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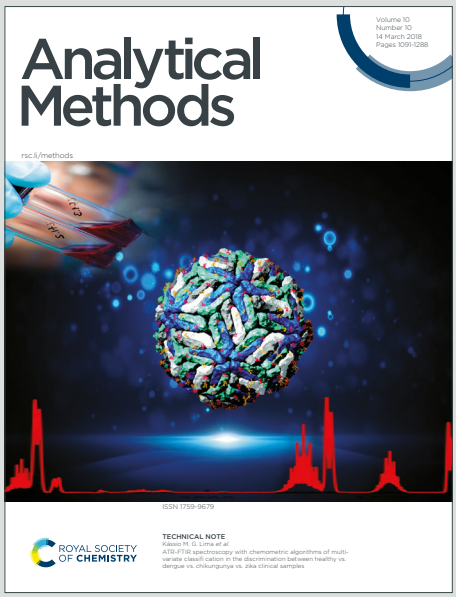

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ARTICLE

Determination of perfluoroalkyl acids in different tissues of graminaceous plants

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A method for the determination of 12 perfluoroalkylacids (PFAA) in vegetal samples was proposed. The analytical procedure was developed to optimize the detention of short-chain PFAA (C<8) due to their higher potential to be translocated and bioaccumulated in plants than long-chain congeners. The method, based on ultrasonic extraction, clean-up and HPLC-MS/MS analysis, determined PFAA in the different plant tissues allowing to study the PFAA distribution and partition in vegetal compartments. The performance of this analytical procedure was validated by analysing samples (root, stem and leaf) of reed grass. The validated method was then applied to graminaceous plants from an agricultural area impacted by a fluorochemical plant discharge (Northern Italy). The PFAA congeners were detected in the most of samples with ΣPFAA concentrations in whole plant ranging from < LOD to 10.4 ng g^{-1} ww and with a greater rate of PFAA accumulation in corn cob than corn kernel. The proposed approach is particularly relevant in edible plant investigation because PFAA levels recorded in the comestible fractions provide information for human risk assessment due to vegetable consumption. Furthermore data on the remaining not edible parts, intended for breeding forage, are also useful for the assessment of the PFAA transfer in the breeding trophic chain.

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a chemical class characterised by a a linear or branched carbon chain which is fully or partially fluorinated[1]. This group includes thousands man-made substances that have been used in numerous industrial and commercial applications, mainly as fluorinated surfactants and fluoropolymer processing aids, since the 1950s[2]. Due to their physicochemical properties, PFAS are persistent, can undergo long-range transport and have possible adverse effects on living organisms[3,4]. The most diffused PFAS in the environment are the perfluoroalkylacids (PFAA), which include perfluoroalkyl carboxylic acids (PFCA) and perfluoroalkyl sulfonic acids (PFSA)[1] and can bioaccumulate both in animals[5] and vegetals[6]. Due to their occurrence in plants, recognized as a non-negligible source of PFAS intake for humans [7–9], the development of reliable methods to determine the PFAS in edible vegetables became essential and urgent[10]. The attention on this issue increased in the last years as well as the number of published studies [11], especially in East Asia [12–15] where vegetables account for a relatively high proportion of the diet. The obtained results are an important contribution to the human exposure assessment, but a greater knowledge of PFAS uptake and tissue distribution in different types of vegetables and plants in real environments is still needed[16].

Despite the lack of official methods of analysis for PFAS in vegetal samples several procedures have been developed. According to Zabaleta et al.[17], the main approaches used for the extraction of PFAS from vegetal matrices are (i) ionpairing, (ii) alkaline digestion and (iii) solid-liquid extraction by an organic solvent enhanced by the application of an energy source such as ultrasound. All these methods need a clean-up step on graphitized carbon black (e.g., ENVI-Carb) or by solid phase extraction (SPE), on polymeric phases such as WAX, MAX and HLB[17]. This step could be replaced by an on-line purification system based on turbulent flow chromatography (TFC)[18]. Recently one-step QuEChERS extraction and clean-up was applied to a variety of edible vegetables[10].

Some of the analytical methods applied to determine the PFAA in vegetals have been designed starting from those developed for solid samples such animal tissue, which were usually optimized to determine long-chain PFAAs. On the contrary analytical methods for vegetables shall be focused on short-chain congeners which are more water soluble and have the highest potential to be translocated to and bioaccumulated in edible plants[19]. Moreover vegetal tissues are a challenging matrix because some co-extracted components such as e.g. cellulose and cuticular waxes can interfere in the extraction phase and induce significant matrix effects in the electrospray ionization[20].

In this study, a method to determine 12 PFAAs in vegetal samples was developed and validated for the different parts of graminaceous plants which were individually analysed to study the PFAA uptake and distribution in the different vegetable

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compartments. The analysed species (*Phragmites australis*), and *Zea mays*) are characterized by high and rigid stem and the maize leaves are covered by waxes. Similar difficult matrices have been rarely analysed for PFAS[21]. The optimized and validated method was then applied to reed grass and maize samples collected in an agricultural area in Northern Italy where the irrigation waters were significantly impacted by PFAA discharges from a fluorochemical plant.

Materials and methods

Chemicals and solvents

A mixed standard of twelve PFAAs (PFAC-MXB Stock Solution) including nine PFCAs, perfluoro-n-butanoic acid (PFBA), perfluoro-n-pentanoic acid (PFPeA), perfluoro-n-hexanoic acid (PFHxA), perfluoro-n-heptanoic acid (PFHpA), perfluoro-n-octanoic acid (PFOA), perfluoro-n-nonanoic acid (PFNA), perfluoro-n-decanoic acid (PFDA), perfluoro-n-undecanoic acid (PFUnDA), perfluoro-n-dodecanoic acid (PFDoDA), and three PFSAs, perfluoro-1-butanesulfonate (PFBS), perfluoro-1-hexanesulfonate (PFHxS), perfluoro-1-octanesulfonate (PFOS), with equal concentrations, was purchased from Wellington Laboratories, Inc. (Guelph, Ontario, Canada). Stable isotope labelled PFCAs and PFSAs used as internal standard compounds (SIL-IS) ($^{13}C_4$-PFBA, $^{13}C_5$-PFPeA, $^{13}C_2$-PFHxA, $^{13}C_2$-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) were purchased from Wellington Laboratories (Guelph, ON, Canada) in solution mixtures (mass-labelled MPFAC-MXA solution at 2 µg mL^{-1} and mass-labelled M3PFPeA solution at 50 µg mL^{-1}).

The phospholipid removal solid-phase extraction (SPE) cartridges (Phree™) (30 mg, 1 mL SPE Tubes) were obtained by Phenomenex (Torrance, California, USA). Hybrid-SPE cartridges were prewashed with 3 mL of acetonitrile (ACN) containing 0.05 % of formic acid according to Honda et al. [22].

All reagents were analytical reagent grade. LC-MS grade Chromasolv methanol (MeOH), LC-MS grade Chromasolv ACN, ammonium acetate (99%), and concentrated formic acid were purchased from Sigma-Aldrich. Water (<18MΩcm resistivity) was produced by a Millipore Direct-QUV water purification system (Millipore, Bedford, MA, USA). Sodium chloride (NaCl) and anhydrous magnesium sulfate $MgSO_4$ (purity ≥99.0%) were purchased from Carlo Erba (Milan, Italy) and Honeywell (Charlotte, North Carolina, USA), respectively.

Preparation of standard solutions

A mixed solution (PFAC-MXB Stock Solution) containing the 12 selected analytes at 2 µg mL^{-1} was diluted in ACN to prepare calibration standard solutions (0-50 µg L^{-1}). The SIL-IS solution was diluted to 40 µg L^{-1} with MeOH. All standard solutions were stored at 4°C.

Sample preparation

At harvest, each plant was split in their different parts: root, stem, leaf and, in case of maize plant, corn cob and corn kernel. Root was washed using tap water and deionized water to remove soil residues and dried with cleaned paper. Each part of plant was considered as a single sample, which was divided in two portions. A few grams of sample were dried in oven at 105°C for 24h to determine the percentage of humidity. The remaining sample was transferred into a food storage bag and placed in freezer at -20 °C. Shortly before extraction phase, a sufficient portion of frozen sample was thawed in oven at 60°C until the complete drying (constant weight), then crushed by grinder and wrapped in aluminium foil until analysis.

Sample extraction

The extraction was carried out according to Mazzoni et al.[23] with minor modifications. For PFAA quantification, about 1 g (2 g for corn kernel) of dry crushed sample was placed into a PP tube and spiked with 100 µL of 40 µg L^{-1} SIL-IS. The extraction was then performed with the addition of 10 mL of a mixture of water and ACN (10:90 v/v) and 140 µL of formic acid followed by vortex agitation for 30 sec, sonication for 15 min and then centrifugation for 12 min (8000 rpm, 10°C). The same steps were repeated twice more by adding 5 mL of ACN and 70 µL of formic acid. After each centrifugation, supernatant was transferred within a single PP tube where 0.5 g of NaCl and 2 g of anhydrous $MgSO_4$ were added later. The PP tube was immediately shaken to prevent coagulation of $MgSO_4$, centrifuged and stored at −4◦C for one night. After that, the extract was concentrated to 1 mL under a gentle stream of nitrogen, purified using a prewashed phospholipid removal SPE cartridge (Phree™) and transferred into a glass vial. 0.2 mL of extract were then transferred into an Eppendorf tube and evaporated to dryness under a gentle nitrogen stream. The residue was dissolved in 0.2 mL of a mixture of a buffer solution (2 mM ammonium acetate/5% MeOH) and MeOH (95:5 v/v). The extract was then agitated by vortex for 30 s, sonicated for 15 min and centrifuged for 2 min (3200 rpm, 10°C). After that, 100 µL of sample were transferred into a micro-vial and acidified by adding 5 µL of formic acid before the injection.

Procedural blanks were included during analyses and handled in the same manner of samples.

Instrumental analysis

All samples were analysed by UHPLC-MS/MS (Thermo Accela 1250 coupled to TSQ Quantum Access MAX, Thermo Scientific, USA) equipped with Water Acquity UPLC BEH C18 column (50 × 2.1 mm id, 1.7 µm particle size). Injection volume was 20 µL. Mobile phases A and B were 2 mM ammonium acetate/5% MeOH and MeOH, respectively. The chromatographic separation was achieved in 12 min with a constant flow rate of 0.3 ml min^{-1}. The mobile phase composition varied according to the gradient program reported in Table 1.

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Table 1. Elution gradients used by the analytical pump. Mobile phases: (A) 2 mM $CH_3CO_2NH_4$ + 5%MeOH; (B) MeOH. Flow rate: 0.3 ml min^{-1}.

Time (min)	Analytical pump	
	A%	B%
0.00	97.5	2.5
2.00	30	70
5.50	10	90
8.00	0	100
11.00	0	100
12.00	97.5	2.5
18.50	97.5	2.5

Table 2. The parameters of heated-electrospray ionization (HESI-II) source.

Parameter	Value
spray voltage	3500 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
collision gas: Argon	1.5 mTorr

Table 3. LC/MS/MS parameters for all target analytes and internal standards.

Target analytes	RT min	Precursor ion (*m/z*)	Product ions (*m/z*)	Collision energy
PFBA	2.60	212.9	168.9	11
PFPeA	3.30	262.9	69.0	39
			218.9	11
PFHxA	3.60	312.9	119.1	22
			268.9	11
PFHpA	3.80	362.9	169.0	18
			318.9	12
PFOA	4.00	412.9	169.0	19
			368.9	13
PFNA	4.50	462.9	218.9	18
			418.9	13
PFDA	4.90	512.9	268.9	18
			468.9	13
PFUnDA	5.30	562.9	268.8	20
			518.8	14
PFDoDA	5.70	612.9	318.8	20
			568.9	14
PFBS	3.30	298.9	80.2	44
			99.1	32
PFHxS	3.80	398.9	80.1	38
			98.9	34
PFOS	4.40	498.9	80.3	45
			99.1	45
$^{13}C_4$-PFBA	2.60	216.9	171.9	11
$^{13}C_5$-PFPeA	3.30	265.9	221.9	11
$^{13}C_2$-PFHxA	3.60	314.9	269.9	11
$^{13}C_4$-PFOA	4.00	416.9	371.9	13
$^{13}C_5$-PFNA	4.50	467.9	422.9	13
$^{13}C_2$-PFDA	4.90	514.9	469.9	13
$^{13}C_2$-PFUnDA	5.30	564.9	519.8	14
$^{13}C_2$-PFDoDA	5.70	614.9	569.9	14
$^{18}O_2$-PFHxS	3.80	402.9	103.0	34
$^{13}C_4$-PFOS	5.00	502.9	99.1	45

A triple quadrupole mass spectrometer equipped with a heated-electrospray ionization (HESI-II) probe operating in negative mode was used. The source parameters were reported in Table 2. The mass spectrometer operated at a resolution of 0.7 Da in negative multiple reaction monitoring (MRM) mode. Table 3 lists the MS/MS transitions and collision energies applied for the different target analytes and isotope labelled standards. The Xcalibur 4.0 (Thermo Scientific) was used for instrument control, data acquisition and processing.

Confirmation and Quantification

Compound identification was performed by comparing their retention times (RT) with those of the SIL-IS (deviation ≤ 0.25%) or with the RT of the reference standards if SIL-IS was unavailable. Except for PFBA, for each analyte one precursor and two product ions were monitored (Table 3).

Quantification was performed by using the isotopic dilution method. Calibration curves were prepared using ACN standard solutions with concentration of 0, 0.5, 1, 2, 5, 10, 20 µg L^{-1}. 100 µL of each standard solution were transferred in micro-vial and spiked with 10 µL of 40 µg L^{-1} SIL-IS and evaporated to dryness under a gentle nitrogen streamfollowing the same procedure adopted for the sample extracts. The residue was redissolved in 100 µL of a mixture of the buffer solution and MeOH (95:5 v/v) and finally acidified with 5 µL of formic acid before injection. Standard calibration curves were acquired before and at the end of each analytical sequence.

Solvent blank samples were injected every five samples. Procedural blanks were injected at the beginning and at the end of each analytical sequence.

Results and discussion

Method development

After harvest, plants were sectioned into their different parts which were individually analysed to study PFAA distribution in vegetable compartments. This approach is particularly relevant in edible plant investigation because PFAA concentrations in the comestible fractions provide fundamental information to assess the human risk due to vegetable consumption. Data on the remaining not edible parts, intended for breeding forage, are also useful for the assessment of the PFAA transfer in the breeding trophic chain. For this reason, maize plants were also split into corn cob and corn kernel.

According to Yamazaki and co-workers[24], samples were dried before extraction. The introduction of this step brought considerable advantages. First of all, dry samples are easier to

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crush than the wet one. Moreover, percentage of humidity in the analysed sample was very variable, ranging from 2% to 72%. The water content in specimen affects some extraction aspects such as the final volume of the supernatant obtained after centrifugation and the amount of salts to be added. In particular, drying of samples allowed to standardized these quantities, to obtain a smaller volume of supernatant, speeding up then its concentration, lower the interference of water in the pretreatment procedures[25] and reduce the amount of salts to add.

The extraction procedure of Mazzoni et al.[23] was developed for the detection of PFAA in animal tissues and sediments and its application on dry plant samples required some changes. Compared to Mazzoni et al.[23] procedure, the extraction was repeated once more adding a higher total amount of ACN (19 mL instead of 9.5 mL) because vegetal sample tends to absorb extraction solvents. Moreover- a higher amount of salts (0.5 g of NaCl and 2 g of $MgSO_4$ instead of 0.2 g and 0.6 g, respectively) were required to prevent coagulation of $MgSO_4$ in the supernatant. In both methods, phospholipids were removed passing the concentrated extract through Phree™ cartridge. Indeed, phospholipids are essential components of biological membranes and signal transduction cascades in plants but can interfere with the analysis of PFAS[22,26,27].

In-line clean up procedure of the extracts by turbulence flow chromatography (TFC)[23] was avoided and the plant extracts were analysed by direct injection. The latter injection method was adopted to improve the recovery of early-eluting short-chain PFAAs, which are more bioaccumulable in edible plants[19]. To achieve optimal separation efficiency of short-chain PFAAs, the mobile phase gradient was optimized (Table 1). In particular, at the beginning of the chromatographic run, the mobile phase was composed by a 97.5% buffer solution to improve the shapes of early-eluting peaks.

Method validation

The method was subjected to the validation procedure by analysing samples (root, stem and leaf) of reed grass (*Phragmites australis*). The obtained results are showed and discussed in the following paragraphs.

Matrix effect The matrix effect (ME) is usually caused by interferences from co-extracted matrix components during the ionization and detection steps[10]. This effect is not negligible in MS analysis, particularly for complicated matrices such as vegetables[28]. In this study, ME was evaluated by comparing extracts of uncontaminated samples fortified at 10 µg L^{-1} with a standard solution at the same concentration. Relative ME (ME%) for each compound was calculated as the ratio of the peak area of native analyte in fortified extract to that in standard solution averaged over 5 replicates:

$$ME\ (\%) = (Peak\ Area_{reed\ grass} / mean\ peak\ Area_{Std}) * 100$$

A ME(%) below 100% means that ionization suppression is present, while values above 100% indicate a ionization enhancement[23]. To consider also sample variability, ME evaluation was replicated 6 times by analysing extracts of reed grass collected in different seasons and sampling sites in order to include diverse vegetative conditions (affected by e.g. growth stage, water availability, air temperature).

Experimental results, reported in Table 4, suggested that suppression of analytes with ≤ 10 C-atoms decreases as the number of C-atoms in compounds increases. Indeed, in leaf samples ME ranged from 12% for PFBA to 116% for PFDA with a gradual increase from shorter- to longer-chain compounds. In the same way, the lowest ME value in stem samples was recorded for PFPeA (25%) while the highest value was estimated for PFDA (113%). In roots ME was more effective for short-chain compounds (ME ≤30% for analytes with ≤ 5 C-atoms) than for long-chain compounds (ME ≥90% for analytes with C8-C10-atoms). Significant ionization suppression for polyfluoroalkyl substances has been already evidenced in maize leaves[21], and total dissolved sugars have been identified as the crucial factor for ME in the determination of PFSA in various vegetables and grains[25].

Regarding the longest-chain compounds (C11-C12), a different situation was observed. In leaf and stem samples, ME for PFUnDA and PFDoDA were highly variable (standard deviation from 81 to 102%). Ionization of long-chain compounds in these two plant fractions is affected by matrix composition. Indeed, the epidermis of leaves and aerial parts of many vascular plants, such as reed grass, are covered by cuticle, which is a film of lipid polymers impregnated with waxes. During extraction of plant fraction wrapped by cuticular waxes, a phospholipid bilayer of the cell membrane is often damaged, which results in presence of intracellular components in the final extract[29]. On the contrary, standard deviations of ME (%) for PFUnDA and PFDoDA were 45% and 24%, respectively, in root samples.

Table 4. Validation parameters: Matrix effect.

Analytes	Matrix effect (%)					
	Root		Stem		Leaf	
	mean	*st. dev.*	mean	*st. dev.*	mean	*st. dev.*
PFBA	27.9	*11*	49.5	*17*	11.5	*3*
PFPeA	28.6	*8*	25.4	*2*	13.8	*5*
PFHxA	68.5	*47*	35.5	*4*	23.8	*2*
PFHpA	60.8	*31*	61.5	*2*	34.8	*2*
PFOA	93.1	*39*	93.6	*14*	69.9	*8*
PFNA	99.4	*37*	99.5	*33*	87.3	*21*
PFDA	90.3	*56*	113.4	*55*	115.9	*59*
PFUnDA	69.9	*45*	128.8	81	101.8	102
PFDoDA	46.2	*24*	139.3	96	116.8	96
PFBS	30.4	*7*	34.5	3	25.6	*6*
PFHxS	88.9	*28*	81.8	*2*	46.1	*8*
PFOS	98.2	*55*	90.3	*38*	83.1	*28*

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According to these results, the present method cannot be considered reliable for PFUnDA and PFDoDA determination in leaf and stem matrices. However, several studies demonstrated that the long-chain compounds are usually retained by roots and do not transfer in the epigeal parts including leaves[30–33].

Repeatability Repeatability of all analytical steps was determined by three replicated analyses of 6 different samples. We included one pool of roots, one of stems and one of leaves of reed grass collected in a sampling site highly contaminated by PFAS (called environmental samples in Table 5). In addition, one sample of each tissue from a reference uncontaminated area was fortified at 10 µg g^{-1} ww the day before the extraction, and analysed. Each final extract was injected three times. Mean concentrations and their CVs of fortified and environmental samples are reported in Table 5. In general, a moderate reproducibility[34] (coefficient of variation, CV, ranging from 11% to 66%) was recorded for the majority of compounds detected in all fortified samples. CV was under 10% (weak variability)[34] only for PFBA in root and for PFPeA and PFHxA in stem samples. Environmental samples generally presented low concentrations of selected PFAAs with CV ranging from 2 to 143%. The worst data have been obtained for unspiked stem samples for which it is more difficult to obtain homogeneous samples. High reproducibility for this kind of difficult matrices has been also obtained for artichoke skin (RSD 87%)[21].

Table 5. Validation parameters: Repeatability is expressed as coefficient of variation (CV). Average concentration recorded in replicates is reported (mean). Fortified samples were spiked at 10 ng g^{-1} ww.

Analytes	Coefficient of variation (%)					
	Fortified samples					
	Root		Stem		Leaf	
	mean ng g^{-1} ww	*CV*	mean ng g^{-1} ww	*CV.*	mean ng g^{-1} ww	*CV*
PFBA	8.5	*8*	8.4	*13*	8.0	*15*
PFPeA	9.3	*21*	8.7	*9*	7.1	*11*
PFHxA	7.5	*13*	7.8	*7*	9.3	*16*
PFHpA	5.2	*20*	8.3	*20*	19.6	*25*
PFOA	6.8	*14*	6.0	*14*	6.7	*19*
PFNA	7.7	*11*	7.2	*15*	9.0	*33*
PFDA	9.2	*28*	8.5	*15*	11.3	*33*
PFUnDA	9.2	*24*	9.4	*21*	n.a.	*n.a.*
PFDoDA	9.2	*40*	7.8	*12*	13.4	*39*
PFBS	16.2	*17*	15.4	*16*	12.5	*16*
PFHxS	9.3	*15*	6.4	*12*	7.1	*17*
PFOS	5.4	*36*	4.6	*35*	5.7	*66*
	Environmental samples					
	Root		Stem		Leaf	
	mean ng g^{-1} ww	*CV*	mean ng g^{-1} ww	*CV.*	mean ng g^{-1} ww	*CV*
PFBA	< LOD	*n.a.*	1.2	*31*	12.2	*9*
PFPeA	4.2	*7*	0.5	*125*	2.1	*16*
PFHxA	32.2	*9*	< LOD	*n.a.*	0.9	*41*
PFHpA	1.4	*8*	0.6	*60*	0.7	*58*
PFOA	< LOD	*n.a.*	< LOD	*n.a.*	1.3	*38*
PFNA	< LOD	*n.a.*	0.5	*70*	2.5	*74*
PFDA	1.0	*124*	< LOD	*n.a.*	6.7	*60*
PFUnDA	< LOD	*n.a.*	2.3	*104*	13.4	*69*
PFDoDA	12.0	*2*	2.5	*143*	10.7	*21*
PFBS	< LOD	*n.a.*	< LOD	*n.a.*	3.5	*21*
PFHxS	< LOD	*n.a.*	< LOD	*n.a.*	0.3	*18*
PFOS	< LOD	*n.a.*	< LOD	*n.a.*	1.2	*87*

n.a.= not available data

Recovery Recovery was calculated as the ratio of the measured concentration to the expected concentration in fortified samples. Fortified concentrations have been calculated by subtracting those measured in the unspiked samples, generally close to LOD. Recoveries (Table 6) were between 60 and 134% for PFCAs (excluding PFHpA) and between 46 and 93 % for selected PFSAs (excluding PFBS). These recovery data are comparable with those obtained in a wide study on 257 samples of vegetables (from 22% for PFBA to 112% for PFDoDA)[35], demonstrating that the large differences in physico-chemical characteristics of PFAAs lead also to differences in recovery if a single analytical method is used. Because of its high variability, PFUnDA recovery in leaves was not calculated. Anomalous recoveries of PFBS (125-162%) and PFHpA (52-196%) can be explained by considering that they were not calibrated by using their own SIL-IS but by $^{13}C_5$-PFPeA and $^{13}C_4$-PFOA, respectively. The recovery estimation suffers from the problem that the labelled surrogate suffers from different matrix effects respect to the native compound. In fact, the highest PFHpA recovery (196.1%) was measured in leaves, where the ME was 35% for PFHpA and 70% for PFOA (Table 4).

Table 6. Validation parameters: Recovery. Fortified samples were spiked at 10 ng g^{-1} ww. Due to the high variability, PFUnDA recovery in leaf was not calculated.

Analytes	Recovery (%)					
	Root		Stem		Leaf	
	mean	*st. dev.*	mean	*st. dev.*	mean	*st. dev.*
PFBA	84.8	*7*	84.5	*11*	80.2	*12*
PFPeA	93.3	*19*	86.8	*8*	70.7	*8*
PFHxA	75.2	*10*	77.7	*5*	93.0	*14*
PFHpA	51.9	*15*	82.7	*17*	196.1	*49*
PFOA	68.0	*9*	59.4	*8*	66.7	*13*
PFNA	76.9	*8*	65.3	*11*	90.4	*30*
PFDA	92.0	*25*	76.6	*13*	112.9	*38*
PFUnDA	89.9	*22*	83.3	*19*	n.a.	*n.a.*
PFDoDA	92.5	*36*	69.1	*9*	134.4	53
PFBS	162.1	*27*	154.4	*25*	125.4	*20*
PFHxS	92.9	*14*	61.7	*8*	70.6	*12*
PFOS	54.0	*20*	46.1	*16*	57.5	*38*

n.a.= not available data

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Linearity and sensitivity Linearity of all target chemicals was determined by a seven-point calibration curve which was set up for each compound by injecting 7 levels of the multicomponent standard solution spiked with SIL-IS at 4 µg L^{-1}. In the considered concentration range, the response of the mass spectrometer was linear with a coefficients of determination (R^2) higher than 0.98 for all the target compounds (Table 7).

Limits of detection (LODs) and limits of quantification (LOQs) were estimated according to the ISO 6107-2:2006 standard as threefold and tenfold the standard deviation of the lowest standard, respectively. The values were expressed in ng g^{-1} ww by referring to the wet weight of extracted sample (1 g of dry weight with an average humidity of 15.8% in root, 27.0% in stem and 19.4% in leaf samples). LODs ranged from 0.1 to 0.7 ng g^{-1} ww while LOQ values were between 0.5 and 3.5 ng g^{-1} ww (Table 7).

Table 7. Validation parameters: Linearity (0-20 µg L^{-1}) and Sensitivity expressed as LOD (ng g^{-1} ww) and LOQ (ng g^{-1} ww)

Analytes	Linearity	Sensitivity					
		Root		Stem		Leaf	
	R^2	LOD	LOQ	LOD	LOQ	LOD	LOQ
PFBA	0.997	0.4	1.9	0.4	1.2	0.4	1.3
PFPeA*	0.996	0.3	1.5	0.3	0.9	0.3	1.0
PFHxA	0.991	0.3	1.5	0.3	0.9	0.3	1.0
PFHpA**	0.994	0.2	0.8	0.2	0.5	0.2	0.6
PFOA**	0.995	0.3	1.2	0.2	0.7	0.2	0.8
PFNA	0.996	0.4	1.8	0.3	1.1	0.4	1.2
PFDA	0.986	0.6	3.1	0.6	1.9	0.6	2.1
PFUnDA	0.996	0.7	3.4	0.6	2.1	0.7	2.3
PFDoDA	0.982	0.7	3.5	0.6	2.1	0.7	2.4
PFBS*	0.984	0.3	1.3	0.2	0.8	0.3	0.9
PFHxS	0.998	0.2	0.7	0.1	0.5	0.2	0.5
PFOS	0.990	0.5	2.4	0.5	1.5	0.5	1.7

*Analytes calibrated by SIL-IS PFPeA;
** Analyte calibrated by SIL-IS PFOA;

Application to environmental samples

The developed method has been applied to vegetal samples taken during the monitoring activity carried out in Northern Italy, in an agricultural area where the surface waters, used for irrigation, are severely polluted by PFAAs[36,37]. The monitoring plan included 10 monitoring stations, where a ubiquitous aquatic vegetal species (*Phragmites australis*), used also for phytodepuration, and a cultivated plant (*Zea mays*) were sampled. The method shown in this article was applied on vegetables collected in the selected sampling sites in 2 different seasons (summer and late autumn) in 2 years (2019-2020). The results of the ΣPFAA determination in 31 reed grass and 13 maize samples are shown as box plots in Figure 1.

The sum of measured PFAA congeners (ΣPFAA) in the whole plant was calculated as the sum of PFAA concentrations determined in each fraction, considering their biomass fraction. In general, PFAA congeners were detected in most of the samples with a detection frequency of 97%. The ΣPFAA concentrations in the two vegetables (as whole plants) ranged from < LOD to 10.4 ng g^{-1} ww. In particular, the median values of PFAA concentrations were 1.6 ± 2.5 ng g^{-1} ww in reed grass and 1.0 ± 2.7 ng g^{-1} ww in maize.

To investigate the partition of these chemicals between fractions, the ΣPFAA concentrations in each part of the plant were reported in Figure 2. The majority of PFAA contamination in reed grass was detected in the leaf samples (46.7%), while the less contaminated fraction of maize was the hypogeal part (9.2%). The field results show that corn cob has a greater rate of PFAA accumulation than corn kernel, confirming what Krippner and co-workers demonstrated in laboratory trials[38]. Moreover, PFAA distribution in each vegetal fraction was evaluated. As Figure 3 clearly shows, long-chain analytes were the dominant contaminants in reed grass root and steam (56%) while the leaves were mainly polluted by short-chain compounds. On the contrary, short-chain PFAA represented the dominant analytes in root, stem and corn cob in maize, while leaf and corn kernel mainly accumulated long-chain congeners. A significantly higher uptake of short-chain PFAAs by maize roots was also observed in a nutrient solution experiment carried out by Krippner et al.[38] PFOA and PFOS were separately plotted because they are classified as accumulable substances of high concern for humans and biota[11]. Nevertheless, both these analytes represented a very small contribution (from 0 to 9%) to the total PFAA contamination in the whole plants.

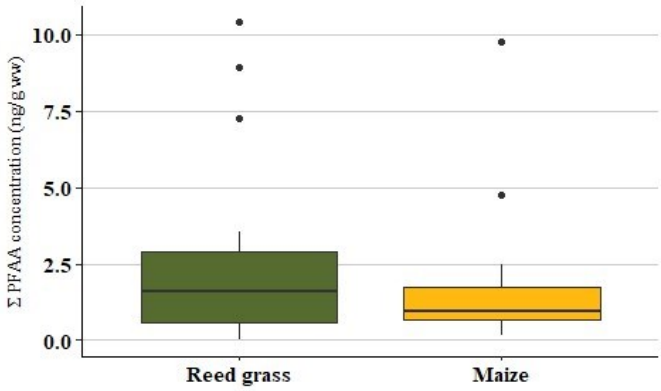

Figure 1. ΣPFAA in whole plants (ng g^{-1} ww) of reed grass (N=31) and maize (N=13).

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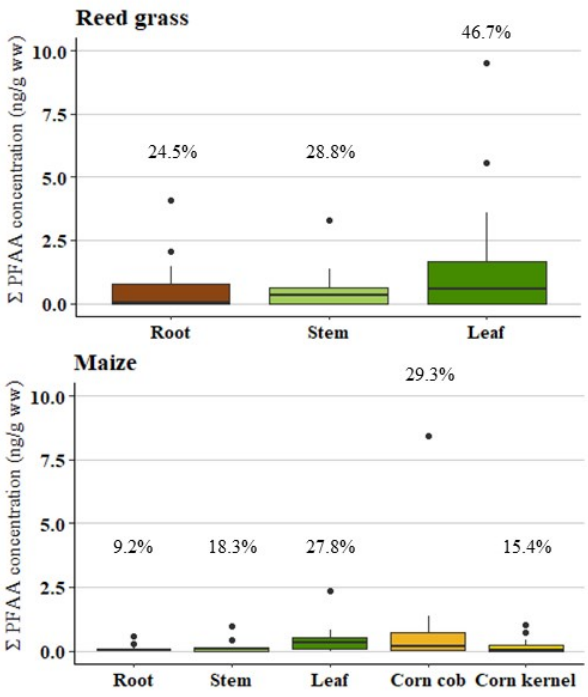

Figure 2. ΣPFAA in the different plant fractions (ng g^{-1} ww) of reed grass (N=31) and maize (N=13).

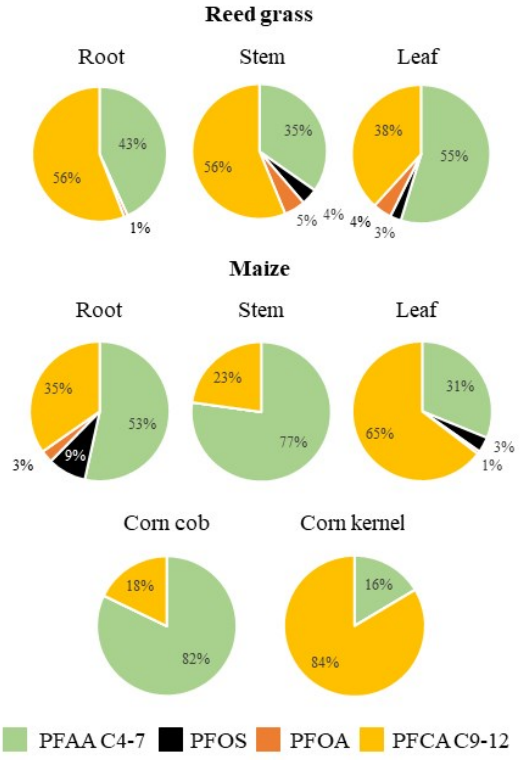

Figure 3. Percentage distribution of PFAAs in each fraction of the collected plants. Short-chain PFCAs (4-7 carbon atoms) and PFBS are in green, PFOS in black, PFOA in orange, and long-chain PFCAs (9-12 carbon atoms) in yellow.

Conclusions

In the present work, a method for the determination of 12 perfluoroalkyl acids in vegetal samples was validated. This analytical procedure was developed to optimize the detection of short-chain PFAA (C<8) due to their higher potential to be translocated to and bioaccumulated in plants than long-chain congeners[19]. The present method has been validated for each plant fraction (root, stem and leaves) of a ubiquitous graminaceous species (reed grass). The procedure was verified to be satisfactory in term of matrix effect, recovery, repeatability, linearity and sensitivity. The obtained results highlighted also the matrix effects of vegetal components on the PFAS extraction and determination, mainly due to the complex composition of the vegetal tissues (e.g., cuticular waxes wrapped on the aerial parts).

The optimized and validated method was then applied to reed grass and maize samples collected during the monitoring activity carried out in Northern Italy, in an agricultural area impacted by a fluorochemical plant. The PFAA congeners were detected in most of the samples with ΣPFAA concentrations in whole plant ranging from <LOD to 10.4 ng g^{-1} ww.

As shown by the monitoring results, the main advantage of this method is the opportunity to determinate PFAA concentration in each part of a vegetal sample. This approach is particularly relevant in plant investigation because PFAA uptake in the edible fractions provides information for human risk assessment due to vegetable consumption, while PFAA concentrations in the remaining parts, used as forage for breeding, can warn on their possible transfer to the trophic chain of farm animal.

Author contributions

CF, SP and SV developed the method and wrote the manuscript, CF performed analysis, SP, MB and RL planned the project, MB, RL revised the paper.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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