, B. van Bavel, A. Kärrman, G. Lindström, S. van Leeuwen, Environ. Sci. Technol. 38 (2004) 248A.

[26] S. Valsecchi, M. Rusconi, S. Polesello, Anal. Bioanal. Chem. 405 (2013) 143-157.

[27] L.E. Sojo, G. Lum, P. Chee, Analyst 128 (2003) 51.

[28] H.R. Liang, R.L. Foltz, M. Meng, P. Bennett, Rapid Commun. Mass Spectrom. 17 (2003) 2851.

[29] C.G. Enke, Anal. Chem. 69 (1997) 4885

[30] N.B. Cech, C.G. Enke, Anal. Chem. 73 (2001) 4632.

[31] S. Valsecchi, M. Rusconi, M. Mazzoni, G. Viviano, R. Pagnotta, C. Zaghi, G. Serrini, S. Polesello, Chemosphere, DOI: 10.1016/j.chemosphere.2014.07.044, Available online 6 August 2014.

Occurrence and sources of perfluoroalkyl acids in Italian river basins

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HIGHLIGHTS

- PFAA concentrations and loads of the main Italian river basins, 40% of Italian area.
- Total concentrations of PFAA ranged from < LOD to 8 µg L^{-1}.
- The most discharged compounds are PFBS (39%) and PFOA (32%).
- Urban load (0.09 t y^{-1}) is 1% of the total PFAA load to sea (7.9 t y^{-1}).
- The main PFAA sources of Italy are two chemical plants (57% of the total load).

GRAPHICAL ABSTRACT
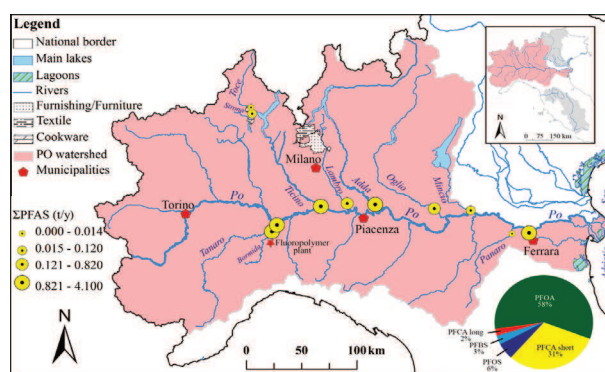
ABSTRACT

This paper presents a survey on the occurrence and sources of 11 perfluoroalkyl acids (PFAA) in the main river basins in Italy, covering about 40% of the Italian surface area and 45% of the Italian population. Total concentrations of PFAA ranged from < LOD to 8 µg L^{-1}, the highest concentrations being measured in the rivers impacted by industrial discharges. Among the rivers directly flowing into the sea, Brenta, Po and Arno present significant concentrations, while concentrations in Tevere and Adige, which are not impacted by relevant industrial activities, are almost all below the detection limits.

The total estimated PFAA load of the five rivers was 7.5 t y^{-1} with the following percentage distribution: 39% PFBS, 32% PFOA, 22% short chain perfluorocarboxylic acids (PFCA), 6% PFOS and 1% long chain PFCA. PFOA and PFOS loads, evaluated in the present work, represent 10% and 2% of the estimated European loads, respectively.

In Italy the most important sources of PFAA are two chemical plants which produce fluorinated polymers and intermediates, sited in the basin of rivers Po and Brenta, respectively, whose overall emission represents 57% of the total estimated PFAA load. Both rivers flow into the Adriatic Sea, raising concern for the marine ecosystem also because a significant PFOS load (0.3 t y^{-1}) is still present.

Among the remaining activities, tanneries and textile industries are relevant sources of respectively PFBS and PFOA, together with short chain PFCA. As an example, the total PFAA load (0.12 t y^{-1}) from the textile district of Prato is equivalent to the estimated domestic emission of the whole population in all the studied basins.

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1. Introduction

Because of their peculiar physical and chemical characteristics, perfluoroalkyl substances (PFAS) find wide application in several industrial processes and products, such as surface treatment of textiles and paper, building paints, cosmetics, insecticide formulations, firefighting foams, and the production of fluoropolymer (Kissa, 2001; Prevedouros et al., 2006; Buck et al., 2011). However, their characteristics, such as resistance to hydrolysis, photolysis, microbial degradation (Giesy and Kannan, 2001; Prevedouros et al., 2006) make these substances highly persistent and widespread in all environmental compartments. Water is the major reservoir of these compounds in the environment, as well as the most important medium for their transport (Prevedouros et al., 2006; Mc Lachlan et al., 2007).

PFAS include thousands of chemicals but environmental studies mainly concentrated on perfluoroalkyl acids (PFAA), such as perfluoroalkylsulphonic acids (PFSA) and perfluoroalkylcarboxylic acids (PFCA). PFSA and PFCA are low molecular weight surfactants, consisting of homologous series of completely fluorinated carbon chains. The two PFAA, most commonly used and found in the environment, are perfluorooctanesulphonate (PFOS) and perfluorooctanoate (PFOA). They have been widely employed in different industrial processes (Kissa, 2001; Prevedouros et al., 2006). Their persistence in the environment together with their bioaccumulation in the trophic chain raised concern about the risks for consumers, including humans, so that European Commission recently included PFOS in the list of priority hazardous substances under the Water Framework Directive (WFD), setting an Environmental Quality Standard (EQS) of $0.65\ \text{ng}\,\text{L}^{-1}$ for freshwater (EC, 2013). At the moment for PFOA, no limits have been established in the aquatic environment, but only a provisional threshold for drinking waters proposed by US Environmental Protection Agency (EPA) of $0.4\ \mu\text{g}\,\text{L}^{-1}$ (EPA, 2009). The regulatory restrictions on the use of PFOS and PFOA (EC, 2006; EPA, 2006) induced the major fluorochemical producers to search substitutes for these compounds, especially among homologues with shorter chain lengths (Wang et al., 2013).

Manufacturing facilities of fluorochemicals are the greatest point sources of PFAS (Prevedouros et al., 2006; Davis et al., 2007) while emissions of municipal and industrial wastewater treatment plant (WWTP) are less relevant (Boulanger et al., 2005; Prevedouros et al., 2006; Schultz et al., 2006; Sinclair and Kannan, 2006).

The first comprehensive study evaluating the concentrations and loads of PFAA in main European rivers was carried out in the framework of the EU project PERFORCE (Mc Lachlan et al., 2007); this paper was followed over the years by several studies investigating their occurrence in surface waters both along big rivers (Ahrens et al., 2009; Loos et al., 2010; Möller et al., 2010) and at regional scale (Ericson et al., 2008; Clara et al., 2009; Rostkowski et al., 2009; Kwadijk et al., 2010; Sanchez-Avila et al., 2010; Muller et al., 2011; Dufkova et al., 2012). Furthermore, a pan-European screening of 122 water samples coming from several streams and rivers was also carried out (Loos et al., 2009). Published data about PFAA occurrence in Italian waters are currently available (Mc Lachlan et al., 2007; Loos et al., 2007, 2008; Castiglioni et al., 2014). The first study measured four PFCA at River Po basin closure, pointing out that Po had the highest concentration of PFOA in Europe (Mc Lachlan et al., 2007). The second study, which determined four PFCA and PFOS along river Po and its tributaries, identified a fluoropolymer plant, sited in river Tanaro basin, as the main PFOA source in river Po basin (Loos et al., 2008).

Our work is the first comprehensive study carried out on the main river basins in Italy, covering more than 40% of the national surface area. We obtained a map of occurrence and emissions of PFAA in the main Italian rivers and highlighted hot spots correlated with specific sources and industrial emissions. Streams and rivers, which drain areas of specific industrial districts, such as textile, tannery, furnishing and household districts, were also sampled.

The collected data allowed us to evaluate the uses and emissions of different PFAA homologues, including molecules that could be potential substitutes of PFOA and PFOS in industrial processes.

From the average concentrations measured at the basin closure, the PFAA loads discharged by rivers into the sea, which is the last receptor of these extremely persistent molecules, have been estimated.

2. Material and methods

2.1. Study areas

Five river basins of the longest Italian rivers which cover the most industrialised areas of Italy were surveyed (Table 1).

River Adige, located in north-eastern Italy, is the second longest river in Italy and flows into the Adriatic Sea (Fig. 1). Its basin is one of the least densely populated regions of Italy (the most populated city is Verona with 258 893 residents) and the main economic activity is agriculture. The last tributary is the river Alpone-Chiampo which flows through the most important tannery district in Italy (Arzignano and Valle del Chiampo).

River Brenta, originating from the lakes of Levico and Caldonazzo (Trentino region), flows into the northern Adriatic Sea, just south of the lagoon of Venezia (Fig. 1). The prevailing activity in its basin is agriculture but a cluster of SME is spread along its catchment. Just before the mouth, it receives the waters of River Bacchiglione, which drains waters from cities of Vicenza (113 352 residents) and Padova (210 914 residents), and River Fratta-Gorzone, which collects treated wastewaters from a textile district, a tannery district and a fluorochemical factory.

River Po is the longest river in Italy (652 km) and has the greatest discharge ($1470\ \text{m}^3\,\text{s}^{-1}$). It flows across the entire northern Italy and it has a drainage area of 74 000 km^2, (about one fourth of the whole Italian surface), of which 45 000 km^2 are in mountainous environments and 29 000 km^2 on the plain (Fig. 2).

The river flows through many important Italian towns, including Torino (901 286 residents), Piacenza (102 225 residents) and Ferrara (130 837 residents), but its catchment, which includes 141 tributaries, covers the main industrialised and populated areas of the country, such as the metropolitan area of Milano (4.4 million inhabitants). More than 16 million people live in the whole basin, nearly one third of the Italian population. The Po basin generates nearly 40% of the Italian Gross Domestic Product by intensive industry and other economic activities. Industries are concentrated in the urban areas of Torino, Milano, Brescia, Mantova and Ferrara with a long-standing tradition in automobile, motor and mechanics manufacturing as well as fine and bulk chemical production. The moist and fertile flood plain is exploited mainly for agriculture.

River Tevere rises in the Apennine Mountains in central Italy and, after a course in a generally southern direction, flows into the Tyrrhenian Sea soon after having crossed Roma, the most populated city of Italy (2 651 040 residents) (Fig. 3). The Tevere valley is partly hilly and partly a fertile plain where agriculture, crafts and animal husbandry are the main sources of income. Industrial development in the region is limited to the areas south of Roma outside the Tevere basin.

River Arno originates on Mount Falterona, (Apennines Mountains, central Italy), passes near Arezzo (98 537 residents), through Firenze (365 539 residents), Empoli (47 912 residents) and Pisa (86 591 residents), and flows into the Tyrrhenian Sea at Marina

Table 1
Characteristics of the investigated river basins.

Basin	River length (km)	Average discharge (m^3 s^{-1})	Area (km^2)	Inhabitants (residents)	Mouth
River Adige	409	235	12 100	1.368.156	Adriatic Sea
River Arno	241	80	8228	2.042.339	Tyrrhenian Sea
River Brenta	174	93	5840	1.692.643	Adriatic Sea
River Po	650	1470	74 000	15.831.080	Adriatic Sea
River Tevere	406	267	17 375	4.412.090	Tyrrhenian Sea

Fig. 1. Map of the basins of River Adige and River Brenta. Yellow bullets represent t the total load (t y^{-1}) of PFAA. The pie chart refers to the percentage composition of the PFAA load of River Brenta at the basin closure. Industrial districts and fluorochemical plant are indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
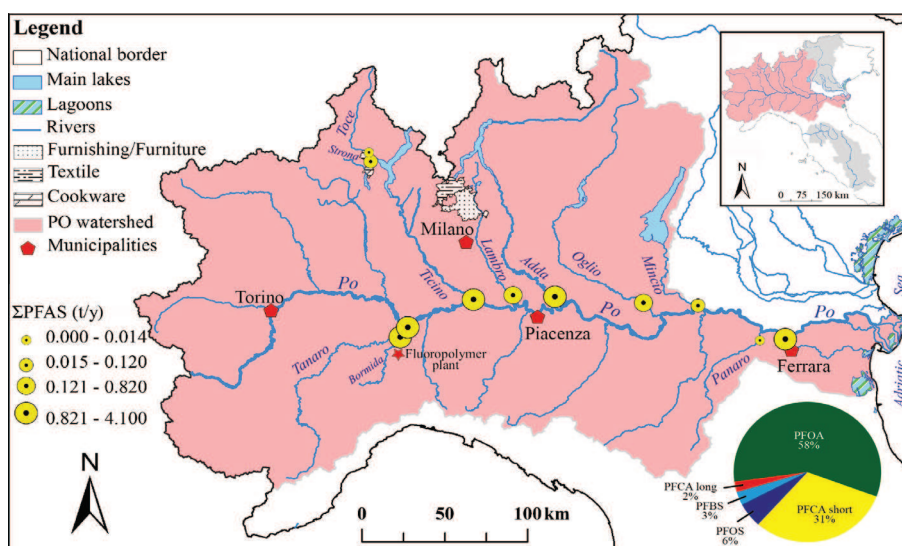
Fig. 2. Map of basin of River Po. Yellow bullets represent the total load (t y^{-1}) of PFAA. The pie chart refers to the percentage composition of the PFAA load of River Po at the basin closure. Industrial districts and fluoropolymer plant are indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

di Pisa (Fig. 3). Tourism is widely diffuse in the basin, though agriculture and livestock are significant economic activities in the upper part of the basin. Downstream Firenze, two Arno's tributaries, Ombrone and Bisenzio, drain the important textile district of Prato, while the tannery district of Santa Croce sull'Arno is located along the terminal part of the River Arno.

2.2. Sampling

20 Rivers were sampled at their basin closure stations (Table SM1). Rivers Arno, Tevere and Po were also sampled along the course of the river (coordinates and descriptions of sampling sites in Table SM2). Sampling campaigns were carried out between 2008 and 2013.

Rivers were sampled by means of a bucket at the centre of the river bed. All samples were collected in 50 mL PP centrifuge tubes and refrigerated at 4 °C until analyses, which was performed within 5 d.

2.3. Chemicals and materials

All reagents were analytical grade. LC–MS Chromasolv® methanol (MeOH), ammonium acetate (99%) and concentrated formic acid (98%) were purchased from Sigma–Aldrich. HPLC grade Milli-Q water was produced by a Millipore Direct-QUV water purification system (Millipore, Bedford, MA, USA). Perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), tetrabutylammonium perfluorobutane sulphonate (PFBS), potassium perfluorohexane sulphonate (PFHxS) and tetrabutylammonium perfluorooctane sulphonate (PFOS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stable isotope-labelled PFCA and PFSA internal standard compounds (SIL-IS) were purchased from Wellington Laboratories in 2 µg mL^{-1} solution mixtures. SIL-IS were $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUnDA, $^{13}C_2$-PFDoDA, $^{18}O_2$-PFHxS and $^{13}C_4$-PFOS.

2.4. On-line-SPE-UHPLC-MS/MS and QA/QC

Water samples were centrifuged and analysed by on-line-SPE-UHPLC-MS/MS according to the already published method (Castiglioni et al., 2014). A Thermo EQuan system with a polar end-capped C18 pre-concentration column (Thermo Hypersil GOLD aQ 12 µm, 20 × 2.1 mm), and a Thermo Hypersil GOLD PFP analytical column (1.9 µm, 50 × 2.1 mm) were used. Before injection, standards and samples were acidified to pH 3 and spiked with SIL-IS by adding 50 µL of concentrated formic acid and 25 µL of the diluted SIL-IS solution (40 ng mL^{-1}) to 10 mL of sample. Five mL of centrifuged and acidified sample are loaded onto the pre-concentration column; the trapped analytes are eluted from the pre-concentration column to the analytical column by a gradient of 2 mM ammonium acetate (5% methanol) and methanol at 300 µL min^{-1}. A QqQ mass spectrometer (Thermo TSQ Quantum Access MAX) equipped with a heated-electrospray ionisation (Thermo HESI-II) probe was used. The mass spectrometer operated in the negative SRM mode. Quantification was done using the isotopic dilution method and calibration curves were made freshly before each analytical run.

The on-line SPE-UHPLC-MS/MS method gave recoveries between 87% and 134%. Total matrix effect ranged between −34% and +39%. Linearity was good (R^2 higher than 0.95) in the LOQ – 200 ng L^{-1} range for all the target compounds. Limits of detection (LODs) ranged from 0.2 to 5.0 ng L^{-1} and LOQs from 1 to 20 ng L^{-1}. The performance of the on-line-SPE-UHPLC-MS/MS method was verified in the "PFOS and PFOA in surface water" proficiency test organised in 2013 by the PT-WFD network (http://www.pt-wfd.eu). Analytical results were satisfactory with calculated *z*-scores of +1.43 and +1.70 respectively for PFOS and PFOA.

2.5. Yearly load calculation

The main problem of the monitoring campaigns is the availability of reliable river discharge data on the sampling date. In the present study daily discharge data have been available only for few rivers (rivers Po, Bormida and Tanaro) from Regional Environmental Agencies (www.arpa.emr.it and www.arpa.piemonte.it). For other rivers no complete series of data have been retrieved. In order to discuss comparable results for all rivers, yearly loads were calculated by multiplying the average concentration by the annual average flow rates (Table SM1) calculated from historical data (Method A). In the case of river Po at the basin closure (Ferrara, Table SM2) it is possible to compare yearly loads obtained
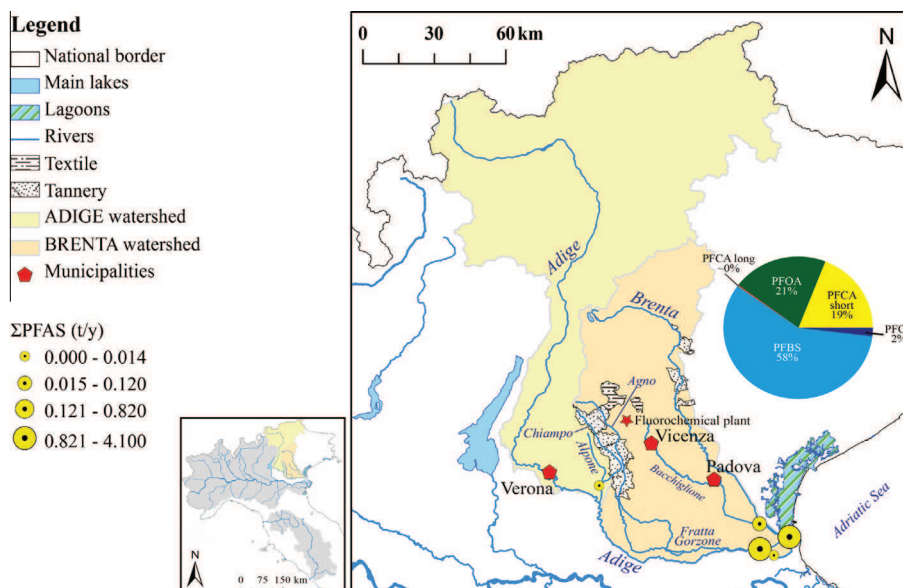

Fig. 3. Map of the basins of River Arno and River Tevere. Yellow bullets represent the total load (t y^{-1}) of PFAS. The pie chart refers to the percentage composition of the PFAA load of River Arno at the basin closure. Industrial districts are indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
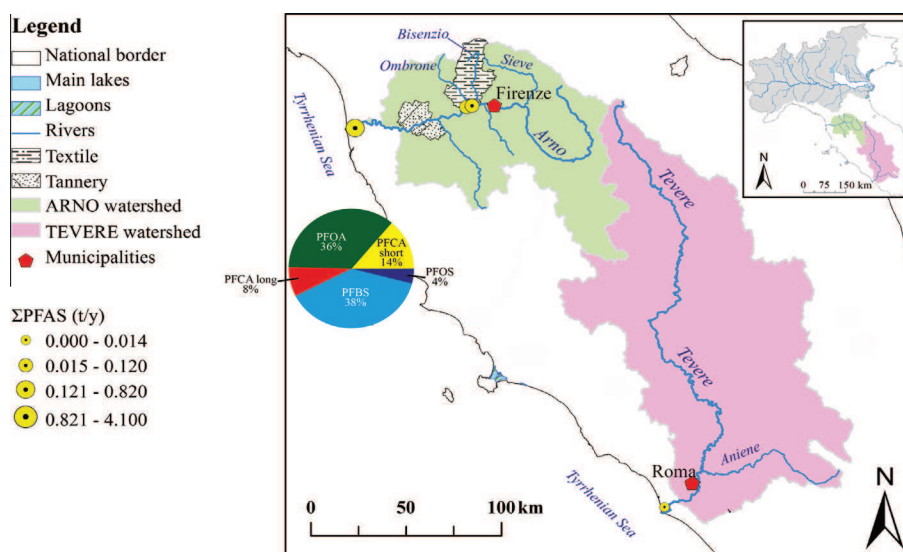
with Method A with those obtained by averaging the loads calculated for each sampling date using the daily discharge (Method B) in the period 2011–2013 (Table SM4): Method A yields 3.7 t y^{-1} of total PFAA (1.2 t y^{-1} short chain PFCA, 2.1 t y^{-1} PFOA, 0.1 long chain PFCA, 0.3 PFSA); Method B yields 2.9 t y^{-1} (0.9 t y^{-1} short chain PFCA, 1.6 t y^{-1} PFOA, 0.1 long chain PFCA, 0.3 PFSA), while. The difference, about 25%, can be considered a roughly estimate of the uncertainty of these procedures.

3. Results

3.1. Concentrations in basins

The occurrence of 11 perfluoroalkyl acids has been surveyed in five important Italian river basins which account for about 40% of the Italian surface area and 45% of the Italian population. 20 rivers, among which there are 10 out of the 13 longest rivers in Italy, have been sampled at their basin closures for a total of 80 samples. Means, medians and concentration ranges of measured PFAA are shown in Table 2. Raw data are available in Supplementary Materials (Tables SM3 and SM4). Further 54 samples have been collected along the course of the main rivers (Po, Arno and Tevere), upstream and downstream the most important tributaries or sources of pollution (Table SM2) and data are presented in Table SM4. The choice of the sampling sites was driven by pressure analysis, particularly by the presence of specific industrial plants or districts which could be a source of PFAA.

Tevere and Adige were the least impacted by PFAA among the main Italian rivers. All the concentrations measured in the urban tract of the river Tevere in Rome (Tables 2 and SM4) were under the detection limits (LOD), even downstream the WWTPs of Rome. Concentrations at the basin closure of the river Adige were also very close to or below the LOD. In the catchment of river Adige a limited input of PFAA was carried by its last tributary (River Alpone-Chiampo) which drains the Chiampo valley where one of the most important Italian tannery districts is located (Fig. 1). However, it is worth noting that both Tevere and Adige have a significant average discharge (about 250 m^3 s^{-1}) which allows minor inputs of PFAA, that are present in the basin, to be diluted.

River Po presents a very different situation: notwithstanding the highest average discharge (1470 m^3 s^{-1}), total PFAA mean concentration, at basin closure, was 80 ng L^{-1} ranging from 51 to 146 ng L^{-1} (Table 2). Mean PFOS concentration in Po (5 ng L^{-1}) was lower than the average of 122 European rivers measured in a pan-European survey (39 ng L^{-1}; Loos et al., 2009), while the highest concentrations were measured for PFOA (mean: 46 ng L^{-1}) and short chain PFCA (25 ng L^{-1}). PFOA and short chain PFCA mean concentrations were higher than 90th percentile values calculated in the EU survey (respectively 26 and 15 ng L^{-1}; Loos et al., 2009). The concentrations at the mouth of the river Po were in the range of rivers draining the most densely populated and impacted areas of northern and central Europe: e.g. PFOA concentrations were about 20 ng L^{-1} both in rivers Thames and Danube (Mc Lachlan et al., 2007; Loos et al., 2010), while in river Scheldt the mean PFOA concentration was 34.1 ng L^{-1} (Möller et al., 2010). PFOA mean concentration (46 ng L^{-1}) was from 1.3 to 4.3 times lower than those measured in previous campaigns on the same river (60–200 ng L^{-1}; Mc Lachlan et al., 2007; Loos et al., 2008), suggesting a possible decrease in PFOA use in fluoropolymer production. Nevertheless, the fluoropolymer plant, sited in Piedmont on the river Bormida, a tributary of river Tanaro, (Fig. 2) is still the most significant source of PFOA and short chain PFCA (PFPeA and PFHxA), as already identified by Loos et al. (2008). Maximum PFOA concentrations of 2.2 and 6.5 µg L^{-1} were measured respectively in the rivers Tanaro and its tributary Bormida from July 2008 to July 2013.

After Tanaro, no further right bank tributaries were sampled till river Panaro, because they are mainly intermittent rivers coming from Apennines and flowing through agricultural areas.

On the contrary, significant sources of PFAA in river Po were the left bank tributaries coming from Lombardy.

River Ticino, which is protected and inserted in a fluvial park, is a source of PFOS (mean concentration: 79 ng L^{-1}, Table 2) because it receives waters from industrial channels and small streams which collect discharges from industrialised areas in the province of Varese.

River Lambro, which flows through the city of Milano and its metropolitan area (about 4.4 million inhabitants), brought about 100 ng L^{-1} of PFAA into river Po, evenly divided among the different classes of compounds. As analysed in detail in a previous paper (Castiglioni et al., 2014), Lambro receives discharges from many municipal and industrial WWTPs, such as those of Milano. But, as highlighted in the cited paper, the highest concentrations were measured in the northern part of the basin where a dense clusters of SME, especially active in textile and silk finishing and furniture production, are present (IPI, 2009) (Fig. 2).

River Adda and, to a lesser extent, river Oglio showed significant concentrations of short chain PFCA (up to 300 ng L^{-1} in river Adda), but unfortunately their source could not be identified because in those basins the various small industrial activities are spread along the agricultural area. Finally the endmost tributaries of river Po, river Mincio on the left bank and river Panaro on the right one, did not significantly contribute to the PFAA load of river Po.

Because not all tributaries have been sampled, in order to identify further possible sources of PFAA, four sampling campaigns along the river Po from Torino to the basin closure (Tables SM2 and SM4) were carried out. The histogram of the average concentrations measured in 2011–2013 campaigns is plotted in Fig. SM1.

The first sampling point was downstream the town of Torino and its WWTP (river km 115) where the metropolitan area had a moderate but significant emission of PFAA in river Po, as evidenced by the total PFAA concentration of 26 ng L^{-1}. A sudden increase in concentrations (total PFAA 87 ng L^{-1}) was measured downstream river Tanaro (km 235), with PFOA (68 ng L^{-1}) as the main pollutant. In the Lombardy tract of the river, from km 300 (downstream Ticino, total PFAA 61 ng L^{-1}) to km 520 (downstream Mincio, total PFAA 91 ng L^{-1}) the concentrations constantly increased notwithstanding the concurrent increase in river flow rates, from 737 to 1000 m^3 s^{-1}. The second evident raising step in concentrations was the inflow of river Adda (km 420) which brought short chain PFCA, whose concentrations raised from 4 to 20 ng L^{-1}. From km 520 (downstream Mincio, total PFAA 91 ng L^{-1}) to the basin closure station (Ferrara, km 572, total PFAA 80 ng L^{-1}) no further sources of PFAA were present and concentrations were diluted by the increase in river flow rate.

Looking at data collected in the 2011–2013 campaigns (Table SM4), total PFAA as well PFOA concentrations appear to be generally decreasing in the last years, but, as it was shown in the case of Ferrara station (Fig. SM2a), the high variability of river discharges at the sampling times (Table SM4) does not allow reliable conclusions to be inferred. Nevertheless, if the PFAA loads, calculated with Method B (see Section 2.5), are plotted, no clear trend can be evidenced (Fig. SM2b).

In order to evaluate the impact of specific districts devoted to textile and tannery manufacturing, we surveyed two further rivers and their basins, river Brenta in Veneto and river Arno in Tuscany (Figs. 1 and 3).

River Brenta at the basin closure showed the highest mean concentration for PFBS (713 ng L^{-1}, PFHxS was always < LOD) in Italy, together with significant concentrations of PFOA (255 ng L^{-1}) and short chain PFCA (235 ng L^{-1}). In the last tract before flowing into the Adriatic Sea, river Brenta receives waters from the moderately

Table 2
PFAA concentrations at basin closure of the investigated rivers.

Basin	River	N		ΣPFCA short (<8C) (ng L^{-1})	PFOA (ng L^{-1})	ΣPFCA long (>8C) (ng L^{-1})	PFBS + PFHxS (ng L^{-1})	PFOS (ng L^{-1})	ΣPFAA (ng L^{-1})
Adige	Adige	3	Mean	<LOD	<LOD	<LOD	<LOD	1	1
	Alpone	2	Mean	2	3	5	2	4	16
			Range	<LOD-4	2–4	<LOD-11	<LOD-4	3–4	7–26
Arno	Arno	2	Mean	11	28	6	30	3	79
			Range	11–11	16–40	3–10	29–31	0–6	59–99
	Bisenzio	1		49	85	32	<LOD	6	173
	Ombrone	1		69	222	97	12	18	418
Brenta	Brenta	2	Mean	235	255	3	713	24	1230
			Range	46–424	218–292	<LOD-6	56–1370	9–38	330–2130
	Bacchiglione	1		18	83	<LOD	23	7	131
	Fratta-Gorzone	2	Mean	490	1316	2	1060	18	2886
			Range	488–493	348–2284	<LOD-3	454–1666	16–21	2525–3246
Po	Po	7	Mean	25	46	2	2	5	80
			Median	24	45	1	1	6	74
			Range	9–45	17–93	<LOD-9	<LOD-6	<LOD-10	51–146
	Bormida	21	Mean	264	1613	25	2	8	1911
			Median	110	1353	6	<LOD	<LOD	1531
			Range	<LOD-1487	253–6480	<LOD-157	<LOD-17	<LOD-109	291–8041
	Tanaro	11	Mean	55	559	8	2	1	625
			Median	33	299	4	<LOD	<LOD	341
			Range	2–244	109–2219	<LOD-25	<LOD-8	<LOD-6	111–2495
	Strona	1		<LOD	<LOD	<LOD	30	14	45
	Toce	2	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Ticino	2	Mean	2	20	7	3	79	111
			Range	1–3	12–28	<LOD-14	3–4	8–150	43–179
	Lambro	5	Mean	11	30	20	22	18	101
			Median	10	29	16	23	19	103
			Range	9–12	17–50	10–39	14–30	12–22	66–143
	Adda	4	Mean	174	17	2	6	7	206
			Median	135	14	2	6	6	158
			Range	110–318	8–33	<LOD-3	3–7	<LOD-17	135–373
	Oglio	3	Mean	41	9	1	2	2	54
			Range	5–71	<LOD-16	0–1	1–2	<LOD-5	29–73
	Mincio	3	Mean	1	6	1	6	<LOD	13
			Range	<LOD-2	<LOD-10	<LOD-1	<LOD-15	<LOD	<LOD-18
	Panaro	1		1	11	2	<LOD	<LOD	14
Tevere	Tevere	4	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

impacted river Bacchiglione and from the highly polluted channel Fratta-Gorzone (Table 2) which collects discharges from textile and tannery districts of the province of Vicenza as well as from a factory which produces fluorochemical intermediates (Fig. 1). In the latter water body total PFAA concentration reached 3 µg L^{-1}, with PFOA and PFBS as the main compounds.

At the mouth of river Arno, the total PFAA concentration was 79 ng L^{-1}, being PFOA (28 ng L^{-1}) and short chain PFSA (30 ng L^{-1}) the main compounds. The measurements carried out on its right bank tributaries (Bisenzio and Ombrone), which receive treated sewage from the most important textile district in Italy (Fig. 3), showed that they are important sources of PFOA and other PFCA. In order to find further sources, two sampling campaigns along the river course were carried out: Fig. SM3 shows the histogram of the campaign in July 2013, when PFAA were measured in 8 sampling sites (Tables SM2 and SM4). The first sampling point was upstream the confluence of river Sieve, at km 107, where the total PFAA concentration was 12 ng L^{-1}. The river Sieve (km 119) and the town of Firenze, with its WWTP (km 137), were limited sources of PFCA and the total PFAA concentration raised only to 20 ng L^{-1}. Downstream Firenze, total PFAA concentration in river Arno raised up to 86 ng L^{-1} because the river receives the waters of rivers Bisenzio (km 148) and Ombrone (km 155) which drain the textile district of Prato, bringing mainly PFOA (43 ng L^{-1}) with short (20 ng L^{-1}) and long chain PFCA (16 ng L^{-1}) (Table 2). The second important source of PFAA in the Arno course was the tannery district of the province of Pisa, where an increase in PFBS (from 8 to

24 ng L^{-1}) was measured in the sampling points sited at km 186 and 202, downstream the two most important tannery WWTPs.

The relationships between measured compounds and anthropic activities are discussed in the next section.

4. Discussions

4.1. Emission of PFAA from specific anthropic activities

Urban emissions. PFAA are present in many consumer products of daily use and thereby the urban conglomerations can be considered both point and diffuse sources (e.g. by run-off) of PFAA for the receiving water bodies. In a previous work (Castiglioni et al., 2014) domestic emission factors (E_F) of 5 and 10 µg d^{-1} per capita for PFOA and total PFAA, respectively, were estimated by averaging outputs of three different WWTPs of Milano, characterised by a very low industrial component (<4%). These E_F are lower than those estimated from river concentrations in industrialised countries (Pistocchi and Loos, 2009; Muller et al., 2011), but very close to E_F (3.5 µg d^{-1} per capita for PFOA) measured in domestic Korean WWTPs (Kim, 2012). Assuming these domestic E_F, we predict the concentrations in rivers downstream big towns, in order to verify the contribution of urban discharges when no industrial activities are present. In the case of Tevere, downstream Roma (2.3×10^6 inhabitants and annual average discharge of 267 m^3 s^{-1}), the predicted concentration is 1 ng L^{-1} for total PFAA, which is actually

under the LOD of our analytical method, and is consistent with the measured data (Table 2) and the absence of intensive industrial activities. The predicted contribution of Firenze (3.65×10^5 inhabitants and $50 \; \text{m}^3 \; \text{s}^{-1}$) in Arno is $1 \; \text{ng} \; \text{L}^{-1}$ which is of the same order of magnitude of the difference ($4 \; \text{ng} \; \text{L}^{-1}$) between the measured concentrations down- and up-stream Firenze (Fig. SM3, Table SM4). The predicted concentrations in Po downstream the metropolitan area of Torino (2.2×10^6 inhabitants with automotive industries; $23 \; \text{m}^3 \; \text{s}^{-1}$) are $11 \; \text{ng} \; \text{L}^{-1}$ for total PFAA and $5.5 \; \text{ng} \; \text{L}^{-1}$ for PFOA. We did not measure PFAA upstream the metropolitan area of Torino, but, from the analysis of pressures, we can assume that there are negligible sources of PFAA in this initial river tract at the foot of the mountain. The comparison of predicted data with average measured data of 22 and $10 \; \text{ng} \; \text{L}^{-1}$ respectively for total PFAA and PFOA (Fig. SM1) allows to estimate that the domestic contribution is about 50% of the total urban emission to the river Po in Torino.

Finally, if we assume that all the inhabitants in the whole Po basin (16×10^6 people) have the same E_f, a concentration of $1.3 \; \text{ng} \; \text{L}^{-1}$ of total PFAA, which should represent the contribution of urban sources, is calculated at the river Po basin closure, considering the average discharge of $1470 \; \text{m}^3 \; \text{s}^{-1}$: this value is negligible with respect to the average measured concentration ($80 \; \text{ng} \; \text{L}^{-1}$, Table 2), showing that industrial emissions heavily prevail on domestic ones in the river Po basin.

Fluorochemical and fluoropolymer plants. In the studied basins we identified two industrial plants which produce fluorochemical intermediates and fluoropolymers. The fluoropolymer plant, which discharges into the river Bormida, was already identified as the main contributor of PFOA to the river Po (Loos et al., 2008). If we consider the average PFOA concentration in the Bormida river ($1613 \; \text{ng} \; \text{L}^{-1}$, Table 2) and the dilution factor between Bormida and Po rivers (36.8 calculated from annual average flow rates reported in Table SM1), we estimate a PFOA concentration at the Po basin closure of $44 \; \text{ng} \; \text{L}^{-1}$, very close to the average measured concentration of $46 \; \text{ng} \; \text{L}^{-1}$, despite the rough approximations in river discharges (Table 1). The accordance between estimated and measured concentrations suggests that PFOA emitted by the fluoropolymer plant is conservative and the load of river Po is mostly due to this single source. Observing concentration data in the period 2008–2013 (Fig. SM4), the use of PFOA in fluoropolymer production was not decreasing since the annual average concentrations in river Bormida varied between 1.3 and $2.0 \; \mu\text{g} \; \text{L}^{-1}$ in this time range.

The second important fluorochemical plant is located in Trissino, VI, Veneto region (Fig. 1) and produces fluorinated molecules, such as PFOA and PFBS. The plant discharges into the municipal WWTP whose output is mixed with the outputs of other four WWTPs and carried by a single sewer pipeline (called Collettore ARICA) to the river Fratta-Gorzone. These five WWTPs collect waters from domestic sewage and industrial districts (mainly textile and tannery industries) of the province of Vicenza (Fig. 1). Therefore it is difficult to distinguish the contribution of the fluorochemical plant from those of the neighbour industrial activities. Nevertheless, we had the opportunity to compare PFAA composition of the primary discharge of the fluorochemical plant with those of the output of the main sewer pipeline (Collettore ARICA) and the river Fratta-Gorzone at the basin closure (Fig. SM5): it is evident that the PFAA pattern in the river and in the pipeline output is very similar to that shown in the output of the fluorochemical plant, with PFBS (68%), PFHxA (11%), PFOA (10%) and PFPeA (8%) being the main fluorocompounds.

Industrial activities which could employ PFAA. As shown in Figs. 1–3, rivers, which drain areas of specific industrial districts, were sampled.

As mentioned above, it was not possible to identify the PFAA emissions pattern of the textile and tannery districts in the province of Vicenza because of the coexistence of a point source fluorochemical plant (Fig. 1).

The PFAA emissions of the textile districts north of Milano, discharging in the river Lambro basin (Fig. 2), were already discussed in a previous paper (Castiglioni et al., 2014). They were equivalent to that coming from the metropolitan area of Milano (4×10^6 inhabitants) as regards PFCA. The furnishing and furniture districts of the provinces of Como and Monza-Brianza was also identified as significant sources of PFOA and short and long chain PFCA in this basin.

The very low (mainly < LOD) concentrations measured in rivers Strona and Toce (Table 2), in whose catchment renowned companies of cookware (e.g. Alessi, Lagostina, Bialetti) are located (Fig. 1), suggest that this very specialised but narrow household and fitting districts was not a significant source of PFAA for the Ticino and Po basins.

A very favourable situation for distinguishing the emissions of textile and tannery activities is present in the river Arno basin. The two specific districts are well separated by each other and close to the principal course of the river (Fig. 3). The textile district of Prato, whose WWTPs discharge into the tributaries Bisenzio and Ombrone, was a significant source of PFOA and short and long chain PFCA, while the contribution of PFSA was more limited (Table 2). The yearly loads of the Prato district, mostly from textile industries, are 66 kg of PFOA and 125 kg of PFAA. On the contrary a significant input of PFSA, mainly PFBS, came from the tannery district in the province of Pisa, few kilometres from the mouth (Fig. 3), so that the amounts of PFBS and PFOA discharged in Tyrrhenian sea by river Arno were rather equivalent (Table 2).

4.2. PFAA loads discharged by rivers into the sea

The PFAA loads to the sea from the studied rivers, estimated by Method A (see Section 2.5) and their percentage compositions are shown in Fig. 1–3. The PFAA loads into the Tyrrhenian sea were limited, because the Tevere contribution was negligible, while Arno discharged $0.2 \; \text{t} \; \text{y}^{-1}$ constituted by 36% PFOA, 38% PFBS, 14% short chain PFCA, 8% long chain PFCA and only 4% PFOS (Fig. 3), the last molecule being the most bioaccumulable in the marine trophic chain.

On the Adriatic side the situation was quite different: though the contribution from river Adige was negligible, rivers Brenta and Po brought to the sea more than $7.3 \; \text{t} \; \text{y}^{-1}$ of PFAA. The load from Brenta (about $3.6 \; \text{t} \; \text{y}^{-1}$) was composed by 58% PFBS, 21% PFOA, 19% short chain PFCA and only 2% PFOS. The load from Po (about $3.7 \; \text{t} \; \text{y}^{-1}$) was composed by 58% PFOA, 31% short chain PFCA, 3% PFBS, 6% PFOS and only 2% long chain PFCA. These data allow to estimate that about $2.9 \; \text{t} \; \text{y}^{-1}$ of PFOA, $2.2 \; \text{t} \; \text{y}^{-1}$ of PFBS, $1.8 \; \text{t} \; \text{y}^{-1}$ of short chain PFCA and $0.3 \; \text{t} \; \text{y}^{-1}$ of PFOS were discharged in the Adriatic Sea by the two rivers. PFHxS was always under the detection limit in these rivers. Because runoff of the rivers Po, Adige and Brenta constitutes about 84% of the total river discharge to the Adriatic Sea (Cozzi and Giani, 2011), the estimated load can be considered representative of the total PFAA load to the sea. The PFAA loads from river Po ($2.1 \; \text{t} \; \text{y}^{-1}$ for PFOA and $0.5 \; \text{t} \; \text{y}^{-1}$ for PFHxA) significantly decreased respect to previous available data ($9.5 \; \text{t} \; \text{y}^{-1}$ for PFOA and $0.8 \; \text{t} \; \text{y}^{-1}$ for PFHxA; Mc Lachlan et al., 2007).

Analyses of PFAA in Adriatic seawater taken 16 km off-shore from Venezia (Italy) were carried out in 2011–2012 (Loos et al., 2013); results showed that PFOA and PFOS were still the predominant compounds together with short chain PFCA (PFPeA and PFHxA) and in a single sample PFHxS. PFBS was not measured because of analytical interferences. Concentrations measured in

the Adriatic Sea are of the same order of North Sea concentrations, where highly impacted rivers, such as Rhine, Elbe and Meuse, discharge (Möller et al., 2010); it is also evident that PFOS and PFOA are still present at significant concentrations, but the use of short chain PFSA and PFCA is increasing.

5. Conclusions

The present study allowed to obtain an overview of the concentrations and loads of perfluoroalkyl acids in five of the main river basins in Italy, covering about 40% of the Italian surface area and 45% of the Italian population. Total concentrations of PFAA ranged from < LOD to 8 µg L^{-1}, the highest concentrations being measured in the rivers impacted by industrial discharges. Among the rivers directly flowing into sea, Brenta, Po and Arno present significant concentrations, while concentrations in Tevere and Adige, which are not impacted by relevant industrial activities, are almost all below the detection limits.

Considering a conservative behaviour of the studied PFAA, low concentrations measured at the basin closure of river Tevere and Adige are consistent with domestic emission factor of 10 µg d^{-1} per capita of total PFAA, calculated from the outputs of municipal sewage treatment plant of Milano. Using these emission factors the load of the whole population living in the studied basins is only 0.09 t y^{-1} which is 1.2% of the total estimated PFAA load (7.5 t y^{-1}). The percentage distribution of the PFAA homologues emitted by the studied river basins is about 39% PFBS, 32% PFOA, 22% short chain PFCA, 6% PFOS and 1% long chain PFCA. PFOA and PFOS loads, evaluated in the present work, represent respectively 10% and 2% of the European estimated loads (Mc Lachlan et al., 2007; Pistocchi and Loos, 2009).

In Italy the most important sources of PFAA are two chemical plants which produce fluorinated polymers and intermediates, sited in the basin of rivers Po and Brenta, respectively, whose overall emission represents 57% of the total estimated PFAA load. Both rivers flow into the Adriatic Sea, raising concern for the marine ecosystem also because a significant PFOS load (0.3 t y^{-1}) is still present notwithstanding the adopted restrictions (EC, 2006).

Among the remaining activities, tanneries and textile industries are relevant sources of PFBS and PFOA, respectively, together with short chain PFCA. As an example, the total PFAA load (0.12 t y^{-1}) from the textile district of Prato (700 km^2) is equivalent to the domestic emissions of the whole population in all the studied basins. Future work will be devoted to estimate emission factors from WWTPs of specific districts and industrial discharges in order to complete the inventory of the PFAA emissions in Italy.

Acknowledgements

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.07.044.

References

Ahrens, L., Felizeter, S., Sturm, R., Xie, Z., Ebinghaus, R., 2009. Polyfluorinated compounds in waste water treatment plant effluents and surface waters along the River Elbe, Germany. Mar. Pollut. Bull. 58, 1326–1333.

Boulanger, B., Vargo, J.D., Schnoor, J.L., Hornbuckle, K.C., 2005. Evaluation of perfluorooctane surfactants in a wastewater treatment system and in a commercial surface protection product. Environ. Sci. Technol. 39, 5524–5530.

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr. Environ. Assess. Manag. 7, 513–541.

Castiglioni, S., Valsecchi, S., Polesello, S., Rusconi, M., Melis, M., Palmiotto, M., Manenti, A., Davoli, E., Zuccato, E., 2014. Sources and fate of perfluorinated compounds in the aqueous environment and in drinking water of a highly urbanized and industrialized area in Italy. J. Hazard. Mater.. http://dx.doi.org/10.1016/j.jhazmat.2014.06.007.

Clara, M., Gans, O., Weiss, S., Sanz-Escribano, D., Scharf, S., Scheffknecht, C., 2009. Perfluorinated alkylated substances in the aquatic environment: an Austrian case study. Wat. Res. 43, 4760–4768.

Cozzi, S., Giani, M., 2011. River water and nutrient discharges in the Northern Adriatic Sea: current importance and long term changes. Cont. Shelf Res. 31, 1881–1893.

Davis, K.L., Aucoin, M.D., Larsen, B.S., Kaiser, M.A., Hartten, A.S., 2007. Transport of ammonium perfluorooctanoate in environmental media near a fluoropolymer manufacturing facility. Chemosphere 67, 2011–2019.

Dufkova, V., Cabala, R., Sevcik, V., 2012. Determination of C(5)–C(12) perfluoroalkyl carboxylic acids in river water samples in the Czech Republic by GC-MS after SPE preconcentration. Chemosphere 87, 463–469.

EC, 2006. European Commission, Directive 2006/122/EC of the European Parliament and of the Council of 12 December 2006 Amending for the 30th Time Council Directive 76/769/EEC on the Approximation of the Laws, Regulations and Administrative Provisions of the Member States Relating to Restrictions on the Marketing and use of Certain Dangerous Substances and Preparations (Perfluorooctane Sulfonates). *Official Journal of the European Union*, 2006, L372/32.

EC, 2013. European Commission, Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as Regards Priority Substances in the Field of Water Policy. *Official Journal of the European Union*, 2013, L226/1.

EPA, 2006. Environmental Protection Agency, PFOA Stewardship Program, 2006. <http://www.epa.gov/opptintr/pfoa/pubs/stewardship/pfoastewardshipbasics.html> (Accessed July 2014).

EPA, 2009. Environmental Protection Agency (EPA), Provisional Health Advisories for Perfluorooctanoic acid (PFOA) and Perfluorooctane Sulfonate (PFOS). <http://water.epa.gov/action/advisories/drinking/upload/2009_01_15_criteria_drinking_pha-PFOA_PFOS.pdf>. (Accessed July 2014).

Ericson, I., Nadal, M., van Bavel, B., Lindstrom, G., Domingo, J.L., 2008. Levels of perfluorochemicals in water samples from Catalonia, Spain: is drinking water a significant contribution to human exposure? Environ. Sci. Poll. Res. Int. 15, 614–619.

Giesy, J.P., Kannan, K., 2001. Global distribution of perfluorooctane sulfonate in wildlife. Environ. Sci. Technol. 35, 1339–1342.

IPI, 2009. Istituto per la Promozione Industriale, I distretti individuati dalle Regioni, Rome. <http://www.attivitaproduttive.regione.lombardia.it/shared/ccurl/868/981/i%20distretti%20individuati%20dalle%20regioni%20agg.IPI-2008.pdf> (Accessed July 2014).

Kim, S.K., 2012. Watershed-based riverine discharge loads and emission factor of perfluorinated surfactants in Korean peninsula. Chemosphere 89, 995–1002.

Kissa, E., 2001. Fluorinated Surfactants and Repellents. Marcel Dekker Inc., New York.

Kwadijk, C.J.A.F., Koryt, P., Koelmans, A.A., 2010. Distribution of perfluorinated compounds in aquatic systems in The Netherlands. Environ. Sci. Technol. 44, 3746–3751.

Loos, R., Wollgast, J., Huber, T., Hanke, G., 2007. Polar herbicides, pharmaceutical products, perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and nonylphenol and its carboxylates and ethoxylates in surface and tap waters around Lake Maggiore in Northern Italy. Anal. Bioanal. Chem. 387, 1469–1478.

Loos, R., Locoro, G., Huber, T., Wollgast, J., Christoph, E.H., de Jager, A., Manfred Gawlik, B., Hanke, G., Umlauf, G., Zaldivar, J.M., 2008. Analysis of perfluorooctanoate (PFOA) and other perfluorinated compounds (PFCs) in the River Po watershed in N-Italy. Chemosphere 71, 306–313.

Loos, R., Gawlik, B.M., Locoro, G., Rimaviciute, E., Contini, S., Bidoglio, G., 2009. EU-wide survey of polar organic persistent pollutants in European river waters. Environ. Pollut. 157, 561–568.

Loos, R., Locoro, G., Contini, S., 2010. Occurrence of polar organic contaminants in the dissolved water phase of the Danube River and its major tributaries using SPE-LC-MS(2) analysis. Wat. Res. 44, 2325–2335.

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Loos, R., Tavazzi, S., Paracchini, B., Canuti, E., Weissteiner, C., 2013. Analysis of polar organic contaminants in surface water of the northern Adriatic Sea by solid-phase extraction followed by ultrahigh-pressure liquid chromatography-QTRAP(R) MS using a hybrid triple-quadrupole linear ion trap instrument. Anal. Bioanal. Chem. 405, 5875–5885.

Mc Lachlan, M.S., Holmstrom, K.E., Reth, M., Berger, U., 2007. Riverine discharge of perfluorinated carboxylates from the European continent. Environ. Sci. Technol. 41, 7260–7265.

Möller, A., Ahrens, L., Surm, R., Westerveld, J., van der Wielen, F., Ebinghaus, R., de Voogt, P., 2010. Distribution and sources of polyfluoroalkyl substances (PFAS) in the River Rhine watershed. Environ. Pollut. 158, 3243–3250.

Muller, C.E., Gerecke, A.C., Alder, A.C., Scheringer, M., Hungerbuhler, K., 2011. Identification of perfluoroalkyl acid sources in Swiss surface waters with the help of the artificial sweetener acesulfame. Environ. Pollut. 159, 1419–1426.

Pistocchi, A., Loos, R., 2009. A map of European emissions and concentrations of PFOS and PFOA. Environ. Sci. Technol. 43, 9237–9244.

Prevedouros, K., Cousins, I.T., Buck, R.C., Korzeniowski, S.H., 2006. Sources, fate and transport of perfluorocarboxylates. Environ. Sci. Technol. 40, 32–44.

Rostkowski, P., Taniyasu, S., Yamashita, N., Falandysz, J.J., Zegarowski, L., Chojnacka, A., Pazdro, K., Falandysz, J., 2009. Survey of perfluorinated compounds (PFCs) in surface waters of Poland. J. Environ. Sci. Health. Part A Toxic/Hazard. Subst. Environ. Eng. 44, 1518–1527.

Sanchez-Avila, J., Meyer, J., Lacorte, S., 2010. Spatial distribution and sources of perfluorochemicals in the NW Mediterranean coastal waters (Catalonia, Spain). Environ. Pollut. 158, 2833–2840.

Schultz, M.M., Barofsky, D.F., Field, J.A., 2006. Quantitative determination of fluorinated alkyl substances by large-volume-injection liquid chromatography tandem mass spectrometry – characterization of municipal wastewaters. Environ. Sci. Technol. 40, 289–295.

Sinclair, E., Kannan, K., 2006. Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. Environ. Sci. Technol. 40, 1408–1414.

Wang, Z., Cousins, I.T., Scheringer, M., Hungerbühler, K., 2013. Fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and their potential precursors. Environ. Int. 60, 242–248.

SUPPLEMENTARY MATERIALS

Occurrence and sources of perfluoroalkyl acids in Italian river basins

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Table SM1. Main characteristics of the investigated rivers and locations of sampling sites at the basin closure.

river	basin	River length km	annual average flow rate m³/s	year	town/municipality	Latitude N	Longitude E
Adda	Po	313	187	2011-2012	Maccastorna LO	45.15512	9.85357
Adige	Adige	410	235	2011-2013	Caverzere VE	45.11297	12.18852
Alpone-Chiampo	Adige	38	3	2012-2013	San Bonifacio VR	45.39397	11.26762
Arno	Arno	241	80	2011-2013	Pisa	43.68396	10.33553
Bacchiglione	Brenta	119	28	2012	Correzzola PD	45.23212	12.11931
Bisenzio	Arno	49	4.1	2013	Signa FI	43.78258	11.10092
Bormida	Po	154	40	2008-2013	Alessandria	44.92777	8.66924
Brenta	Brenta	174	93	2011-2012	Chioggia VE	45.17953	12.27734
Fratta-Gorzone	Brenta	100	23	2011-2012	Cavarzere VE	45.13893	12.11762
Lambro	Po	130	69	2011-2012	Orio Litta LO	45.16590	9.53170
Mincio	Po	194	60	2011-2012	Roncoferraro MN	45.09080	10.94838
Oglio	Po	280	130	2011-2012	Marcaria MN	45.11444	10.53180
Ombrone	Arno	41	7.8	2013	Signa FI	43.77696	11.06482
Panaro	Po	148	37	2011	Bondeno FE	44.88295	11.41432
Po	Po	652	1470	2010-2013	Ferrara	44.88819	11.6086
Strona	Po	28	13	2013	Gravellona Toce VB	45.925491	8.43278
Tanaro	Po	273	123	2008-2013	Rivarone AL	44.98405	8.72730
Tevere	Tevere	406	267	2011	Fiumicino RM	41.77127	12.24985
Ticino	Po	248	348	2011-2012	Linarolo PV	45.14300	9.22920
Toce	Po	84	70	2012	Ornavasso VB	45.976208	8.418871

Table SM2. Location of the sampling sites along the Tevere, Arno e Po Rivers.

river	Distance from the Source km	year	N	town/municipality	Latitude N	Longitude E	Description
Tevere	325	2011	1	Monterotondo RM	42.08698	12.60199	upstream Roma
Tevere	347	2011	1	Roma	41.98728	12.49981	upstream Aniene
Tevere	356	2011	1	Roma	41.93986	12.48590	downstream Aniene
Tevere	366	2011	1	Roma	41.89011	12.47725	town centre
Tevere	381	2011	1	Roma	41.80717	12.41804	downstream Roma
Tevere	400	2011	1	Fiumicino RM	41.77127	12.24985	basin closure
Arno	56	2011	1	Capolona AR	43.51993	11.83139	upstream Arezzo
Arno	107	2011-2013	2	Rignano sull' Arno FI	43.72155	11.45462	upstream Sieve
Arno	119	2011-2013	2	Pontassieve FI	43.77064	11.42364	downstream Sieve, upstream Firenze
Arno	137	2013	1	Firenze	43.77992	11.21823	downstream Firenze
Arno	148	2011-2013	2	Lastra a Signa Fi	43.77304	11.09295	downstream Bisenzio
Arno	155	2011-2013	2	Capraia e Limite FI	43.76682	11.03437	downstream Ombrone
Arno	186	2011-2013	2	Castelfranco di sotto PI	43.69428	10.74869	downstream Tannery WWTP
Arno	202	2013	1	Calcinaia PI	43.68123	10.61547	downstream Usciana channel and Tannery district
Arno	236	2011-2013	2	Marina di Pisa, PI	43.68396	10.33553	basin closure
Po	115	2010-2012	4	San Mauro Torinese TO	45.10367	7.76308	downstream Torino
Po	215	2010-2013	5	Valenza AL	45.0512	8.6318	upstream Tanaro
Po	235	2010-2013	6	Isola Sant'Antonio AL	45.0364	8.8211	downstream Tanaro
Po	300	2011-2012	3	Pieve Porto Morone PV	45.0943	9.4510	downstream Ticino
Po	350	2010-2013	6	Piacenza	45.06159	9.69732	downstream Lambro
Po	420	2010-2013	6	San Daniele Po CR	45.0418	10.1816	downstream Adda
Po	500	2010-2013	5	San Benedetto Po MN	45.0740	10.9336	downstream Oglio
Po	520	2010-2012	4	Revere MN	45.0558	11.13532	downstream Mincio
Po	572	2010-2013	7	Ferrara	44.88819	11.6086	basin closure

Table SM3. Concentrations of PFAA in the sampling sites at the basin closure (LOD: PFBA 5 ng/L; PFPeA 2 ng/L; PFHxA 0.2 ng/L; PFHpA 0.2 ng/L; PFOA 0.5 ng/L; PFNA 0.5 ng/L; PFDA 0.5 ng/L; PFUnDA 0.5 ng/L; PFDoDA 1 ng/L; PFBS 1 ng/L; PFHxS 5 ng/L; PFOS 2.5 ng/L) n.d.:not determined; n.a.: not avaible

Water body	Town	Sampling date	Latitude	Longitude	River length km	Flow rate (at sampling) m³/s	PFBA ng/L	PFPeA ng/L	PFHxA ng/L	PFHpA ng/L	PFOA ng/L	PFNA ng/L	PFDA ng/L	PFUnDA ng/L	PFDoDA ng/L	PFBS ng/L	PFHxS ng/L	PFOS ng/L
Adda	Maccastorna LO	01/03/2011	45.15512	9.85357	313	n.a.	<LOD	59.4	46.0	4.4	16.5	1.6	1.2	<LOD	<LOD	5.5	<LOD	<LOD
Adda	Maccastorna LO	13/07/2011	45.15512	9.85357	313	n.a.	401.0	174.7	137.2	6.3	32.6	0.6	1.1	<LOD	<LOD	3.2	<LOD	17.2
Adda	Maccastorna LO	18/01/2012	45.15512	9.85357	313	n.a.	107.6	89.7	46.0	6.0	11.4	1.5	1.0	<LOD	<LOD	7.4	<LOD	<LOD
Adda	Maccastorna LO	30/05/2012	45.15512	9.85357	313	n.a.	145.0	69.9	54.1	3.3	7.6	<LOD	<LOD	<LOD	<LOD	5.9	<LOD	11.3
Adige	Cavarzere VE	04/05/2011	45.11297	12.18852	410	n.a.	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Adige	Cavarzere VE	24/10/2012	45.11297	12.18852	410	n.a.	13.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Adige	Cavarzere VE	30/04/2013	45.10385	11.48895	410	n.a.	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.6
Alpone	San Bonifacio VR	24/10/2012	45.34084	11.27152	38	n.a.	<LOD	2.4	1.6	<LOD	2.3	<LOD	2.1	2.7	6.0	4.3	<LOD	4.4
Alpone	San Bonifacio VR	14/02/2013	45.39397	11.26762	38	n.a.	<LOD	<LOD	<LOD	<LOD	3.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.1
Arno	Pisa PI	25/07/2013	43.68396	10.33553	241	n.a.	21.9	<LOD	5.0	5.8	40.5	3.4	6.3	<LOD	<LOD	31.4	<LOD	6.4
Arno	Pisa PI	02/05/2013	43.68396	10.33553	241	n.a.	15.2	<LOD	6.4	4.9	15.8	3.0	<LOD	<LOD	<LOD	29.3	<LOD	<LOD
Bacchiglione	Correzzola PD	24/10/2012	45.23212	12.11931	119	n.a.	35.8	4.5	10.1	3.6	83.0	<LOD	<LOD	<LOD	<LOD	23.1	<LOD	6.8
Bisenzio	Signa FI	24/07/2013	43.78258	11.10092	49	n.a.	10.3	9.9	17.8	21.7	85.5	19.4	12.4	<LOD	<LOD	<LOD	<LOD	6.0
Bormida	Alessandria AL	01/07/2008	44.92777	8.66924	154	13.0	n.d.	<LOD	29.0	33.0	1465.0	2.0	2.0	<LOD	<LOD	<LOD	n.d.	<LOD
Bormida	Alessandria AL	13/10/2009	44.92777	8.66924	154	6.8	n.d.	26.0	82.0	133.0	3882.0	17.9	<LOD	<LOD	<LOD	<LOD	n.d.	<LOD
Bormida	Alessandria AL	12/11/2009	44.92777	8.66924	154	33.6	n.d.	<LOD	22.0	16.0	253.0	<LOD	<LOD	<LOD	<LOD	<LOD	n.d.	<LOD
Bormida	Alessandria AL	17/02/2010	44.92777	8.66924	154	22.0	n.d.	22.6	17.7	26.0	583.0	4.5	<LOD	<LOD	<LOD	<LOD	n.d.	6.0
Bormida	Alessandria AL	20/03/2010	44.92777	8.66924	154	56.3	n.d.	8.5	8.6	14.3	298.9	4.2	<LOD	<LOD	<LOD	<LOD	n.d.	2.0

Water body	Town	Sampling date	Latitude	Longitude	River length km	Flow rate (at sampling) m³/s	PFBA ng/L	PFPeA ng/L	PFHxA ng/L	PFHpA ng/L	PFOA ng/L	PFNA ng/L	PFDA ng/L	PFUnDA ng/L	PFDoDA ng/L	PFBS ng/L	PFHxS ng/L	PFOS ng/L
Bormida	Alessandria AL	21/04/2010	44.92777	8.66924	154	22.0	n.d.	16.6	18.3	31.5	561.0	3.9	<LOD	<LOD	<LOD	<LOD	n.d.	<LOD
Bormida	Alessandria AL	21/05/2010	44.92777	8.66924	154	19.6	n.d.	<LOD	<LOD	<LOD	1415.5	<LOD	<LOD	<LOD	<LOD	<LOD	n.d.	<LOD
Bormida	Alessandria AL	30/06/2010	44.92777	8.66924	154	7.7	n.d.	7.5	25.6	77.2	1945.0	4.3	<LOD	<LOD	<LOD	<LOD	n.d.	<LOD
Bormida	Alessandria AL	08/07/2010	44.92777	8.66924	154	6.0	n.d.	8.5	74.8	118.0	3592.0	9.9	<LOD	<LOD	<LOD	<LOD	n.d.	<LOD
Bormida	Alessandria AL	24/08/2010	44.92777	8.66924	154	5.3	n.d.	6.2	72.4	101.2	1828.0	18.4	11.6	6.0	<LOD	<LOD	n.d.	<LOD
Bormida	Alessandria AL	13/10/2010	44.92777	8.66924	154	8.8	n.d.	<LOD	23.0	32.1	377.9	<LOD	<LOD	<LOD	<LOD	<LOD	n.d.	<LOD
Bormida	Alessandria AL	28/02/2011	44.92777	8.66924	154	34.0	<LOD	28.1	44.9	59.6	1262.4	29.6	7.7	5.3	<LOD	<LOD	<LOD	<LOD
Bormida	Alessandria AL	11/07/2011	44.92777	8.66924	154	3.1	<LOD	237.5	134.0	156.2	1300.1	18.5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Bormida	Alessandria AL	17/01/2012	44.92777	8.66924	154	5.3	<LOD	167.2	191.4	372.0	2188.7	70.3	31.5	36.2	19.1	17.0	<LOD	109.2
Bormida	Alessandria AL	16/05/2012	44.92777	8.66924	154	24.4	<LOD	<LOD	42.5	60.0	860.9	2.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Bormida	Alessandria AL	15/06/2012	44.92777	8.66924	154	17.21	<LOD	<LOD	20.4	8.2	267.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	37.6
Bormida	Alessandria AL	06/07/2012	44.92777	8.66924	154	11.97	<LOD	584.7	185.8	131.5	1899.9	12.5	<LOD	<LOD	<LOD	10.5	<LOD	<LOD
Bormida	Alessandria AL	26/07/2012	44.92777	8.66924	154	3.3	205.1	255.7	76.9	65.5	1353.1	30.7	31.9	44.4	<LOD	<LOD	<LOD	<LOD
Bormida	Alessandria AL	29/08/2012	44.92777	8.66924	154	2.5	234.5	195.1	346.2	946.0	6479.9	37.6	22.3	11.1	<LOD	2.6	<LOD	<LOD
Bormida	Alessandria AL	16/10/2012	44.92777	8.66924	154	18.53	172.7	<LOD	16.1	30.1	476.1	2.8	0.9	2.0	<LOD	<LOD	<LOD	<LOD
Bormida	Alessandria AL	17/07/2013	44.92777	8.66924	154	n.a.	85.4	23.2	62.2	50.3	1580.9	6.2	3.2	3.3	1.0	5.9	<LOD	4.5
Brenta	Chioggia VE	04/05/2011	45.17953	12.27734	174	n.a.	<LOD	189.7	200.8	33.1	292.4	1.4	3.4	0.9	<LOD	1370.4	<LOD	38.1
Brenta	Chioggia VE	24/10/2012	45.17953	12.27734	174	n.a.	216.1	9.9	24.3	11.9	218.5	<LOD	<LOD	<LOD	<LOD	56.0	<LOD	9.3
Fratta-Gorzone	Cavarzere VE	05/05/2011	45.13893	12.11762	100	n.a.	<LOD	189.0	253.7	45.1	347.6	1.1	2.1	<LOD	<LOD	1665.7	<LOD	20.8
Fratta-Gorzone	Cavarzere VE	24/10/2012	45.15534	12.20982	100	n.a.	333.2	105.9	213.3	173.6	2283.7	<LOD	<LOD	<LOD	<LOD	418.0	35.6	16.0

Distribuzione e valutazione del rischio di composti perfluorurati in bacini fluviali italiani

Water body	Town	Sampling date	Latitude	Longitude	River length km	Flow rate (at sampling) m³/s	PFBA ng/L	PFPeA ng/L	PFHxA ng/L	PFHpA ng/L	PFOA ng/L	PFNA ng/L	PFDA ng/L	PFUnDA ng/L	PFDoDA ng/L	PFBS ng/L	PFHxS ng/L	PFOS ng/L
Lambro	Oriolitta LO	01/03/2011	45.16590	9.53170	130	n.a.	<LOD	2.5	4.4	5.5	29.3	9.0	5.6	1.6	<LOD	14.5	<LOD	16.3
Lambro	Oriolitta LO	11/07/2011	45.16590	9.53170	130	n.a.	<LOD	<LOD	4.7	4.4	49.6	8.5	18.0	9.5	3.2	23.2	<LOD	22.2
Lambro	Oriolitta LO	17/01/2012	45.16590	9.53170	130	n.a.	<LOD	<LOD	4.2	7.9	17.2	4.9	3.8	1.5	<LOD	14.0	<LOD	12.3
Lambro	Oriolitta LO	18/11/2012	45.16590	9.53170	130	n.a.	<LOD	2.4	4.7	3.3	28.5	7.7	4.4	3.3	3.4	26.7	<LOD	18.6
Lambro	Oriolitta LO	20/11/2012	45.16590	9.53170	130	n.a.	<LOD	6.2	3.3	<LOD	27.4	6.1	4.6	2.1	3.5	29.6	<LOD	20.9
Mincio	Roncoferraro MN	03/03/2011	45.09080	10.94838	194	n.a.	<LOD	<LOD	1.7	<LOD	9.7	0.6	<LOD	<LOD	<LOD	2.1	<LOD	<LOD
Mincio	Roncoferraro MN	13/07/2011	45.09080	10.94838	194	n.a.	<LOD	<LOD	<LOD	<LOD	7.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Mincio	Roncoferraro MN	18/01/2012	45.09080	10.94838	194	n.a.	<LOD	<LOD	1.6	<LOD	<LOD	<LOD	0.7	0.6	<LOD	15.1	<LOD	<LOD
Oglio	Marcaria MN	03/03/2011	45.11444	10.53180	280	n.a.	<LOD	25.9	18.8	1.4	10.6	<LOD	<LOD	<LOD	<LOD	2.0	<LOD	<LOD
Oglio	Marcaria MN	13/07/2011	45.11444	10.53180	280	n.a.	<LOD	<LOD	5.0	<LOD	15.8	<LOD	1.3	<LOD	<LOD	1.2	<LOD	5.4
Oglio	Marcaria MN	18/01/2012	45.11444	10.53180	280	n.a.	39.2	48.7	20.0	2.8	<LOD	<LOD	0.6	<LOD	<LOD	1.4	<LOD	<LOD
Ombrone	Signa FI	24/07/2013	43.77696	11.06482	41	n.a.	40.3	<LOD	40.3	29.1	221.9	30.1	51.3	10.9	4.4	12.4	<LOD	17.6
Panaro	Bondeno FE	03/03/2011	44.88295	11.41432	148	38.1	<LOD	<LOD	0.5	0.5	11.3	0.8	0.76	<LOD	<LOD	<LOD	<LOD	<LOD
Po	Ferrara FE	21/07/2010	44.88819	11.6086	652	747	n.d.	21.5	16.1	7.8	93.0	0.7	<LOD	<LOD	<LOD	n.d.	n.d.	6.5
Po	Ferrara FE	14/10/2010	44.88819	11.6086	652	1390	n.d.	14.6	12.0	7.2	17.0	<LOD	<LOD	<LOD	<LOD	n.d.	n.d.	<LOD
Po	Ferrara FE	03/03/2011	44.88819	11.6086	652	1607	<LOD	13.0	8.3	2.6	44.5	1.2	<LOD	<LOD	<LOD	4.2	<LOD	<LOD
Po	Ferrara FE	13/07/2011	44.88819	11.6086	652	662	<LOD	13.1	13.0	1.0	64.8	0.6	<LOD	<LOD	<LOD	5.2	<LOD	7.7
Po	Ferrara FE	18/01/2012	44.88819	11.6086	652	808	17.4	11.6	7.4	<LOD	55.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Po	Ferrara FE	28/04/2013	44.88819	11.6086	652	2739	<LOD	10.8	4.5	<LOD	19.7	<LOD	2.7	4.4	1.8	1.5	<LOD	7.1

Water body	Town	Sampling date	Latitude	Longitude	River length km	Flow rate (at sampling) m^3/s	PFBA ng/L	PFPeA ng/L	PFHxA ng/L	PFHpA ng/L	PFOA ng/L	PFNA ng/L	PFDA ng/L	PFUnDA ng/L	PFDoDA ng/L	PFBS ng/L	PFHxS ng/L	PFOS ng/L
Po	Ferrara FE	02/10/2013	44.88819	11.6086	652	1160	9.6	<LOD	8.1	0.7	28.2	<LOD	<LOD	0.7	1.5	6.2	<LOD	10.3
Strona	Gravellona Toce VB	16/01/2013	45.92549	8.43278	28	n.a.	19.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	30.4	<LOD	14.3
Tanaro	Rivarone AL	01/07/2008	44.98405	8.72730	273	82.1	n.d.	<LOD	4.0	3.0	181.0	<LOD	<LOD	<LOD	<LOD	n.d.	n.d.	<LOD
Tanaro	Rivarone AL	30/06/2010	44.98405	8.72730	273	108.6	n.d.	2.0	<LOD	<LOD	109.0	<LOD	<LOD	<LOD	<LOD	n.d.	n.d.	<LOD
Tanaro	Rivarone AL	19/07/2010	44.98405	8.72730	273	38.8	n.d.	4.5	19.0	40.6	901.0	2.6	<LOD	<LOD	<LOD	n.d.	n.d.	<LOD
Tanaro	Rivarone AL	24/08/2010	44.98405	8.72730	273	52.1	n.d.	<LOD	13.0	20.0	299.0	4.0	5.0	<LOD	<LOD	n.d.	n.d.	<LOD
Tanaro	Rivarone AL	13/10/2010	44.98405	8.72730	273	53.2	n.d.	<LOD	6.2	10.0	141.0	0.0	<LOD	<LOD	<LOD	n.d.	n.d.	<LOD
Tanaro	Rivarone AL	28/02/2011	44.98405	8.72730	273	130	<LOD	12.7	23.0	12.0	416.5	10.7	5.5	2.8	<LOD	2.0	<LOD	<LOD
Tanaro	Rivarone AL	11/07/2011	44.98405	8.72730	273	34	<LOD	2.9	18.9	62.6	843.4	6.9	3.7	1.7	1.2	<LOD	<LOD	6.1
Tanaro	Rivarone AL	17/01/2012	44.98405	8.72730	273	37.9	<LOD	<LOD	20.5	45.3	636	4.4	2.4	1.9	<LOD	1.9	<LOD	<LOD
Tanaro	Rivarone AL	29/08/2012	44.98405	8.72730	273	8.17	<LOD	38.8	59.2	145.6	2219	13.0	6.3	5.5	<LOD	7.8	<LOD	<LOD
Tanaro	Rivarone AL	16/10/2012	44.98405	8.72730	273	51.4	<LOD	<LOD	5.1	13.4	215	1.1	0.5	2.0	<LOD	<LOD	<LOD	<LOD
Tanaro	Rivarone AL	17/07/2013	44.98405	8.72730	273	57.4	39.4	<LOD	16.7	10.9	184	1.0	0.8	<LOD	<LOD	<LOD	<LOD	4.3
Tevere	Fiumicino RM	11/05/2011	41.77127	12.24985	406	n.a.	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ticino	Linarolo PV	11/07/2011	45.14300	9.22920	248	n.a.	<LOD	<LOD	1.5	2.0	28.1	<LOD	<LOD	<LOD	<LOD	3.7	<LOD	8.2
Ticino	Linarolo PV	17/01/2012	45.14300	9.22920	248	n.a.	<LOD	<LOD	1.4	<LOD	11.7	6.1	5.7	1.9	<LOD	2.7	<LOD	149.5
Toce	Ornavasso VB	08/11/2012	45.97621	8.418871	84	n.a.	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Distribuzione e valutazione del rischio di composti perfluorurati in bacini fluviali italiani

Table SM4. Concentrations of PFAA in the sampling sites along the Tevere. Arno e Po rivers (LOD: PFBA 5 ng/L; PFPeA 2 ng/L; PFHxA 0.2 ng/L; PFHpA 0.2 ng/L; PFOA 0.5 ng/L; PFNA 0.5 ng/L; PFDA 0.5 ng/L; PFUnDA 0.5 ng/L; PFDoDA 1 ng/L; PFBS 1 ng/L; PFHxS 5 ng/L; PFOS 2.5 ng/L) n.d.:not determined; n.a.: not avaible

Water body	Town	Sampling date	Latitude	Longitude	Km from the source	Flow rate (at sampling) m³/s	PFBA ng/L	PFPeA ng/L	PFHxA ng/L	PFHpA ng/L	PFOA ng/L	PFNA ng/L	PFDA ng/L	PFUnDA ng/L	PFDoDA ng/L	PFBS ng/L	PFHxS ng/L	PFOS ng/L
Tevere	Monterotondo RM	11/05/2011	42.08698	12.60199	325	n.a.	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Tevere	Roma RM	11/05/2011	41.98728	12.49981	347	n.a.	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Tevere	Roma RM	11/05/2011	41.93986	12.48590	356	n.a.	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Tevere	Roma RM	11/05/2011	41.89011	12.47725	366	n.a.	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Tevere	Roma RM	11/05/2011	41.80717	12.41804	381	n.a.	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Tevere	Fiumicino RM	11/05/2011	41.77127	12.24985	400	n.a.	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Arno	Capolona AR	02/05/2011	43.51993	11.83139	56	n.a.	15.9	2.1	2.0	2.0	2.8	1.9	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Arno	Rignano sull' Arno FI	02/05/2011	43.72155	11.45462	107	n.a.	11.2	6.9	2.4	2.1	5.4	1.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Arno	Pontassieve FI	02/05/2011	43.77064	11.42364	119	n.a.	18.4	5.5	3.7	2.2	7.0	2.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Arno	Lastra a Signa FI	02/05/2011	43.77304	11.09295	148	n.a.	8.8	4.8	4.4	3.6	18.9	3.8	<LOD	<LOD	<LOD	<LOD	<LOD	3.0
Arno	Capraia e Limite FI	02/05/2011	43.76682	11.03437	155	n.a.	21.4	<LOD	5.7	5.7	21.3	6.1	<LOD	<LOD	<LOD	<LOD	<LOD	3.5
Arno	Castelfranco di sotto PI	02/05/2011	43.69428	10.74869	186	n.a.	20.4	<LOD	5.5	4.3	24.7	6.9	1.8	<LOD	<LOD	12.5	<LOD	3.3
Arno	Pisa PI	02/05/2011	43.68396	10.33553	236	n.a.	15.2	<LOD	6.4	4.9	15.8	3.0	<LOD	<LOD	<LOD	29.3	<LOD	<LOD
Arno	Rignano sull' Arno FI	23/07/2013	43.72155	11.45462	107	n.a.	32.5	<LOD	<LOD	0.7	7.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.5
Arno	Pontassieve FI	23/07/2013	43.77064	11.42364	119	n.a.	78.7	14.7	<LOD	<LOD	<LOD	0.6	<LOD	<LOD	<LOD	<LOD	<LOD	2.7
Arno	Firenze FI	24/07/2013	43.77992	11.21823	137	n.a.	10.2	8.4	<LOD	1.4	6.9	0.6	<LOD	<LOD	<LOD	1.7	<LOD	3.4
Arno	Lastra a Signa FI	24/07/2013	43.77304	11.09295	148	n.a.	16.7	<LOD	10.7	9.6	43.2	6.0	5.3	<LOD	<LOD	1.1	<LOD	10.5
Arno	Capraia e Limite FI	24/07/2013	43.76682	11.03437	155	n.a.	14.0	2.3	6.9	7.2	52.5	5.6	8.2	2.4	<LOD	3.7	<LOD	6.6

Water body	Town	Sampling date	Latitude	Longitude	Km from the source	Flow rate (at sampling) m³/s	PFBA ng/L	PFPeA ng/L	PFHxA ng/L	PFHpA ng/L	PFOA ng/L	PFNA ng/L	PFDA ng/L	PFUnDA ng/L	PFDoDA ng/L	PFBS ng/L	PFHxS ng/L	PFOS ng/L
Arno	Castelfranco di sotto PI	24/07/2013	43.69428	10.74869	186	n.a.	20.6	<LOD	12.1	9.5	57.8	5.8	7.7	<LOD	<LOD	8.2	<LOD	8.9
Arno	Calcinaia PI	24/07/2013	43.68123	10.61547	202	n.a.	31.0	<LOD	9.2	7.5	52.3	3.6	<LOD	<LOD	<LOD	23.8	<LOD	7.4
Arno	Pisa PI	25/07/2013	43.68396	10.33553	236	n.a.	21.9	<LOD	5.0	5.8	40.5	3.4	6.3	<LOD	<LOD	31.4	<LOD	6.4
Po	San Mauro Torinese TO	19/7/010	45.10367	7.76308	115	52.0	n.d.	<LOD	1.5	4.3	<LOD	<LOD	<LOD	<LOD	<LOD	n.d.	n.d.	<LOD
Po	Valenza AL	19/7/010	45.0512	8.6318	215	139	n.d.	<LOD	1.6	5.5	3.0	0.6	<LOD	<LOD	<LOD	n.d.	n.d.	<LOD
Po	Isola Sant'Antonio AL	19/7/010	45.0364	8.8211	235	219	n.d.	<LOD	3.6	7.3	124.0	0.9	<LOD	<LOD	<LOD	n.d.	n.d.	5.8
Po	Piacenza PI	21/07/2010	45.06159	9.69732	350	420	n.d.	<LOD	3.6	7.5	97.0	1.0	<LOD	<LOD	<LOD	n.d.	n.d.	6.1
Po	San Daniele Po CR	21/07/2010	45.0418	10.1816	390	455	n.d.	29.9	20.4	6.2	57.0	1.1	<LOD	<LOD	<LOD	n.d.	n.d.	7.1
Po	San Benedetto Po MN	21/07/2010	45.0740	10.9336	500	553	n.d.	22.2	14.4	6.4	67.0	0.6	<LOD	<LOD	<LOD	n.d.	n.d.	5.3
Po	Revere MN	21/07/2010	45.0558	11.13532	520	764	n.d.	20.8	16.0	6.5	74.0	0.6	<LOD	<LOD	<LOD	n.d.	n.d.	3.9
Po	Ferrara FE	21/07/2010	44.88819	11.6086	572	747	n.d.	21.5	16.1	7.8	93.0	0.7	<LOD	<LOD	<LOD	n.d.	n.d.	6.5
Po	Isola Sant'Antonio AL	13/10/2010	45.0364	8.8211	235	193	n.d.	<LOD	4.5	7.4	59.5	<LOD	<LOD	<LOD	<LOD	n.d.	n.d.	<LOD
Po	Ferrara FE	14/10/2010	44.88819	11.6086	572	1390	n.d.	14.6	12.0	7.2	17.0	<LOD	<LOD	<LOD	<LOD	n.d.	n.d.	<LOD
Po	San Mauro Torinese TO	28/02/2011	45.10367	7.76308	115	69	<LOD	<LOD	1.2	1.1	9.4	2.0	2.5	<LOD	<LOD	1.6	<LOD	<LOD
Po	Valenza AL	28/02/2011	45.0512	8.6318	215	172	<LOD	<LOD	1.2	0.7	7.9	1.1	<LOD	<LOD	<LOD	9.3	<LOD	<LOD
Po	Isola Sant'Antonio AL	28/02/2011	45.0364	8.8211	235	327	<LOD	<LOD	1.5	0.8	19.3	0.9	<LOD	<LOD	<LOD	15.9	<LOD	<LOD
Po	Pieve Porto Morone PV	01/03/2011	45.0943	9.4510	300	837	<LOD	<LOD	2.7	2.3	70.1	1.2	0.6	<LOD	<LOD	9.0	<LOD	<LOD
Po	Piacenza PI	01/03/2011	45.06159	9.69732	350	795	<LOD	2.41	3.5	2.6	68.6	1.7	0.8	<LOD	<LOD	7.2	<LOD	<LOD
Po	San Daniele Po CR	01/03/2011	45.0418	10.1816	390	950	<LOD	12.4	10.9	3.5	60.1	2.9	0.9	<LOD	<LOD	6.5	<LOD	<LOD
Po	San Benedetto Po MN	03/03/2011	45.0740	10.9336	500	1291	<LOD	11.0	10.7	2.9	55.6	1.5	<LOD	<LOD	<LOD	6.6	<LOD	<LOD

Distribuzione e valutazione del rischio di composti perfluorurati in bacini fluviali italiani

Water body	Town	Sampling date	Latitude	Longitude	Km from the source	Flow rate (at sampling) m³/s	PFBA ng/L	PFPeA ng/L	PFHxA ng/L	PFHpA ng/L	PFOA ng/L	PFNA ng/L	PFDA ng/L	PFUnDA ng/L	PFDoDA ng/L	PFBS ng/L	PFHxS ng/L	PFOS ng/L
Po	Revere MN	03/03/2011	45.0558	11.13532	520	1490	<LOD	9.9	9.8	2.3	47.9	1.4	<LOD	<LOD	<LOD	5.6	<LOD	<LOD
Po	Ferrara FE	03/03/2011	44.88819	11.6086	572	1607	<LOD	13.0	8.3	2.6	44.51	1.2	<LOD	<LOD	<LOD	4.2	<LOD	<LOD
Po	San Mauro Torinese TO	11/07/2011	45.10367	7.76308	115	50	<LOD	<LOD	1.1	0.3	8.0	0.7	0.6	<LOD	1.1	2.0	<LOD	6.5
Po	Valenza AL	11/07/2011	45.0512	8.6318	215	199	<LOD	2.1	<LOD	<LOD	13.6	0.7	1.9	<LOD	1.0	<LOD	<LOD	<LOD
Po	Isola Sant'Antonio AL	11/07/2011	45.0364	8.8211	235	331	<LOD	<LOD	1.1	1.6	69.1	1.8	2.9	0.7	1.3	6.0	<LOD	12.7
Po	Pieve Porto Morone PV	11/07/2011	45.0943	9.4510	300	637	<LOD	2.3	0.8	<LOD	42.1	0.7	0.7	<LOD	1.1	4.5	<LOD	5.2
Po	Piacenza PI	13/07/2011	45.06159	9.69732	350	500	<LOD	<LOD	0.9	0.3	69.2	0.6	2.2	1.0	<LOD	6.5	<LOD	<LOD
Po	San Daniele Po CR	13/07/2011	45.0418	10.1816	390	552	<LOD	6.7	19.0	<LOD	50.9	<LOD	<LOD	<LOD	<LOD	3.0	<LOD	<LOD
Po	San Benedetto Po MN	13/07/2011	45.0740	10.9336	500	564	<LOD	14.1	18.9	2.3	57.3	1.1	2.1	<LOD	<LOD	6.4	<LOD	16.3
Po	Revere MN	13/07/2011	45.0558	11.13532	520	748	<LOD	11.7	19.1	1.5	57.5	<LOD	0.6	<LOD	<LOD	5.9	<LOD	10.0
Po	Ferrara FE	13/07/2011	44.88819	11.6086	572	662	<LOD	13.1	13.0	1.0	64.8	0.6	<LOD	<LOD	<LOD	5.2	<LOD	7.7
Po	San Mauro Torinese TO	17/01/2012	45.10367	7.76308	115	46.5	<LOD	<LOD	2.6	3.7	23.7	1.4	1.3	2.1	2.1	3.6	<LOD	<LOD
Po	Valenza AL	17/01/2012	45.0512	8.6318	215	136	<LOD	<LOD	1.8	<LOD	<LOD	<LOD	1.0	0.8	1.6	5.8	<LOD	<LOD
Po	Isola Sant'Antonio AL	17/01/2012	45.0364	8.8211	235	261	<LOD	<LOD	5.6	8.8	122.4	1.4	1.4	0.9	<LOD	3.5	<LOD	<LOD
Po	Pieve Porto Morone PV	17/01/2012	45.0943	9.4510	300	451	<LOD	<LOD	2.2	3.2	30.2	<LOD	0.6	<LOD	<LOD	3.1	<LOD	<LOD
Po	Piacenza PI	18/01/2012	45.06159	9.69732	350	530	<LOD	<LOD	<LOD	<LOD	79	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Po	San Daniele Po CR	18/01/2012	45.0418	10.1816	390	577	<LOD	15.1	8.7	<LOD	71	<LOD	<LOD	<LOD	<LOD	1.5	<LOD	3.9
Po	San Benedetto Po MN	18/01/2012	45.0740	10.9336	500	674	<LOD	13.4	11.8	5.9	51.7	1.3	0.9	<LOD	<LOD	5.0	<LOD	<LOD
Po	Revere MN	18/01/2012	45.0558	11.13532	520	762	<LOD	14.5	11.6	6.7	50.1	1.2	1.0	0.8	<LOD	4.8	<LOD	<LOD
Po	Ferrara FE	18/01/2012	44.88819	11.6086	572	808	17.4	11.6	7.4	<LOD	56	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Water body	Town	Sampling date	Latitude	Longitude	Km from the source	Flow rate (at sampling) m³/s	PFBA ng/L	PFPeA ng/L	PFHxA ng/L	PFHpA ng/L	PFOA ng/L	PFNA ng/L	PFDA ng/L	PFUnDA ng/L	PFDoDA ng/L	PFBS ng/L	PFHxS ng/L	PFOS ng/L
Po	Piacenza PI	30/05/2012	45.06159	9.69732	350	1128	<LOD	<LOD	1.4	1.1	12.9	<LOD	<LOD	<LOD	<LOD	3.8	<LOD	3.2
Po	San Daniele Po CR	30/05/2012	45.0418	10.1816	390	1181	<LOD	<LOD	6.7	1.4	10.7	<LOD	<LOD	<LOD	<LOD	3.2	<LOD	5.1
Po	Ferrara FE	28/04/2013	44.88819	11.6086	572	2739	<LOD	10.8	4.5	<LOD	19.7	<LOD	2.7	4.4	1.8	1.5	<LOD	7.1
Po	Valenza AL	17/07/2013	45.0512	8.6318	215	n.a.	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.0
Po	Isola Sant'Antonio AL	17/07/2013	45.0364	8.8211	235	260	15.5	<LOD	1.8	2.8	61.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.8
Po	Piacenza PI	01/10/2013	45.06159	9.69732	350	958	12.8	<LOD	5.1	0.6	15.8	<LOD	<LOD	1.1	2.7	5.8	<LOD	3.3
Po	San Daniele Po CR	01/10/2013	45.0418	10.1816	390	1068	17.2	2.5	11.9	0.9	21.5	<LOD	<LOD	<LOD	1.5	4.4	<LOD	11.9
Po	San Benedetto Po MN	01/10/2013	45.0740	10.9336	500	993	<LOD	<LOD	<LOD	<LOD	17.1	<LOD	<LOD	2.2	3.3	4.0	<LOD	5.4
Po	Ferrara FE	02/10/2013	44.88819	11.6086	572	1160	9.6	<LOD	8.1	0.7	28.2	<LOD	<LOD	0.7	1.5	6.2	<LOD	10.3

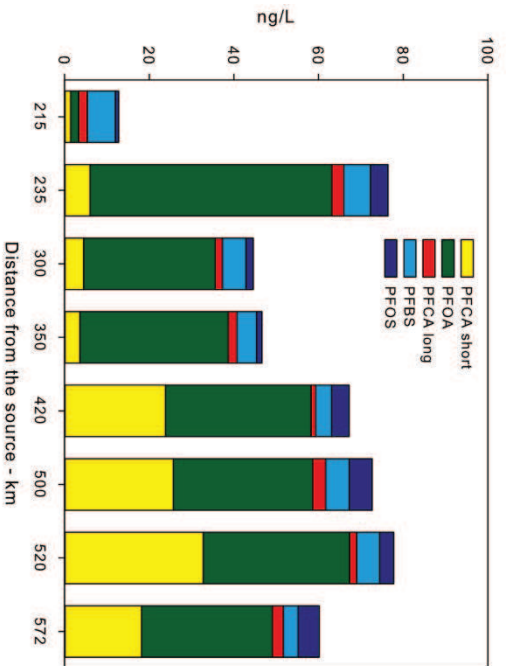*Distribuzione e valutazione del rischio di composti perfluorurati in bacini fluviali italiani*

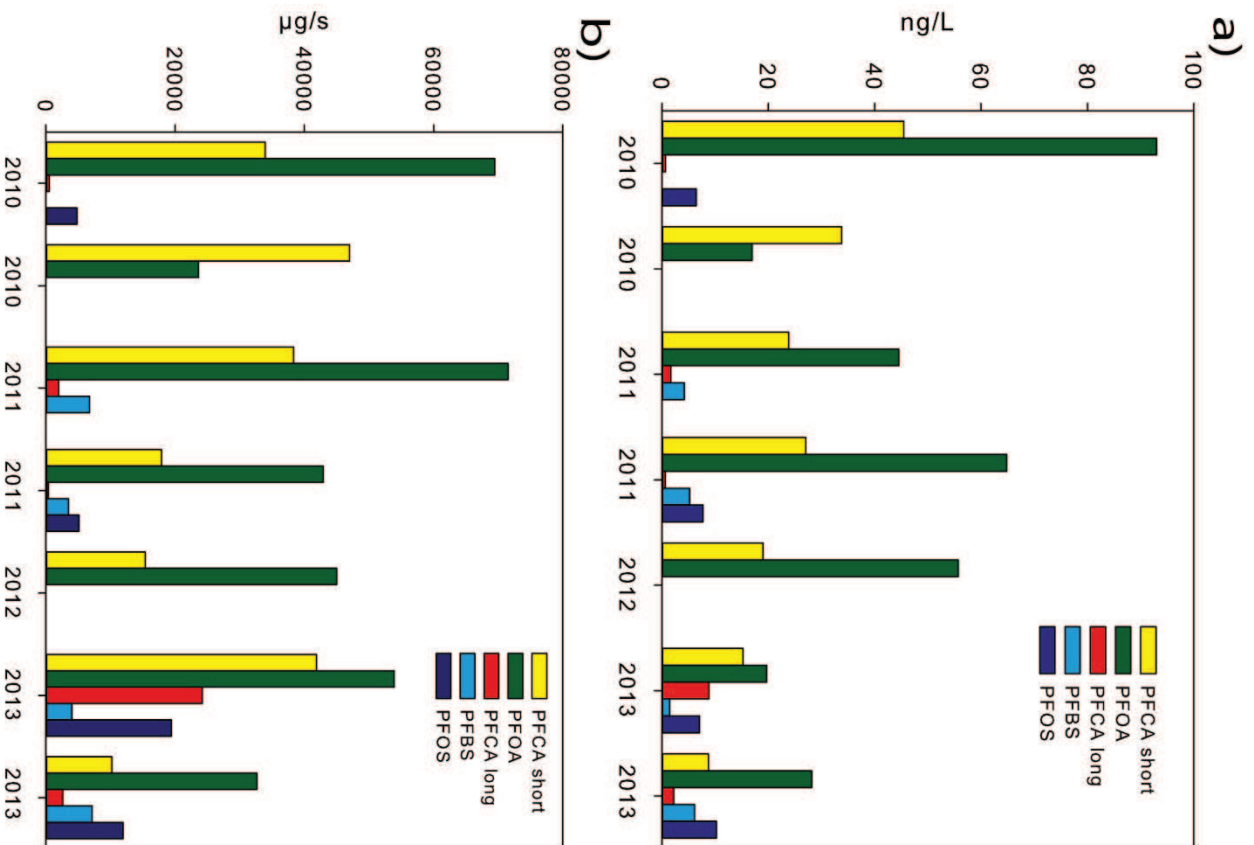Figure SM1. PFAS concentrations along River Po; sample sites are described in table SM2

Figure SM2: Temporal variation of:a) concentrations (in ng/L) and b) loads (in μg/s) of PFAA in river Po at Ferrara (basin closure station) from 2010 to 2013
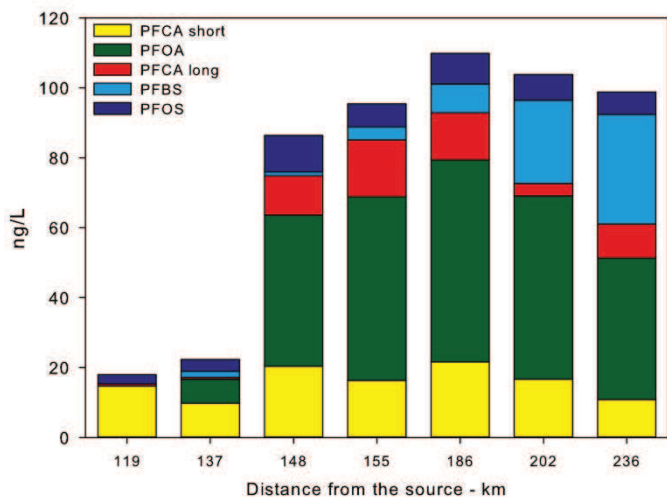
Figure SM3. PFAS concentrations along River Arno; sample sites are described in table SM2
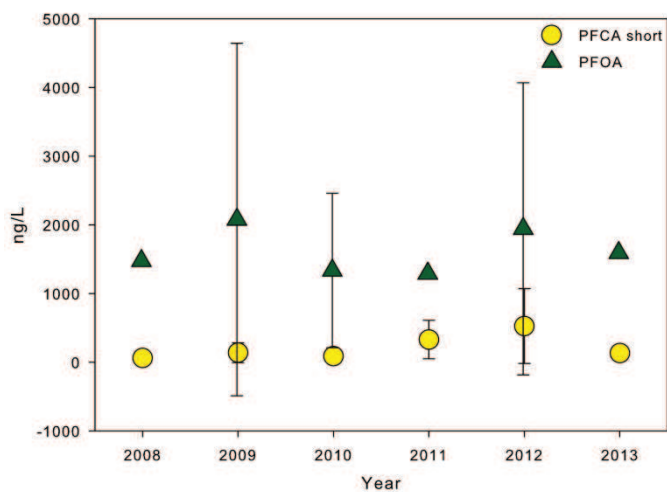

Figure SM4. Temporal evolution of PFOA and short chain PFCA concentrations in River Bormida downstream the discharge of fluoropolymer plant.
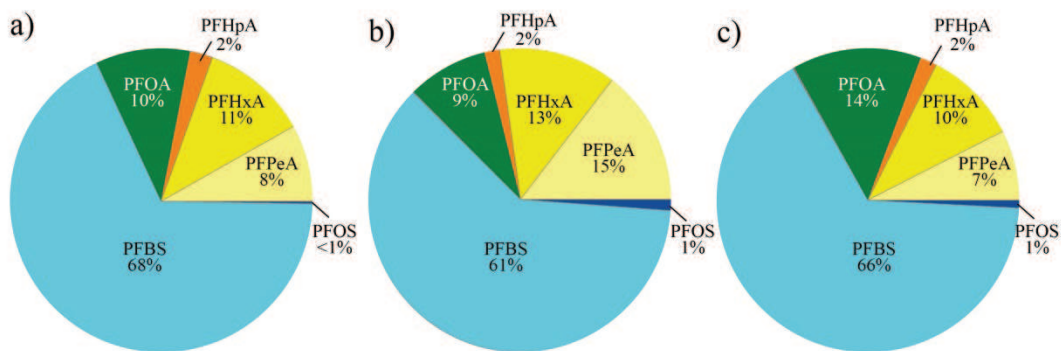

Figure SM5. PFAS percentage composition of (a) effluent of fluorochemical plant, (b) effluent of the sewer pipeline (called Collettore ARICA) and (c) river Fratta-Gorzone at basin closure.