Article

Assessment of Reed Grasses (*Phragmites australis*) Performance in PFAS Removal from Water: A Phytoremediation Pilot Plant Study

Claudia Ferrario [1,*], Cosimo Peruzzi [2], Alessio Cislaghi [2,3], Stefano Polesello [1,*], Sara Valsecchi [1], Roberto Lava [4,5], Francesca Zanon [4], Gianfranco Santovito [6], Alberto Barausse [6] and Marco Bonato [6,7]

1   Water Research Institute—National Research Council of Italy (IRSA-CNR), Via del Mulino 19, 20861 Brugherio, MB, Italy; sara.valsecchi@irsa.cnr.it

2   Department of Agricultural and Environmental Sciences (DiSAA), University of Milan, Via Celoria 2, 20133 Milan, MI, Italy; cosimo.peruzzi@unimi.it (C.P.); alessio.cislaghi@unimi.it (A.C.)

3   Centre of Applied Studies for the Sustainable Management and Protection of Mountain Areas (Ge.S.Di.Mont.), University of Milan, Via Morino 8, Edolo, 25048 Brescia, BS, Italy

4   ARPAV, Regional Environmental Protection Agency of Veneto, Laboratory Department, Via Lissa 6, 30174 Mestre, VE, Italy; roberto.lava@arpa.veneto.it (R.L.); francesca.zanon@arpa.veneto.it (F.Z.)

5   EFSA, European Food Safety Authority, Via Carlo Magno 1A, 43126 Parma, PR, Italy

6   Department of Biology (DiBio), University of Padova, Via Bassi 58/b, 35131 Padova, PD, Italy; gianfranco.santovito@unipd.it (G.S.); alberto.barausse@unipd.it (A.B.)

7   ARPAV, Regional Environmental Protection Agency of Veneto, Dipartimento Regionale Qualità Ambientale, Via della Pace, 73, 45100 Rovigo, RO, Italy; marco.bonato@arpa.veneto.it

*   Correspondence: claudia.ferrario@marionegri.it (C.F.); polesello@irsa.cnr.it (S.P.)

Abstract: Per- and polyfluoroalkyl substances (PFASs) have multiple emission sources, from industrial to domestic, and their high persistence and mobility help them to spread in all the networks of watercourses. Diffuse pollution of these compounds can be potentially mitigated by the application of green infrastructures, which are a pillar of the EU Green Deal. In this context, a phytoremediation pilot plant was realised and supplied by a contaminated well-located in Lonigo (Veneto Region, Italy) where surface and groundwaters were significantly impacted by perfluoroalkyl acids (PFAAs) discharges from a fluorochemical factory. The investigation involved the detection of perfluorobutanoic acid (PFBA), perfluorooctanoic acid (PFOA), perfluorobutanesulfonic acid (PFBS) and perfluorooctanesulfonic acid (PFOS) inside the inlet and outlet waters of the phytoremediation pilot plant as well as in reed grasses grown into its main tank. The obtained results demonstrate that the pilot plant is able to reduce up to 50% of considered PFAAs in terms of mass flow without an evident dependence on physico-chemical characteristics of these contaminants. Moreover, PFAAs were found in the exposed reed grasses at concentrations up to 13 ng g^{-1} ww. A positive correlation between PFAA concentration in plants and exposure time was also observed. In conclusion, this paper highlights the potential efficiency of phytodepuration in PFAS removal and recommends improving the knowledge about its application in constructed wetlands as a highly sustainable choice in wastewater remediation.

Keywords: PFAS removal; *Phragmites australis*; phytoremediation pilot plant; PFAS accumulation; green infrastructure

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a large group of organofluorine compounds. According to the report on the terminology of PFASs recently published under the framework of the Global PFC Group "PFAS are defined as fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it), i.e., with a few noted exceptions, any chemical with at

least a perfluorinated methyl group (-CF$_3$) or a perfluorinated methylene group (-CF$_2$-) is a PFAS" [1]. The presence of the extremely strong C-F bond leading to their high stability and resistance to thermal and (bio)chemical degradation [2–4]. Due to their unique properties, PFASs have been widely employed in everyday products and industrial formulations with many different applications from non-stick coatings (e.g., cookware and food packaging) to fire-fighting foams, pesticides, and cosmetics since the 1940s [5]. At the same time, these characteristics of PFASs make them environmentally persistent, with the potential to undergo long-range transport and damage living organisms [6,7]. Among PFASs, the most diffused compounds in the environment are perfluoroalkyl acids (PFAAs), which include perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs) [8]. PFASs are widely detected around the globe [9] in all environmental compartments, including air [10], water [4,11], soils and sediments [12], animals [13–15], plants [16,17], and human beings [18]. Moreover, they are often associated with toxic effects in organisms including humans [19–21]. These adverse health effects are particularly well-known with regard to long-chain (C8 and above) PFASs that have been consequently phased out from production in the US and Europe. Long-chain PFASs are now restricted in use (e.g., perfluorooctanoic acid, PFOA, perfluorooctanesulfonic acid, PFOS, and their salts and related compounds under the Stockholm Convention, and long-chain PFASs included on the EU REACH candidate list) and many national food and health authorities have established tolerable daily intakes (TDIs) [22–25].

PFASs are a major worldwide concern related to water management [26]. To address PFASs occurrence in wastewater, various treatment processes have been developed and successfully employed [27–32]. Recently, several critical reviews on different approaches for PFASs removal from water have been published [26,33–35]. Shortly, the currently used technologies include ion exchange resins, filtration, Granular Activated Carbon (GAC), chemical oxidation, ultraviolet (UV) irradiation, and incineration. The ion exchange process, using anion exchange resins, exhibited promising potentials for the effective removal of PFOS from wastewater [36]. However, some gaps still need to be filled such as how to safely treat and dispose of the highly concentrated eluate generated from this process or how effective is the ion exchange process in removing PFAAs [37]. Water treatment through physical separation technologies, such as nanofiltration and reverse osmosis membranes [33], is considered the most effective method for PFAAs removal as well as the most expensive one [38]. Additional costs are related to drinking water; indeed, this process removes harmful contaminants but may strip many minerals from treated water, which needs to be remineralized [38]. On the contrary, GAC technology is characterized by lower costs [39]. The efficacy of this method is widely accepted and proven [35], but is quickly lost with time [40,41] and PFAA-saturated GAC should be regenerated by thermal processes at high temperature [42]. On the other hand, chemical oxidation is considered a promising process to degrade contaminants from water [43]. Generally, PFASs are resistant to most oxidants/reductants used in water treatments [44] and incomplete mineralization may cause the creation of toxic intermediates that are more harmful than their precursors [26]. Oxidation process efficiency can be increased including UV irradiation [45] even though the removal of PFCAs and shorter-chain PFSAs by UV system is ineffective [26]. Finally, incineration is one of the most familiar routes to destroy organic compounds that are burnt at 600–1000 °C. In fact, degradation of PFASs requires extremely high temperatures because the carbon-fluorine bonds are severed at 1000 °C [46]. As a consequence, operating costs are high and harmful emissions [47–50] as well as active greenhouse gases [46] can be formed.

In recent years, more attention has been paid on green infrastructure to limit the pollutant diffusion in the environment [51–54]. Natural or constructed wetlands (CWs) are considered a highly sustainable choice in wastewater remediation [55] as well as a cost-effective and easy to operate solution [56]. CWs can transform many of the common pollutants (and less common) into harmless products or essential nutrients that can be used for additional biological productivity and, consequently, extract pollutants from the water-soil medium. The removal of contaminants in CWs is obtained by a synergic work that

mainly involves soil particles, plants, and microbial communities [57]. More specifically, the plants that grow in water-saturated soil play an active key role in stabilizing the bacteria that colonise the root systems and rootstocks [54]. Regarding PFASs, promising results were obtained by Chen et al. (2012) [58] who investigated the removal of PFOA and PFOS from spiked water by four individual pilot-scale planted CW systems. Nevertheless, the knowledge on PFASs fate in CWs and the efficiency of CWs in PFASs removal is still in its infancy [56].

Reed grass, *Phragmites australis* (Cav.) Trin. Ex Steud., is one of the most widely spread plants in the world and it has been used in phytoremediation treatments of different types of wastewater, soil, and sediments since the 1970s [59]. Several studies demonstrated the ability of this species to uptake high levels of metals [35,60] as well as to remove veterinary drugs [61,62], pharmaceutical products [63–65], phthalic acid esters [66], silica [67], dyes [68,69], and pesticides [70,71] from water. Despite this, only a few studies reported data concerning reed grass affinity to PFASs accumulation. In particular, Mudumbi et al. (2014) [72] investigated the susceptibility of this species to uptake PFOA from contaminated riparian sediments while in Wang et al. (2019) [73] bioaccumulation of PFASs was monitored in reed grasses grown in a CW which receives water from wastewater treatment plants (WWTPs). This limited number of works proves that most studies on PFASs uptake are focused on agricultural plants [16] and their aims are mainly connected with food security rather than environmental clean-up [74].

In this context, a phytoremediation pilot plant was realised and supplied by a contaminated well-located in Lonigo (Vicenza Province, Veneto Region, northeastern Italy) where surface and groundwaters were significantly impacted by PFAA discharges from a fluorochemical plant [75,76]. The groundwater contamination plume, which extends over an area of 190 km^2, affects both public waterworks and private wells raising health concerns for residents [77]. Based on this, the present study aims to investigate the efficiency of a phytoremediation pilot system covered by reed grass in PFAA removal and to describe the PFAA uptake and distribution in different vegetable parts.

2. Materials and Methods

2.1. Study Area

The phytoremediation pilot plant was installed inside the "Vivai dall'Ava" garden centre, sited at Lonigo municipality (Vicenza Province, Veneto Region, northeastern Italy). Lonigo is served by a contaminated waterwork, which draws water from a groundwater which is the most impacted PFAA polluted area in Italy [77]. The complete physico-chemical characteristics of waters used in the test are available on the water manager website (www.acquedelchiampospa.it/analisi-acqua/analisi (accessed on 14 March 2022)). As stated in the quarterly report released in November 2018, groundwater collected in Lonigo has a pH of 7.5, conductivity of 580 µS cm^{-1}, alkalinity of 350 mg L^{-1} HCO$_3^-$ and hardness of 22.4 mg L^{-1}. The high level of water contamination by PFASs is well-documented by the results of the regional environmental monitoring plan started in 2013, according to which in Lonigo PFAA levels reached up to 4.7 µg L^{-1} in surface water and 31.1 µg L^{-1} in groundwater (data available on ARPAV website (www.arpa.veneto.it/dati-ambientali (accessed on 14 March 2022)).

During the monitoring campaign in 2018, the groundwater from the private well was mainly contaminated by PFOA, with concentrations ranging from 1.5 to 2.4 µg L^{-1}, and perfluorobutanoic acid (PFBA) that reached up to 1.2 µg L^{-1}. About 12% of the pollution was due to perfluoropentanoic acid (up to 0.8 µg L^{-1}), perfluorobutanesulfonic acid (PFBS, up to 0.7 µg L^{-1}) and perfluorohexanoic acid (up to 0.7 µg L^{-1}) while the other PFAAs represented less than 3% of water contamination (ARPAV). The continuous monitoring of the water quality during this study highlighted that PFAA concentrations were constant over time in the considered well.

2.2. The Phytoremediation Pilot Plant

The disposition of the components forming the phytoremediation pilot plant is sketched in Figure 1. In particular, three plastic tanks formed the core of the pilot plant and addressed the following functions: (i) the first tank (input tank, base of 1 m × 1 m and height of 1 m) was used as water storage and received the contaminated water directly from the well; (ii) the second one (phytoremediation tank, base of 1 m × 3 m and height of 1 m) was the principal tank and it was fed by the input tank with a constant flow rate, while (iii) the last one (output tank, base of 0.43 m × 0.67 m) collected the overflow of the phytoremediation tank. A set of pumps, pipes, and taps linked the three tanks, guaranteeing the hydraulic connection among them. It is important to highlight that the phytoremediation tank had two baffles delimitating two calm water zones thick 0.2 m each (Figure 1), hence the effective length of the phytoremediation tank is 2.6 m. The phytoremediation and output tanks were not covered or shielded during the experimental campaign.
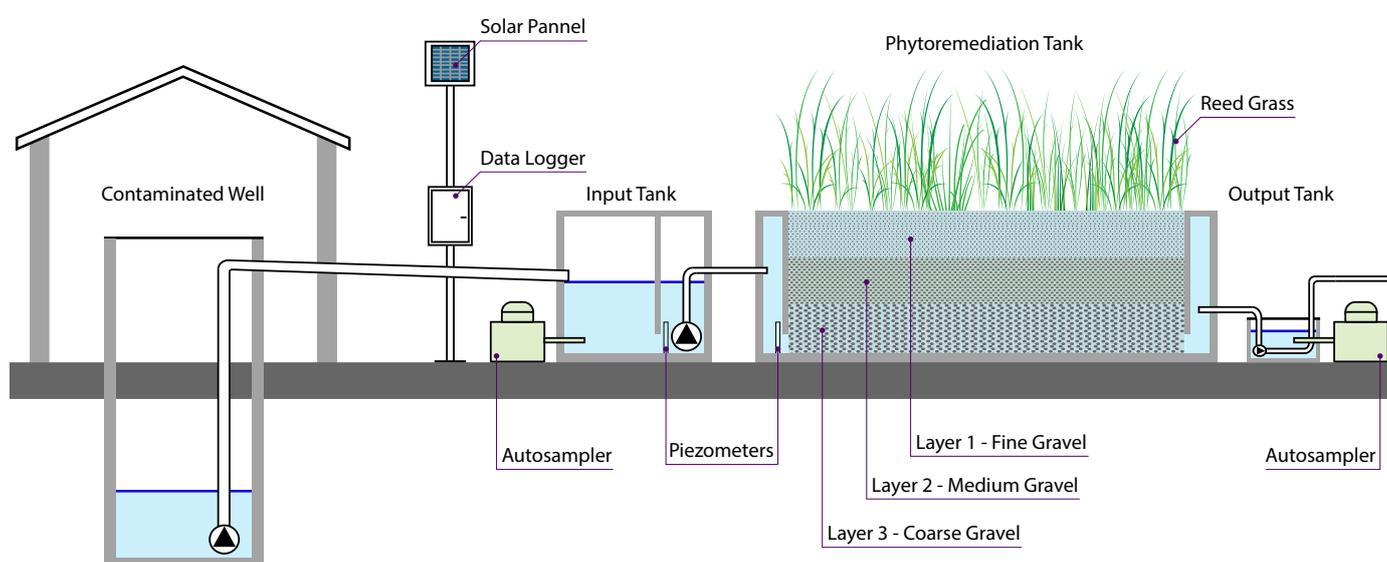

Figure 1. Overview of the Lonigo phytoremediation pilot plant (the dimensions are not in scale).

The phytoremediation tank had a central section containing three layers of gravel with different granulometry over which the reed grasses were planted. Going from the top to the bottom, there was a thin layer of 5 cm of fine gravel (5 mm of diameter), below it there was a layer 25 cm thick of medium gravel (10–20 mm) and further underneath there was a layer of 50 cm composed by coarse gravel (80–100 mm). The fraction of soil in the phytoremediation tank was equal to 1% of the tank volume. This configuration had the purpose to isolate the performance of reed grass in PFAA extraction from a contaminated flow, neglecting the role potentially played by the soil. Indeed, the chosen approach allows us to simplify a typical water treatment process that occurs in a CW to better understand the single contribution due to plant uptake and therefore optimize the management of vegetal fraction in a phytoremediation system.

We decided to use reed grass because it is widely employed in wastewater treatment wetlands since it grows easily whenever abundant water is available [59,78]. Hence, in April 2018, 200 small plants of reed grasses were planted in the fine gravel thin layer. The water level inside the phytoremediation tank was kept such as to completely saturate the entire gravel mass.

Figure 1 reports also the disposition of the in-situ instrumentation involved to monitor the hydraulic quantities of interest during the experimental campaign. Two low-pressure vibrating wire piezometric sensors (piezometers, Encardio Rite, India) recorded the water level inside both the input tank and the phytoremediation tank in a real-time mode and the data were stored in an appropriate data logger, powered without interruption by solar

panels. Instead, inside the outer tank, a graduated rod was mounted to visually quantify the water levels. Moreover, we placed two automatic samplers (autosamplers, Teledyne ISCO, Lincoln, NE, USA), one gathering the water samples for the inlet tank and the other from the outlet tank, to measure the PFAA concentration. Each autosampler contains 24 bottles with a capacity of 1000 mL. Further, the "Vivai dall'Ava" garden centre is equipped with an air temperature sensor placed at 2 m above the ground.

In September 2018, before pursuing the water quality test and after the system reached steady-state conditions, it was possible to estimate the most relevant hydraulic parameters governing the Lonigo pilot plant. The mean inlet discharge to the phytoremediation tank (Q_{IN}) was directly set by the pump located in the input tank and it is equal to 0.275 m^3 day^{-1}. For measuring the mean outlet discharge from the phytoremediation tank (Q_{OUT}), the situation was different since the outflow was not regulated by a pump. Hence, Q_{OUT} was estimated by monitoring the variation in water levels inside the outlet tank during dry conditions, providing an average value of 0.175 m^3 day^{-1}. Since the piezometers revealed a constant water level inside the tanks and no water losses occurred, the difference between Q_{IN} and Q_{OUT} can be attributed to evapotranspiration (*ET*). To validate this hydraulic steady-state condition, the daily *ET* was estimated using both the FAO-56 Penman–Monteith method [79] and the Hargreaves–Samani method [80], using the in situ recorded air temperature and the meteorological observations (i.e., air relative humidity, wind speed, and solar radiation) gathered by the ARPAV station present in the Lonigo municipality. The two methods provided similar results and the modelled Q_{OUT}, estimated as the difference between Q_{IN} and *ET*, supports the measured value of Q_{OUT}, with a discrepancy estimated to be of the order of 25% due to both the evapotranspiration modelling [81,82] and measurement uncertainty (the interested readers can find additional details in Bettio (2018) [83]).

The hydraulic retention time (*HRT*) was measured with a tracer test experiment that uses Rhodamine WT as the tracer. In particular, three doses of 1 g each of Rhodamine WT were directly injected into the phytoremediation tank. At the same time, the Rhodamine WT concentration in the outflow of the tank was measured by means of a submersible fluorimeter (SCUFA, Turner Designs, San Jose, CA, USA). The measurements were taken for 2 weeks and the concentrations were determined every 2 h. Through this procedure, *HRT* was estimated to be 5.1 days. Table 1 resumes the hydraulic parameters pertaining to the phytoremediation tank.

Table 1. Hydraulic Parameters Characterising the Phytoremediation Tank. Q_{IN} is the Inlet Flow Rate, Q_{OUT} is the Outlet Flow Rate, and *HRT* is the Hydraulic Retention Time.

Q_{IN} (m^3 day^{-1})	Q_{OUT} (m^3 day^{-1})	*HRT* (day)
0.275	0.175	5.1

2.3. Experimental Activities

In 2018, two efficiency tests were carried out on 24 September (hereafter indicated as "before PFAA spike") and 1 October (hereafter indicated as "after PFAA spike") taking inlet and outlet water for four consecutive days. The second efficiency test started with the addition of a mixture of PFBA, PFBS, PFOA, and PFOS in the input tank. These chemicals were selected for the following reasons: PFBA and PFOA are the dominant congeners recorded in the water of the well, PFBS represents the commonest PFAA in the Veneto drinking water while PFOS is one of the most frequent and abundant congeners revealed in the serum of Veneto inhabitants [77]. The nominal concentrations of the spiked solution (PFBA 35, PFBS 15, PFOA 25, PFOS 15 µg L^{-1}) were chosen according to the typical discharge concentrations of the wastewater treatment plants in the impacted area in Veneto. The actual concentrations measured in the input tank after adding and mixing 100 mL of the spiking solution (PFBA 350, PFBS 150, PFOA 250, PFOS 150 mg L^{-1}) are reported in Table 2.

Table 2. PFAA Concentration in Water Expressed Both as a Single Congener (PFBA, PFOA, PFBS, PFOS) and the Sum of the Same (ΣPFAA). It is Important to Recall That the Autosampler in the Outlet was Activated Two Days Before that one at the Inlet.

Day	Date	Tank	Concentration (µg L^{-1})				
			PFBA	PFBS	PFOA	PFOS	ΣPFAA
			Before PFAA spike				
1	24 September 2018	Input	1.00	0.52	2.70	0.08	4.30
2	25 September 2018	Input	1.01	0.50	2.71	0.07	4.30
3	26 September 2018	Input	1.02	0.54	2.61	0.07	4.23
4	27 September 2018	Input	0.97	0.52	2.71	0.07	4.26
3	26 September 2018	Output	1.02	0.53	2.6	0.07	4.22
4	27 September 2018	Output	1.00	0.53	2.7	0.07	4.31
5	28 September 2018	Output	0.99	0.54	2.8	0.06	4.38
6	29 September 2018	Output	1.00	0.57	2.8	0.07	4.42
			After PFAA spike				
1	1 October 2018	Input	34.32	15.59	23.62	8.43	81.95
2	2 October 2018	Input	24.92	13.26	20.26	5.53	63.96
3	3 October 2018	Input	16.47	9.29	14.06	3.33	43.14
4	4 October 2018	Input	11.82	6.93	11.64	2.22	32.61
3	3 October 2018	Output	12.12	7.84	11.44	1.34	32.74
4	4 October 2018	Output	14.52	8.31	13.27	1.99	38.08
5	5 October 2018	Output	15.62	8.59	14.47	2.16	40.84
6	6 October 2018	Output	13.09	8.25	12.54	1.80	35.69

The autosampler in the outflow was activated exactly two days after the switching on of the autosampler in the inflow, hence each test lasted for 6 consecutive days. In this way, considering the *HRT* value, the entire evolution of the plume induced by the PFAA spike should be properly caught. In both tests, the samples were collected every 4 h until all the 24 bottles of both autosamplers were filled. After that, four average samples were created by unifying the first sextet of bottles, the second sextet of bottles, the third sextet of bottles and the fourth sextet of bottles, respectively. From each average sample, a bottle of 500 mL was extracted and sent to ARPAV laboratory for chemical analysis. To sum, for each test, eight bottles of 500 mL each (four from the autosampler placed at the input tank and four from the autosampler placed at the output tank) were gathered and analysed (see Sections 2.5 and 2.6 to have more details on the analysis procedure).

The capacity of the pilot plant in subtracting each PFAA congeners from the incoming water was estimated by means of the removal efficiencies, which, expressed on the basis of the mass flow loads, is equal to $RE(\%) = (1 - M_{OUT}/M_{IN}) \times 100$. The calculus must be performed between the first-day inlet data and the fifth-day outlet data since the *HRT* is roughly equal to 5 days (Table 1).

Samples of reed grass were also collected from the phytoremediation tank and sent to the IRSA-CNR laboratory for chemical analysis, according to the methodology reported in Ferrario et al. (2021) [17] (see Section 2.5). One vegetal sample was taken before PFAA addition (12 September 2018) while a pool of plants was collected about at 20 days (24 October 2018, hereafter indicated as 't$_{20}$'), 5 months (7 March 2019, 't$_{150}$'), and 10 months (14 August 2019, 't$_{300}$') "after PFAA spike" in the tank. The characteristics of reed grasses at harvest are reported in Table 3.

Table 3. Characteristics of Samples at Harvest Time. Each Sample Was Constituted by a Pool of Plants. Height Was Expressed as the Average Height of the Reed Grass Belonging to the Same Pool. The Percentage of Moisture Was Estimated in the IRSA-CNR Laboratory (see Section 2.5). The Reed Grass Collected on 12 September (Percentage of Moisture = 49.9%) Was Not Divided into Fractions Because Its Leaves Were Not Yet Unfurled.

Date	Height	Weight	Root		Stem		Leaf	
	(cm)	(g ww)	Moisture (%)	Fraction of Plant (%)	Moisture (%)	Fraction of Plant (%)	Moisture (%)	Fraction of Plant (%)
12 September 2018	128.0	63.7	-	-	-	-	-	-
24 October 2018	140.4	138.3	33.0	44.7	7.6	34.8	3.7	20.5
7 March 2019	163.7	166.1	37.7	31.5	22.3	38.6	14.5	28.9
14 August 2019	187.0	194.0	86.4	18.3	28.3	44.4	15.7	37.3

2.4. Chemical Analysis and Standards

Four perfluoroalkyl acids (two carboxylic and two sulfonic acids) were investigated in water and vegetal samples (Table 4). A mixed standard of native PFAAs (PFAC-MXB Stock Solution) 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) were bought from Wellington Laboratories (Guelph, ON, Canada) in solution mixtures (mass-labelled MPFAC-MXA solution at 2 mg mL^{-1}). All reagents were analytical reagent grade. LC-MS grade Chromasolv acetonitrile (99.7%), LC-MS grade Chromasolv methanol (99.9%), ammonium acetate (99%) and concentrated formic acid (98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Water (<18 MΩ cm resistivity) was produced by a Millipore Direct-QUV water purification system (Millipore, Bedford, MA, USA).

Table 4. List of Considered PFAAs in the Increasing Chain-Length Order.

Abbreviation	Chemical (Common) Name	No. of Perfluorinated Carbons (C_nF_{2n})	Functional Group	Molar Mass (g mol^{-1})	CAS Number
Short-chain PFAA					
PFBA	Perfluorobutanoic acid	3	–COOH	214	375-22-4
PFBS	Perfluorobutanesulfonic acid	4	–SO$_3^-$	300	375-73-5
Long-chain PFAA					
PFOA	Perfluorooctanoic acid	7	–COOH	414	335-67-1
PFOS	Perfluorooctanesulfonic acid	8	–SO$_3^-$	500	1763-23-1

2.5. Sample Extraction

Water samples were analysed according to the Standard Operating Procedures (ASTM D7979-16) adopted by ARPAV laboratory and validated according to ISO/IEC 17025:2005. For plant analysis, sample preparation and extraction were carried out according to Ferrario et al. (2021) [17]. Briefly, at harvest, each plant was split into its different parts (root, stem, and leaf) that were individually analysed. A few grams of sample were dried in an oven at 105 °C for 24 h to determine the percentage of moisture. Shortly before the extraction phase, a sufficient portion of the remaining sample was thawed in the oven at 60 °C until complete drying (constant weight). About 1 g of the dry crushed sample was spiked with 100 mL of 40 μg L^{-1} SIL-IS and then extracted by ultra-sonication with acidified acetonitrile and treatment with MgSO$_4$/NaCl. The evaporated extract (1 mL) was filtered on Phree™ cartridges (Phenomenex, Torrance, CA, USA) before analysis.

2.6. PFAA Determination

PFAA detection in water was performed by HPLC LC-30AD XR Shimadzu coupled with an API 6500 AB Sciex triple quadrupole and with a CTC PAL HTS XT autosampler. The column used was a Phenomenex Kinetex Evo C18 (1.7 μm × 2.1 mm × 100 mm). The water analysis followed the ASTM Standard Method D7979-16 [84].

Regarding plants, the vegetal final extracts were analysed by liquid chromatography coupled to mass spectrometry UHPLC-MS/MS (TSQ Quantum™ Access MAX, Thermo Scientific, Waltham, MA, USA) equipped with a Waters Acquity UPLC BEH C18 column (50 × 2.1 mm id, 1.7 μm particle size) by direct injection. Detailed method for PFAA determination in plants are described in Ferrario et al. (2021) [17].

Quantification was performed using the isotopic dilution method. External standard solutions at different concentrations were prepared by diluting PFAC-MXB Stock Solution to obtain the calibration curves, which were acquired before each analytical run. The correlation coefficients (R2) of the calibration curves were >0.98 for all target compounds in the two matrices. Recoveries ranged from 103 to 123% for water and from 46 to 162% for the plant. Limits of quantification (LOQs) were estimated according to the ISO 6107-2:2006 standard as tenfold the standard deviation of the lowest calibration curve standard. The LOQs of PFAAs in water were 2.5 ng L^{-1}. In plants, the LOQ values were expressed in ng g^{-1} ww by referring to the wet weight of the extracted sample and ranged from 0.2 to 0.7 ng g^{-1} ww. Reported PFAA concentrations were corrected by subtracting the average procedural blank values above LOQs.

3. Results and Discussion

3.1. PFAAs in Water

The investigation involved the detection of four PFAAs (i.e., PFBA, PFOA, PFBS, and PFOS) inside the inlet and outlet water of the phytoremediation pilot plant as well as in reed grasses located within its main tank (Figure 1).

Table 2 shows PFAA concentrations in inlet and outlet water before and after the PFAA spike in the tank. The obtained results highlighted that under not altered conditions (i.e., "before PFAA spike") PFAA concentration in the inlet water was constant over time with a mean concentration of 4.27 ± 0.03 μg L^{-1}. In the same range were also PFAA levels recorded in the outlet water. Based on this, it is possible to conclude that the pilot plant was not able to reduce PFAA concentration in the water under not altered conditions. However, it must be considered that during the test reed grasses were still in the first phases of the growth (see Table 2 and Section 3.2) and that this preliminary conclusion does not assess the removal efficiencies considering the mass flows (see Table 5 and following paragraph). Differently, with the PFAA injection, the level in the output is more than halved with respect to the input concentration. This behaviour is more clearly visible by representing the values of Table 2 in a graphical form, as shown in Figure 2.

Table 5. Removal Efficiencies *RE*(%) Calculated Considering the Mass Flows: $RE(\%) = (1 - M_{OUT}/M_{IN}) \times 100)$.

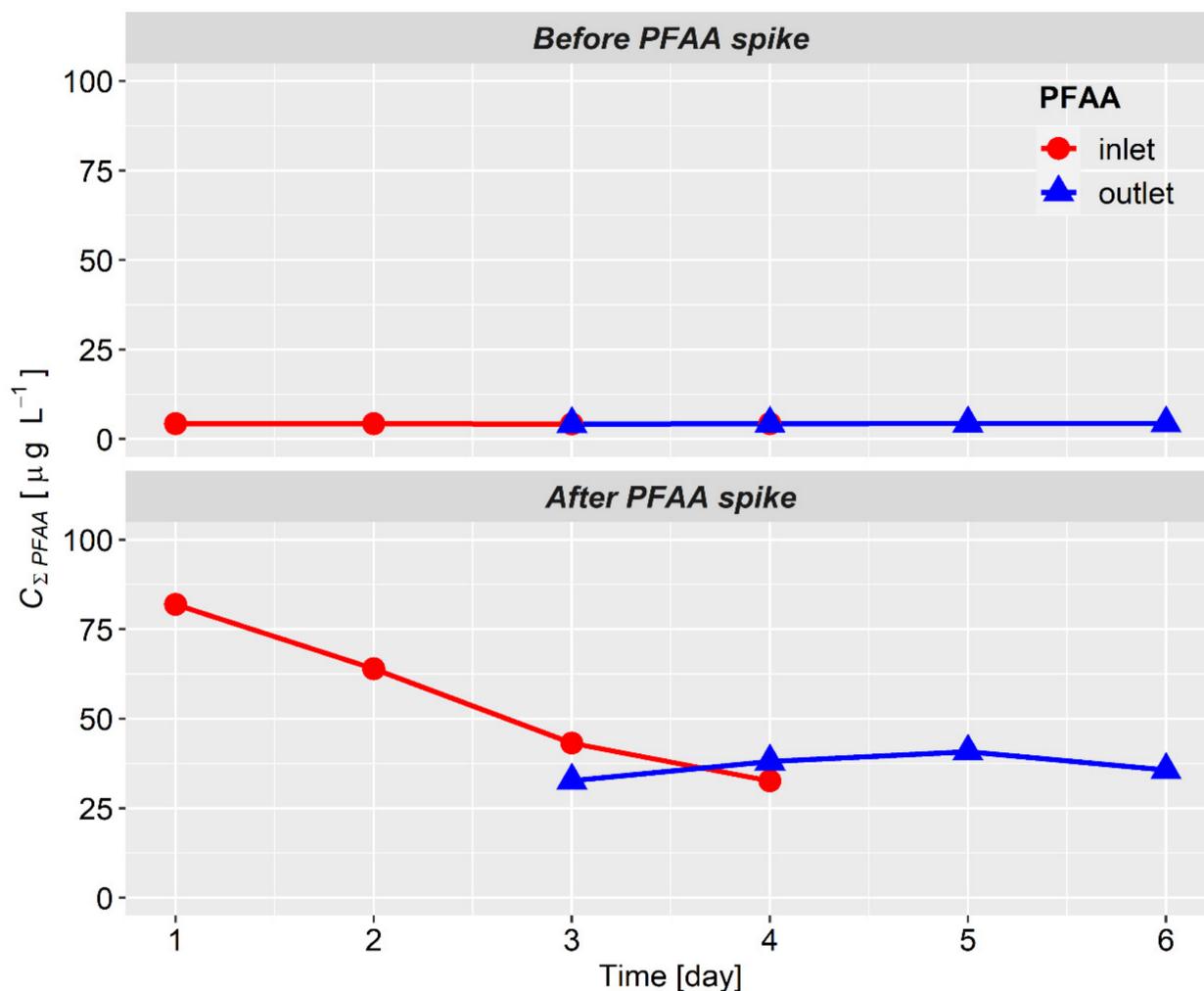|	PFBA	PFBS	PFOA	PFOS	ΣPFAA
Before PFAA spike	36.7	32.8	34.5	50.4	35.1
After PFAA spike	71.0	64.9	61.0	83.7	68.3

Figure 2. PFAA total concentration before the PFAA spike (**top panel**) and after the PFAA spike (**bottom panel**). The red markers indicate the values of the inlet averaged daily samples whereas the blue markers indicate those of the outlet.

It is interesting to note how the concentration peak of the PFAA spike in the inflow is clearly visible in the outflow after around 5 days (Figure 2, bottom panel), as expected, considering the value of *HRT* (Table 1). This is in agreement with the fact that our phytoremediation tank works as a plug flow reactor [85]. Each congener reports a similar trend as for $C_{\Sigma PFAA}$ (Table 2 and Figure 3), without revealing any further information.

By recalling that Q_{OUT} and Q_{IN} (Table 1) are not equal since part of the water is transformed into water vapour due to evapotranspiration, it can be interesting to consider also the mass flow in addition to the concentration, in order to quantify the performance of the phytoremediation pilot plant. The inlet mass flow M_{IN} can be calculated as $M_{IN} = C_{IN} \, Q_{IN}$ and the outlet mass flow M_{OUT} can be similarly estimated (with appropriate modifications). By taking into consideration the *HRT* value and the concentration peak propagation shown in Figures 2 and 3, an acceptable choice is to compare M_{IN} and M_{OUT} between the first and fifth day, respectively (Figure 4). By this way, it is possible to assess that, after the *HRT*, the phytoremediation tank can reduce by 35% the daily ΣPFAAs incoming mass flow in the "before PFAA spike" test and by almost 65% the daily ΣPFAAs incoming mass flow in the "after PFAA spike" test. It is therefore interesting to note that the more the incoming concentrations and mass flow increase, the better the phytoremediation pilot plant works. Furthermore, all the congeners are reduced and stored inside the phytoremediation tank. In the test with low PFAA concentration (Table 5 and Figure 4, left

panel), the PFBA, PFOA, and PFBS congeners are reduced by more than 30% each, while PFOS mass flow is almost halved. Similarly, during the test with high PFAA concentrations (Table 5 and Figure 4, right panel), the reduction in PFBA, PFOA, and PFBS mass flows is always higher than 60%, while the PFOS decreasing reaches values around 80%. The considered compounds have different lengths of fluorinated alkyl chain and functional group (Table 4) that mainly drive their behaviour [86]. Despite this, there is no evidence that the physico-chemical characteristics, such as octanol/water partition coefficients (K_{ow}) and the number of perfluorinated carbons, of the PFAA congeners, influence the removal efficiencies of the pilot plant.
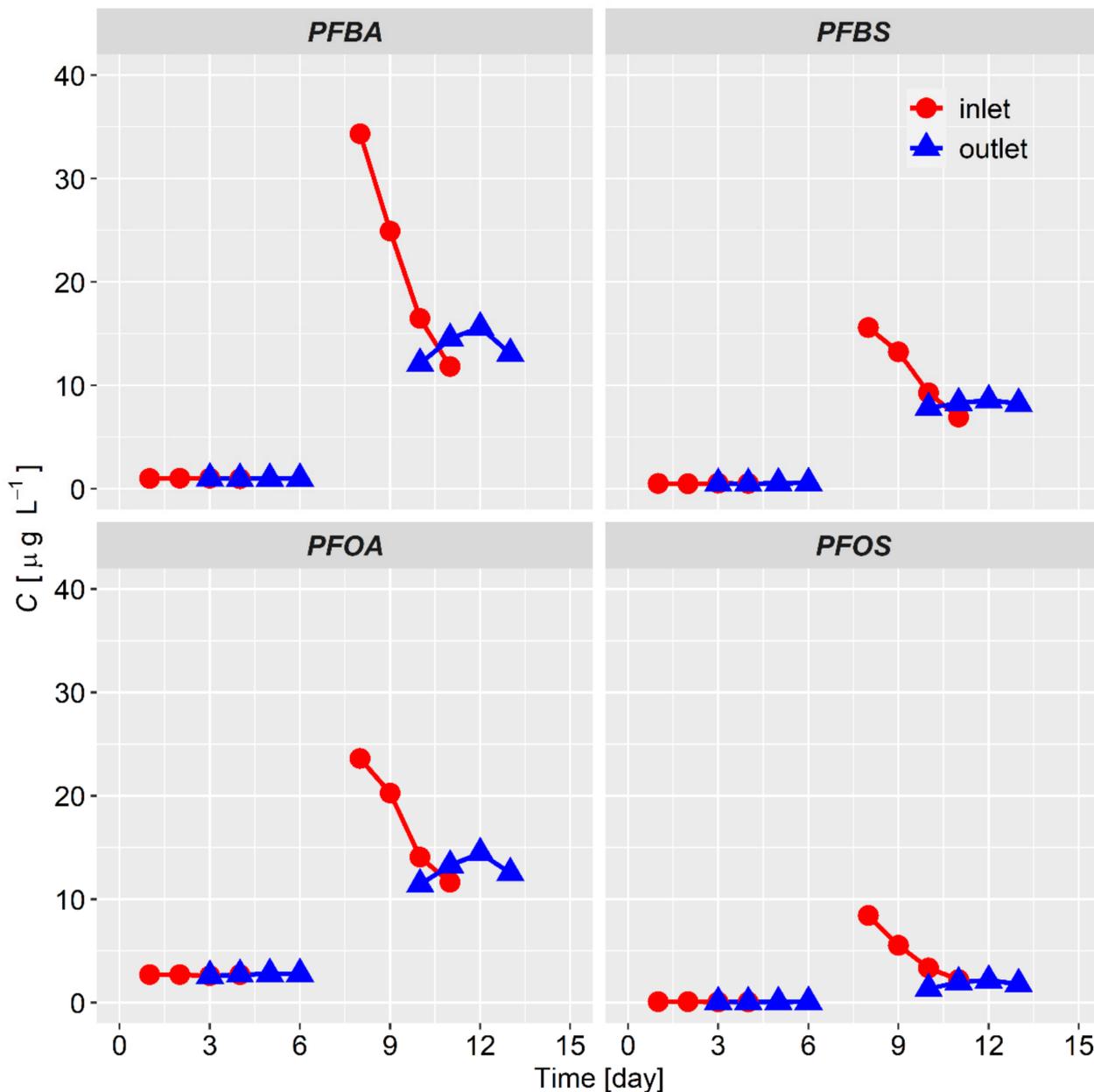
Figure 3. Congener concentrations before the PFAA spike (from day 1 to day 6) and after the PFAA spike (from day 8 to day 13). The red markers indicate the values of the inlet averaged daily samples whereas the blue markers indicate those of the outlet.
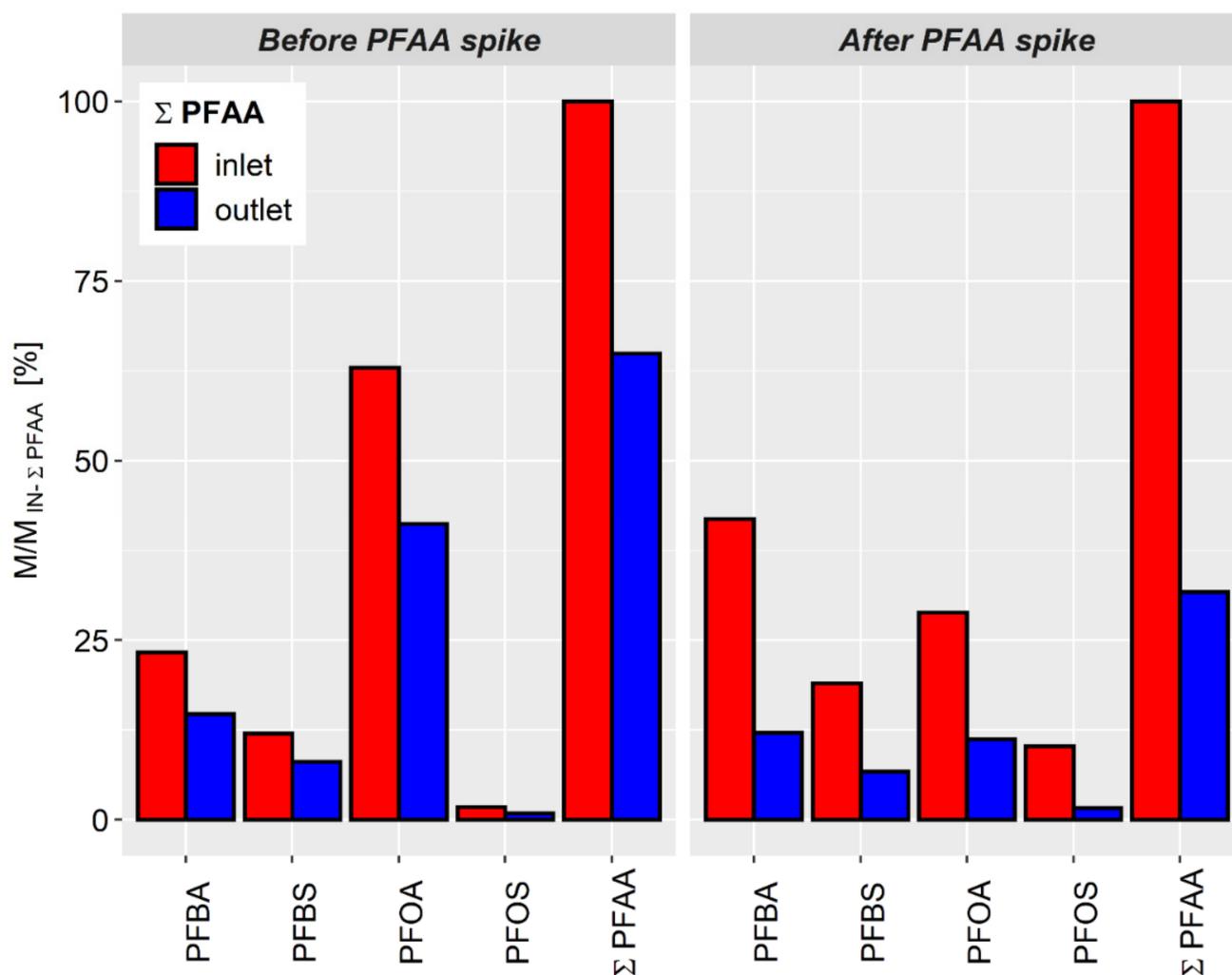
Figure 4. Normalised inlet and outlet mass flow before the PFAA spike (**left panel**) and after the PFAA spike (**right panel**). The comparison was performed between the first-day inlet data and the fifth-day outlet data since the *HRT* is roughly equal to 5 days.

In general, the removal efficiency of CWs is influenced by some external factors including temperature, hydraulic retention time, influent quality, the density of vegetation, and characteristics of microbial communities [87–89]. In addition, the removal efficiency usually decreases with increasing the pollutant load [90] and is quite independent by loading rate [91]. Conversely, we found that an increase in the PFAA load in the influent water causes an enhancement in the removal efficiency of the phytoremediation pilot plant. The same trend of removal performance was observed in pilot plants similar to that in Lonigo, but aimed to treat nitrogen loads. In these cases, an improvement of nitrogen loads resulted in higher removal of nitrate-nitrogen and total nitrogen [91,92].

3.2. PFAAs in Reed Grass

Figure 5 shows the results of the PFAA determination in the three pools of plants collected 20 days (t_{20}), 150 days (t_{150}) and 300 days (t_{300}) after the PFAA spike test in the phytoremediation tank. ΣPFAA in the whole plant was calculated as the sum of PFAA concentrations determined in each vegetal fraction, considering its biomass. Due to this approach, the plant harvested before the addition of the PFAA mixture was not analysed because its stem and leaves were not yet unfurled and then were not distinguishable. Considering the whole plant, ΣPFAA concentration was 2.5 ng g^{-1} ww in the pool of

reed grass collected at t_{20} while levels up to 6.6 and 12.9 ng g^{-1} ww were found in plants harvested at t_{150} and t_{300}, respectively. The obtained results suggested that ΣPFAA concentration in exposed reed grasses increased with exposure time, as observed even for wheat [93], a graminaceous plant as well. This correlation between contaminant uptake and exposure time confirms that bioaccumulation seems limited only by the toxicity threshold at which the chemical damages the plant causing growth inhibition or death [94]. The increase in ΣPFAA concentration found in reed grass collected from the Lonigo plant can be considered constant over time with a rise of 2.6% in the first 130 days after the PFAA spike (from t_{20} to t_{150}) and of 2% in the following 150 days (from t_{150} to t_{300}). This result seems to be in contrast with the seasonal trend of accumulation observed in the same species [95]. Indeed, authors found that the highest uptake of metals and nutrients in reed grass occurs between April and November. However, vegetal samples collected from the Lonigo plant were collected from the end of October to the beginning of August and then the two experimental studies did not cover the same time frame. Moreover, the mentioned work was carried out in Ireland where weather conditions are different from northern Italy.
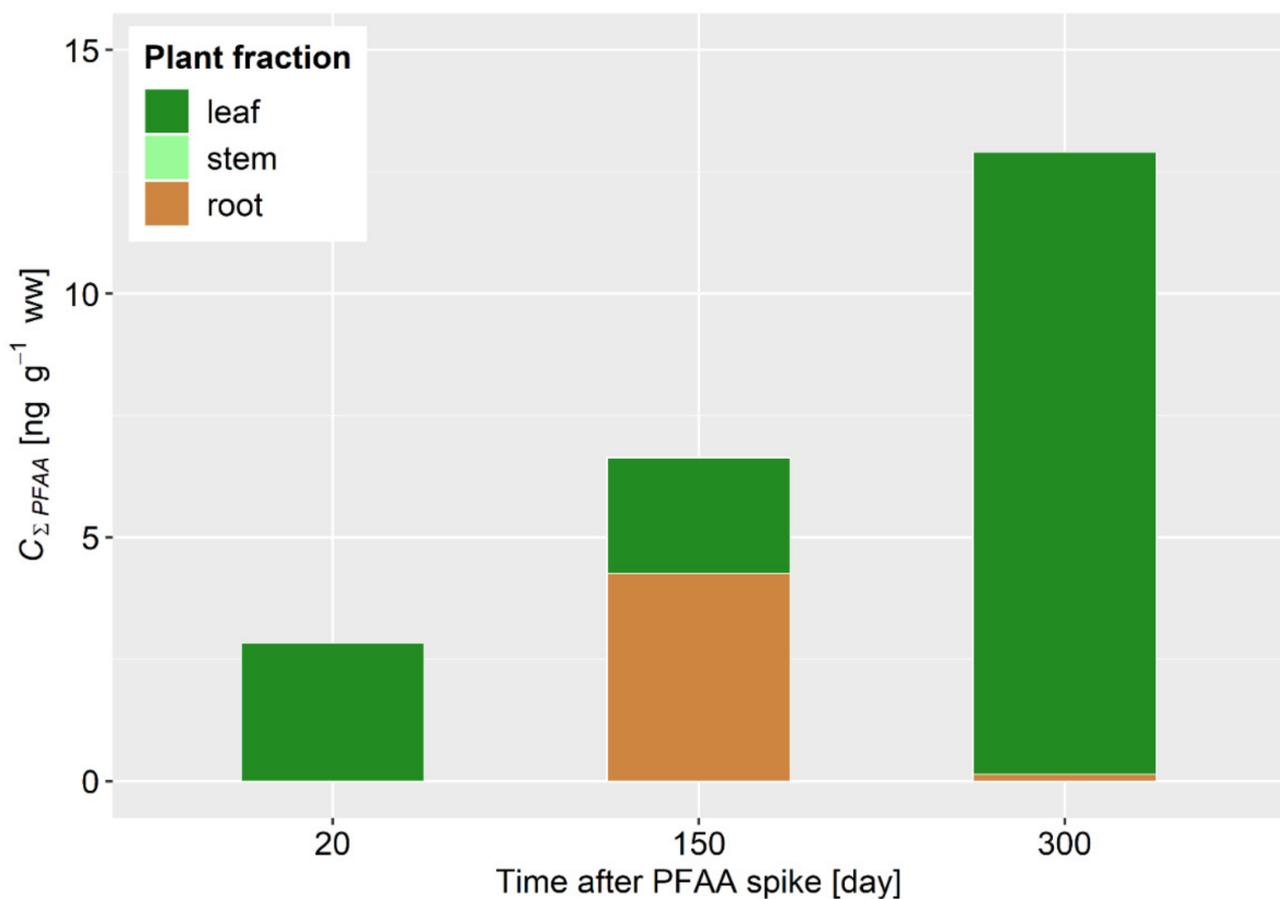

Figure 5. PFAA concentration in plants (ng g^{-1} ww) harvested about 20 days (t_{20}), 5 months (t_{150}) and 10 months (t_{300}) "after PFAA spike" in the tank. Different colours represent ΣPFAA levels in each part of the plant: root (brown), stem (dark green) and leaf (light green).

An uneven partition of compounds between vegetal fractions was also noticed (Figure 5). In particular, stem resulted to be the least polluted part, unable to accumulate PFAA concentrations below the limits of quantification (LOQ) values. On the contrary, most PFAA pollution was generally detected in leaves. This result shows that PFAA partition in plants grown in the pilot system is the same as observed in spontaneous reed grasses [17].

In contrast to these general findings, over 60% of PFAA contamination found in the pool harvested at t_{150} was detected in root and the concentration recorded in its leaf (mean:

2.4 ng g^{-1} ww) was not significantly different from that found in the sample collected at t_{20} (mean: 2.5 ng g^{-1} ww). A possible explanation can be that this pool was collected in early spring (7 March 2019), after the winter season when plants need less water to keep hydrated due to lower temperatures, less solar radiation and then a decrease in evapotranspiration. A seasonal cycle was already observed in reed grass less than 0.5 m tall [96], the same size range as vegetables from the Lonigo pilot plant. As a consequence, a negligible volume of polluted water might have reached leaves from October (t_{20}) to March (t_{150}) while roots were continuously exposed to the contaminated medium. Significant uptake of the reed grass was measured also in plants harvested at t_{300} (Figure 5). This evidence suggested that spiked substances were stored in the growth medium (e.g., residual soil and biofilms on gravels) and absorbed by the roots.

PFAAs are a group of heterogeneous substances with different chemical-physical properties. The behaviour of these compounds is mainly driven by the length of their fluorinated alkyl chain and their functional group [86]. Based on this, the composition of PFAA contamination found in vegetal samples was evaluated to compare the affinity of reed grass to each considered congener. It is important to remind that the pilot plant constantly receives contaminated water from the well (see Sections 2.1 and 2.2) and that levels of congeners in inlet and outlet water after the end of the second test (6 October 2018) are not available. In general, the contamination measured in the reed grasses grown inside the Lonigo pilot plant was dominated by short-chain PFAAs, as also observed in reed grasses grown in CWs that receive water from fluorochemical industrial WWTPs [73]. Such phenomenon is particularly evident in the sample collected at t_{300}. The highest levels of short-chain PFAAs found in these plants can be explained considering that over 95% of reed grass contamination were recorded in leaf. Indeed, this vegetal fraction tends to mainly accumulate short-chain PFAAs rather than long-chain congeners, which have lower mobility [16]. Despite this, PFOA was always detected in harvested reed grasses, probably because this is the dominant PFAA in the well water, which feeds the pilot plant (see Section 2.1).

The behaviour of the different substances can be observed in Figure 6. Bioaccumulation of perfluorocarboxylic acids (PFBA and PFOA), which were less dissociated in the water and whose partition depends more on their lipophilicity, showed a slight increase in the first 20 days and then their concentrations declined plausibly due to the biomass dilution. The behaviour of perfluorosulfonic acids (PFBS and PFOS), which are completely dissociated in water, was more difficult to interpret. Excluding that those substances can be metabolized by the plant [16], we can suppose that the perfluorosulfonate anions were more prone to be exchanged by the plants with the growing environment because they are soluble compounds. PFOS in particular was less accumulated by the plants because it was present at low concentrations in inlet water and was more strongly retained by soil and biofilms on which the plants grew.

One of the problems of the implementation of phytodepuration plant for PFAAs is the disposal of the contaminated plants. The frequency of the mowing procedure should depend on the plant vegetative cycle. Then, the mowed plants which are contaminated by PFAAs should be thermally treated in incinerators at high temperature in order to destroy these very persistent molecules. Decomposition of PFAAs can be obtained at the usual operating temperatures of urban incinerators, but the total defluorination needs higher temperatures, 900–1000 °C [94].
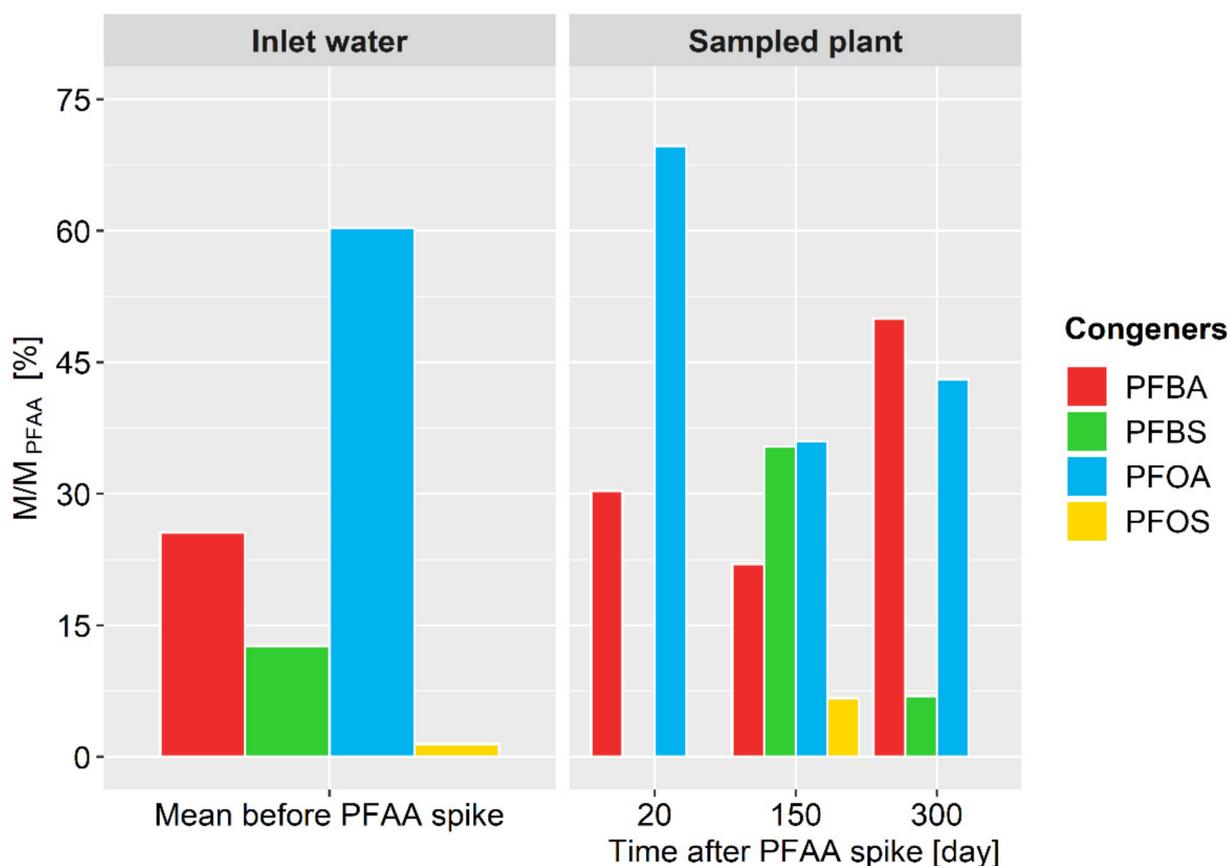
Figure 6. Percentage distribution of PFAAs in inlet water (left panel) "before PFAA spike", and in reed grass samples (right panel) harvested about 20 days (t_{20}), 5 months (t_{150}) and 10 months (t_{300}) "after PFAA spike" in the tank.

4. Conclusions

The green infrastructures are considered a pillar of the EU Green Deal in order to mitigate diffuse pollution. PFASs have multiple emission sources, from industrial to domestic ones, and their high persistence and mobility help them to spread in all the networks of watercourses. These compounds are difficult to control in the point sources, but the application of green infrastructure can help to fulfil this goal.

This paper demonstrates that the reed grass, commonly used in phytoremediation are able to uptake PFASs during their lifetime. In particular, PFASs concentration in exposed reed grasses increased with exposure time, ranging from 2.5 ng g^{-1} ww in plants harvested in October to 12.9 ng g^{-1} ww in those collected in August of the following year. An uneven partition of PFAAs between vegetal fractions was also noticed, confirming that these compounds can be significantly accumulated in leaves. Based on data recorded in outlet water, phytoremediation under controlled conditions in the pilot plant is able to reduce from 30 to 50% of PFASs in terms of mass flow without an evident dependence on their physico-chemical characteristics. Obtained results also highlight that higher concentrations in the inflow water facilitate plant uptake and removal efficiency. Future work will be to test this uptake mechanism and removal efficiency in CWs under real conditions. In general, CWs are considered a highly sustainable choice in wastewater remediation [55] even though the cost-effectiveness of PFASs removal and disposal should be evaluated considering that high-temperature incineration is necessary to dispose PFASs contaminated plants.

References

1. Wang, Z.; Buser, A.M.; Cousins, I.T.; Demattio, S.; Drost, W.; Johansson, O.; Ohno, K.; Patlewicz, G.; Richard, A.M.; Walker, G.W.; et al. A New OECD Definition for Per- and Polyfluoroalkyl Substances. *Environ. Sci. Technol.* **2021**, 1–4. [CrossRef]
2. Krafft, M.P.; Riess, J.G. Per- and polyfluorinated substances (PFASs): Environmental challenges. *Curr. Opin. Colloid Interface Sci.* **2015**, *20*, 192–212. [CrossRef]
3. Liu, J.; Mejia Avendaño, S. Microbial degradation of polyfluoroalkyl chemicals in the environment: A review. *Environ. Int.* **2013**, *61*, 98–114. [CrossRef] [PubMed]
4. Xiao, F. Emerging poly- and perfluoroalkyl substances in the aquatic environment: A review of current literature. *Water Res.* **2017**, *124*, 482–495. [CrossRef]
5. Bonato, M.; Corrà, F.; Bellio, M.; Guidolin, L.; Tallandini, L.; Irato, P.; Santovito, G. Pfas environmental pollution and antioxidant responses: An overview of the impact on human field. *Int. J. Environ. Res. Public Health* **2020**, *17*, 8020. [CrossRef]
6. Ahrens, L.; Bundschuh, M. Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: A review. *Environ. Toxicol. Chem.* **2014**, *33*, 1921–1929. [CrossRef]
7. Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicol. Sci.* **2007**, *99*, 366–394. [CrossRef]
8. 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.J. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integr. Environ. Assess. Manag.* **2011**, *7*, 513–541. [CrossRef] [PubMed]
9. McCarthy, C.; Kappleman, W.; DiGuiseppi, W. Ecological Considerations of Per- and Polyfluoroalkyl Substances (PFAS). *Curr. Pollut. Reports* **2017**, *3*, 289–301. [CrossRef]
10. Fang, X.; Wang, Q.; Zhao, Z.; Tang, J.; Tian, C.; Yao, Y.; Yu, J.; Sun, H. Distribution and dry deposition of alternative and legacy perfluoroalkyl and polyfluoroalkyl substances in the air above the Bohai and Yellow Seas, China. *Atmos. Environ.* **2018**, *192*, 128–135. [CrossRef]
11. Valsecchi, S.; Rusconi, M.; Mazzoni, M.; Viviano, G.; Pagnotta, R.; Zaghi, C.; Serrini, G.; Polesello, S. Occurrence and sources of perfluoroalkyl acids in Italian river basins. *Chemosphere* **2015**, *129*, 126–134. [CrossRef] [PubMed]
12. Ahmed, M.B.; Johir, M.A.H.; McLaughlan, R.; Nguyen, L.N.; Xu, B.; Nghiem, L.D. Per- and polyfluoroalkyl substances in soil and sediments: Occurrence, fate, remediation and future outlook. *Sci. Total Environ.* **2020**, *748*, 141251. [CrossRef] [PubMed]
13. Valsecchi, S.; Babut, M.; Mazzoni, M.; Pascariello, S.; Ferrario, C.; De Felice, B.; Bettinetti, R.; Veyrand, B.; Marchand, P.; Polesello, S. Per- and Polyfluoroalkyl Substances (PFAS) in Fish from European Lakes: Current Contamination Status, Sources, and Perspectives for Monitoring. *Environ. Toxicol. Chem.* **2021**, *40*, 658–676. [CrossRef] [PubMed]
14. Giesy, J.P.; Kannan, K. Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol.* **2001**, *35*, 1339–1342. [CrossRef]
15. Houde, M.; Martin, J.W.; Letcher, R.J.; Solomon, K.R.; Muir, D.C.G. Biological monitoring of polyfluoroalkyl substances: A review. *Environ. Sci. Technol.* **2006**, *40*, 3463–3473. [CrossRef]
16. Ghisi, R.; Vamerali, T.; Manzetti, S. Accumulation of perfluorinated alkyl substances (PFAS) in agricultural plants: A review. *Environ. Res.* **2019**, *169*, 326–341. [CrossRef]

17. Ferrario, C.; Valsecchi, S.; Lava, R.; Bonato, M.; Polesello, S. Determination of perfluoroalkyl acids in different tissues of graminaceous plants. *Anal. Methods* **2021**, *13*, 1643–1650. [CrossRef]

18. Da Silva, B.F.; Ahmadireskety, A.; Aristizabal-Henao, J.J.; Bowden, J.A. A rapid and simple method to quantify per- and polyfluoroalkyl substances (PFAS) in plasma and serum using 96-well plates. *MethodsX* **2020**, *7*, 101111. [CrossRef]

19. Ankley, G.T.; Cureton, P.; Hoke, R.A.; Houde, M.; Kumar, A.; Kurias, J.; Lanno, R.; McCarthy, C.; Newsted, J.; Salice, C.J.; et al. Assessing the Ecological Risks of Per- and Polyfluoroalkyl Substances: Current State-of-the Science and a Proposed Path Forward. *Environ. Toxicol. Chem.* **2021**, *40*, 564–605. [CrossRef]

20. Gredelj, A.; Nicoletto, C.; Valsecchi, S.; Ferrario, C.; Polesello, S.; Lava, R.; Zanon, F.; Barausse, A.; Palmeri, L.; Guidolin, L.; et al. Uptake and translocation of perfluoroalkyl acids (PFAA) in red chicory (*Cichorium intybus* L.) under various treatments with pre-contaminated soil and irrigation water. *Sci. Total Environ.* **2020**, *708*, 134766. [CrossRef]

21. Gredelj, A.; Nicoletto, C.; Polesello, S.; Ferrario, C.; Valsecchi, S.; Lava, R.; Barausse, A.; Zanon, F.; Palmeri, L.; Guidolin, L.; et al. Uptake and translocation of perfluoroalkyl acids (PFAAs) in hydroponically grown red chicory (*Cichorium intybus* L.): Growth and developmental toxicity, comparison with growth in soil and bioavailability implications. *Sci. Total Environ.* **2020, *720*, 137333.** [CrossRef]

22. Brendel, S.; Fetter, É.; Staude, C.; Vierke, L.; Biegel-Engler, A. Short-chain perfluoroalkyl acids: Environmental concerns and a regulatory strategy under REACH. *Environ. Sci. Eur.* **2018**, *30*. [CrossRef]

23. Ritscher, A.; Wang, Z.; Scheringer, M.; Boucher, J.M.; Ahrens, L.; Berger, U.; Bintein, S.; Bopp, S.K.; Borg, D.; Buser, A.M.; et al. Zürich Statement on Future Actions on Per- and Polyfluoroalkyl Substances. *Environ. Health Perspect.* **2018**, *126*, 084502. [CrossRef]

24. Wang, Z.; Dewitt, J.C.; Higgins, C.P.; Cousins, I.T. A Never-Ending Story of Per- and Polyfluoroalkyl Substances (PFASs)? *Environ. Sci. Technol.* **2017**, *51*, 2508–2518. [CrossRef]

25. Schrenk, D.; Bignami, M.; Bodin, L.; Chipman, J.K.; del Mazo, J.; Grasl-Kraupp, B.; Hogstrand, C.; Hoogenboom, L.; Leblanc, J.C.; Nebbia, C.S.; et al. Risk to human health related to the presence of perfluoroalkyl substances in food. *EFSA J.* **2020**, *18*. [CrossRef]

26. Ahmed, M.B.; Alam, M.M.; Zhou, J.L.; Xu, B.; Johir, M.A.H.; Karmakar, A.K.; Rahman, M.S.; Hossen, J.; Hasan, A.T.M.K.; Moni, M.A. Advanced treatment technologies efficacies and mechanism of per- and poly-fluoroalkyl substances removal from water. *Process Saf. Environ. Prot.* **2020**, *136*, 1–14. [CrossRef]

27. Cao, M.H.; Wang, B.B.; Yu, H.S.; Wang, L.L.; Yuan, S.H.; Chen, J. Photochemical decomposition of perfluorooctanoic acid in aqueous periodate with VUV and UV light irradiation. *J. Hazard. Mater.* **2010**, *179*, 1143–1146. [CrossRef]

28. Hori, H.; Yamamoto, A.; Hayakawa, E.; Taniyasu, S.; Yamashita, N.; Kutsuna, S.; Kiatagawa, H.; Arakawa, R. Efficient decomposition of environmentally persistent perfluorocarboxylic acids by use of persulfate as a photochemical oxidant. *Environ. Sci. Technol.* **2005**, *39*, 2383–2388. [CrossRef]

29. Hori, H.; Nagaoka, Y.; Yamamoto, A.; Sano, T.; Yamashita, N.; Taniyasu, S.; Kutsuna, S.; Osaka, I.; Arakawa, R. Efficient decomposition of environmentally persistent perfluorooctanesulfonate and related fluorochemicals using zerovalent iron in subcritical water. *Environ. Sci. Technol.* **2006**, *40*, 1049–1054. [CrossRef]

30. Hori, H.; Yamamoto, A.; Koike, K.; Kutsuna, S.; Osaka, I.; Arakawa, R. Photochemical decomposition of environmentally persistent short-chain perfluorocarboxylic acids in water mediated by iron(II)/(III) redox reactions. *Chemosphere* **2007**, *68*, 572–578. [CrossRef]

31. Hori, H.; Nagaoka, Y.; Murayama, M.; Kutsuna, S. Efficient decomposition of perfluorocarboxylic acids and alternative fluoro-chemical surfactants in hot water. *Environ. Sci. Technol.* **2008**, *42*, 7438–7443. [CrossRef] [PubMed]

32. Lee, Y.C.; Lo, S.L.; Chiueh, P.T.; Liou, Y.H.; Chen, M.L. Microwave-hydrothermal decomposition of perfluorooctanoic acid in water by iron-activated persulfate oxidation. *Water Res.* **2010**, *44*, 886–892. [CrossRef] [PubMed]

33. Kucharzyk, K.H.; Darlington, R.; Benotti, M.; Deeb, R.; Hawley, E. Novel treatment technologies for PFAS compounds: A critical review. *J. Environ. Manag.* **2017**, *204*, 757–764. [CrossRef] [PubMed]

34. Sorlini, S.; Collivignarelli, M.C.; Miino, M.C. Technologies for the control of emerging contaminants in drinking water treatment plants. *Environ. Eng. Manag. J.* **2019**, *18*, 2203–2216.

35. Lu, D.; Sha, S.; Luo, J.; Huang, Z.; Zhang Jackie, X. Treatment train approaches for the remediation of per- and polyfluoroalkyl substances (PFAS): A critical review. *J. Hazard. Mater.* **2020**, *386*, 121963. [CrossRef] [PubMed]

36. Deng, S.; Yu, Q.; Huang, J.; Yu, G. Removal of perfluorooctane sulfonate from wastewater by anion exchange resins: Effects of resin properties and solution chemistry. *Water Res.* **2010**, *44*, 5188–5195. [CrossRef]

37. Dixit, F.; Dutta, R.; Barbeau, B.; Berube, P.; Mohseni, M. PFAS removal by ion exchange resins: A review. *Chemosphere* **2021**, *272*, 129777. [CrossRef]

38. Stoiber, T.; Evans, S.; Temkin, A.M.; Andrews, D.Q.; Naidenko, O.V. PFAS in drinking water: An emergent water quality threat. *Water Solut.* **2020**, *1*, e49.

39. Du, Z.; Deng, S.; Bei, Y.; Huang, Q.; Wang, B.; Huang, J.; Yu, G. Adsorption behavior and mechanism of perfluorinated compounds on various adsorbents-A review. *J. Hazard. Mater.* **2014**, *274*, 443–454. [CrossRef]

40. Kennedy, A.M.; Reinert, A.M.; Knappe, D.R.U.; Ferrer, I.; Summers, R.S. Full- and pilot-scale GAC adsorption of organic micropollutants. *Water Res.* **2015**, *68*, 238–248. [CrossRef]

41. McCleaf, P.; Englund, S.; Östlund, A.; Lindegren, K.; Wiberg, K.; Ahrens, L. Removal efficiency of multiple poly- and perfluoroalkyl substances (PFASs) in drinking water using granular activated carbon (GAC) and anion exchange (AE) column tests. *Water Res.* **2017**, *120*, 77–87. [CrossRef]

42. Sonmez Baghirzade, B.; Zhang, Y.; Reuther, J.F.; Saleh, N.B.; Venkatesan, A.K.; Apul, O.G. Thermal Regeneration of Spent Granular Activated Carbon Presents an Opportunity to Break the Forever PFAS Cycle. *Environ. Sci. Technol.* **2021**, *55*, 5608–5619. [CrossRef]

43. Radjenovic, J.; Duinslaeger, N.; Avval, S.S.; Chaplin, B.P. Facing the Challenge of Poly- And Perfluoroalkyl Substances in Water: Is Electrochemical Oxidation the Answer? *Environ. Sci. Technol.* **2020**, *54*, 14815–14829. [CrossRef]

44. Nzeribe, B.N.; Crimi, M.; Mededovic Thagard, S.; Holsen, T.M. Physico-Chemical Processes for the Treatment of Per- And Polyfluoroalkyl Substances (PFAS): A review. *Crit. Rev. Environ. Sci. Technol.* **2019**, *49*, 866–915. [CrossRef]

45. Sahu, S.P.; Qanbarzadeh, M.; Ateia, M.; Torkzadeh, H.; Maroli, A.S.; Cates, E.L. Rapid Degradation and Mineralization of Perfluorooctanoic Acid by a New Petitjeanite Bi3O(OH)(PO4)2 Microparticle Ultraviolet Photocatalyst. *Environ. Sci. Technol. Lett.* **2018**, *5*, 533–538. [CrossRef]

46. Yamada, T.; Taylor, P.H.; Buck, R.C.; Kaiser, M.A.; Giraud, R.J. Thermal degradation of fluorotelomer treated articles and related materials. *Chemosphere* **2005**, *61*, 974–984. [CrossRef]

47. Stoiber, T.; Evans, S.; Naidenko, O.V. Disposal of products and materials containing per- and polyfluoroalkyl substances (PFAS): A cyclical problem. *Chemosphere* **2020**, *260*, 127659. [CrossRef]

48. Krusic, P.J.; Roe, D.C. Gas-phase NMR technique for studying the thermolysis of materials: Thermal decomposition of ammonium perfluorooctanoate. *Anal. Chem.* **2004**, *76*, 3800–3803. [CrossRef]

49. McKay, G. Dioxin characterisation, formation and minimisation during municipal solid waste (MSW) incineration: Review. *Chem. Eng. J.* **2002**, *86*, 343–368. [CrossRef]

50. Tuppurainen, K.; Halonen, I.; Ruokojärvi, P.; Tarhanen, J.; Ruuskanen, J. Formation of PCDDs and PCDFs in municipal waste incineration and its inhibition mechanisms: A review. *Chemosphere* **1998**, *36*, 1493–1511. [CrossRef]

51. Boano, F.; Caruso, A.; Costamagna, E.; Ridolfi, L.; Fiore, S.; Demichelis, F.; Galvão, A.; Pisoeiro, J.; Rizzo, A.; Masi, F. A review of nature-based solutions for greywater treatment: Applications, hydraulic design, and environmental benefits. *Sci. Total Environ.* **2020**, *711*, 134731. [CrossRef] [PubMed]

52. Rizzo, A.; Tondera, K.; Pálfy, T.G.; Dittmer, U.; Meyer, D.; Schreiber, C.; Zacharias, N.; Ruppelt, J.P.; Esser, D.; Molle, P.; et al. Constructed wetlands for combined sewer overflow treatment: A state-of-the-art review. *Sci. Total Environ.* **2020**, *727*, 138618. [CrossRef] [PubMed]

53. Lavrnić, S.; Alagna, V.; Iovino, M.; Anconelli, S.; Solimando, D.; Toscano, A. Hydrological and hydraulic behaviour of a surface flow constructed wetland treating agricultural drainage water in northern Italy. *Sci. Total Environ.* **2020**, *702*. [CrossRef] [PubMed]

54. Kadlec, R.H.; Wallace, S. *Treatment Wetlands*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2009; ISBN 9781566705264.

55. Colares, G.S.; Dell'Osbel, N.; Wiesel, P.G.; Oliveira, G.A.; Lemos, P.H.Z.; da Silva, F.P.; Lutterbeck, C.A.; Kist, L.T.; Machado, Ê.L. Floating treatment wetlands: A review and bibliometric analysis. *Sci. Total Environ.* **2020**, *714*, 136776. [CrossRef] [PubMed]

56. Arslan, M.; El-Din, M.G. Removal of per- and poly-fluoroalkyl substances (PFASs) by wetlands: Prospects on plants, microbes and the interplay. *Sci. Total Environ.* **2021**, *800*, 149570. [CrossRef] [PubMed]

57. Wang, D.; Bai, Y.; Qu, J. The Phragmites root-inhabiting microbiome: A critical review on its composition and environmental application. *Engineering* **2021**, *in press*. [CrossRef]

58. Chen, Y.C.; Lo, S.L.; Lee, Y.C. Distribution and fate of perfl uorinated compounds (PFCs) in a pilot constructed wetland. *Desalin. Water Treat.* **2012**, *37*, 178–184. [CrossRef]

59. Rezania, S.; Park, J.; Rupani, P.F.; Darajeh, N.; Xu, X.; Shahrokhishahraki, R. Phytoremediation potential and control of *Phragmites australis* as a green phytomass: An overview. *Environ. Sci. Pollut. Res.* **2019**, *26*, 7428–7441. [CrossRef]

60. Huang, X.; Wang, L.; Zhu, S.; Ho, S.H.; Wu, J.; Kalita, P.K.; Ma, F. Unraveling the effects of arbuscular mycorrhizal fungus on uptake, translocation, and distribution of cadmium in *Phragmites australis* (Cav.) Trin. ex Steud. *Ecotoxicol. Environ. Saf.* **2018**, *149*, 43–50. [CrossRef]

61. Carvalho, P.N.; Basto, M.C.P.; Almeida, C.M.R. Potential of *Phragmites australis* for the removal of veterinary pharmaceuticals from aquatic media. *Bioresour. Technol.* **2012**, *116*, 497–501. [CrossRef]

62. Almeida, C.M.R.; Santos, F.; Ferreira, A.C.F.; Gomes, C.R.; Basto, M.C.P.; Mucha, A.P. Constructed wetlands for the removal of metals from livestock wastewater—Can the presence of veterinary antibiotics affect removals? *Ecotoxicol. Environ. Saf.* **2017**, *137*, 143–148. [CrossRef]

63. Liu, X.; Huang, S.; Tang, T.; Liu, X.; Scholz, M. Growth characteristics and nutrient removal capability of plants in subsurface vertical flow constructed wetlands. *Ecol. Eng.* **2012**, *44*, 189–198. [CrossRef]

64. Marsik, P.; Podlipna, R.; Vanek, T. Study of praziquantel phytoremediation and transformation and its removal in constructed wetland. *J. Hazard. Mater.* **2017**, *323*, 394–399. [CrossRef]

65. Topal, M. Uptake of tetracycline and degradation products by *Phragmites australis* grown in stream carrying secondary effluent. *Ecol. Eng.* **2015**, *79*, 80–85. [CrossRef]

66. Wang, A.; Chi, J. Phthalic acid esters in the rhizosphere sediments of emergent plants from two shallow lakes. *J. Soils Sediments* **2012**, *12*, 1189–1196. [CrossRef]

67. Schaller, J.; Brackhage, C.; Paasch, S.; Brunner, E.; Bäucker, E.; Dudel, E.G. Silica uptake from nanoparticles and silica condensation state in different tissues of *Phragmites australis*. *Sci. Total Environ.* **2013**, *442*, 6–9. [CrossRef]

68. Kankiliç, G.B.; Metin, A.Ü.; Tüzün, I. *Phragmites australis*: An alternative biosorbent for basic dye removal. *Ecol. Eng.* **2016**, *86*, 85–94. [CrossRef]

69. Haddaji, D.; Bousselmi, L.; Saadani, O.; Nouairi, I.; Ghrabi-Gammar, Z. Enzymatic degradation of azo dyes using three macrophyte species: Arundo donax, Typha angustifolia and *Phragmites australis*. *Desalin. Water Treat.* **2015**, *53*, 1129–1138.

70. Lv, T.; Zhang, Y.; Zhang, L.; Carvalho, P.N.; Arias, C.A.; Brix, H. Removal of the pesticides imazalil and tebuconazole in saturated constructed wetland mesocosms. *Water Res.* **2016**, *91*, 126–136. [CrossRef]

71. Hechmi, N.; Aissa, N.B.; Abdenaceur, H.; Jedidi, N. Evaluating the phytoremediation potential of *Phragmites australis* grown in pentachlorophenol and cadmium co-contaminated soils. *Environ. Sci. Pollut. Res.* **2014**, *21*, 1304–1313. [CrossRef]

72. Mudumbi, J.B.N.; Ntwampe, S.K.O.; Muganza, M.; Okonkwo, J.O. Susceptibility of Riparian Wetland Plants to Perfluorooctanoic Acid (PFOA) Accumulation. *Int. J. Phytoremediat.* **2014**, *16*, 926–936. [CrossRef]

73. Wang, P.; Zhang, M.; Lu, Y.; Meng, J.; Li, Q.; Lu, X. Removal of perfluoalkyl acids (PFAAs) through fluorochemical industrial and domestic wastewater treatment plants and bioaccumulation in aquatic plants in river and artificial wetland. *Environ. Int.* **2019**, *129*, 76–85. [CrossRef]

74. Li, Y.; He, L.; Lv, L.; Xue, J.; Wu, L.; Zhang, Z.; Yang, L. Review on plant uptake of PFOS and PFOA for environmental cleanup: Potential and implications. *Environ. Sci. Pollut. Res.* **2021**, *28*, 30459–30470. [CrossRef] [PubMed]

75. Castiglioni, S.; Valsecchi, S.; Polesello, S.; Rusconi, M.; Melis, M.; Palmiotto, M.; Manenti, A.; Davoli, E.; Zuccato, E. 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.* **2015**, *282*, 51–60. [CrossRef] [PubMed]

76. World Health Organization (WHO). *Keeping Our Water Clean: The Case of Water Contamination in Veneto Region, Italy*; WHO: Geneva, Switzerland, 2017.

77. Pitter, G.; Da Re, F.; Canova, C.; Barbieri, G.; Jeddi, M.Z.; Daprà, F.; Manea, F.; Zolin, R.; Bettega, A.M.; Stopazzolo, G.; et al. Serum levels of perfluoroalkyl substances (PFAS) in adolescents and young adults exposed to contaminated drinking water in the Veneto region, Italy: A cross-sectional study based on a health surveillance program. *Environ. Health Perspect.* **2020**, *128*, 027007. [CrossRef] [PubMed]

78. Milke, J.; Gałczyńska, M.; Wróbel, J. The importance of biological and ecological properties of *Phragmites australis* (Cav.) Trin. Ex steud., in phytoremendiation of aquatic ecosystems-The review. *Water* **2020**, *12*, 1770. [CrossRef]

79. Allen, R.G.; Pereira, L.S.; Raes, D.; Smith, M. *Crop Evapotraspiration Guidelines for Computing Crop Water Requirements*; FAO: Rome, Italy, 1998; ISBN 9251042195.

80. Hargreaves, G.H.; Samani, Z.A. Reference crop evapotranspiration from temperature. *Appl. Eng. Agric.* **1985**, *1*, 96–99. [CrossRef]

81. Paredes, P.; Pereira, L.S.; Almorox, J.; Darouich, H. Reference grass evapotranspiration with reduced data sets: Parameterization of the FAO Penman-Monteith temperature approach and the Hargeaves-Samani equation using local climatic variables. *Agric. Water Manag.* **2020**, *240*, 106210. [CrossRef]

82. Talebmorad, H.; Ahmadnejad, A.; Eslamian, S.; Ostad-Ali-Askari, K.; Singh, V.P. Evaluation of uncertainty in evapotranspiration values by FAO56-Penman-Monteith and Hargreaves-Samani methods. *Int. J. Hydrol. Sci. Technol.* **2020**, *10*, 135–147. [CrossRef]

83. Bettio, D. Analysis and Modelling of the Functioning of a Phytoremediation Pilot Plant for the Extraction of PFAS COMPOUNDS from Water. Master's Thesis, University of Padova, Padova, Italy, 2018.

84. *ASTM-D7979*; ASTM Standard Test Method for Determination of Perfluorinated Compounds in Water, Sludge, Influent, Effluent and Wastewater by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS). ASTM International: West Conshohocken, PA, USA, 2016.

85. Metcalf, L.; Eddy, H.P. *Wastewater Engineering: Treatment, Disposal, and Reuse*, 4th ed.; McGraw-Hill: New York, NY, USA, 2002.

86. Scher, D.P.; Kelly, J.E.; Huset, C.A.; Barry, K.M.; Hoffbeck, R.W.; Yingling, V.L.; Messing, R.B. Occurrence of perfluoroalkyl substances (PFAS) in garden produce at homes with a history of PFAS-contaminated drinking water. *Chemosphere* **2018**, *196*, 548–555. [CrossRef]

87. Huang, J.; Reneau, R.B., Jr.; Hagedorn, C. Nitrogen removal in constructed wetlands employed to treat domestic wastewater. *Water Res.* **2000**, *34*, 2582–2588. [CrossRef]

88. Knight, R.L.; Payne, V.W.E.; Borer, R.E.; Clarke, R.A.; Pries, J.H. Constructed wetlands for livestock wastewater management. *Ecol. Eng.* **2000**, *15*, 41–55. [CrossRef]

89. Kumari, M.; Tripathi, B.D. Efficiency of *Phragmites australis* and *Typha latifolia* for heavy metal removal from wastewater. *Ecotoxicol. Environ. Saf.* **2015**, *112*, 80–86. [CrossRef]

90. Akratos, C.S.; Tsihrintzis, V.A. Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. *Ecol. Eng.* **2007**, *29*, 173–191. [CrossRef]

91. Rodríguez, M.; Brisson, J. Pollutant removal efficiency of native versus exotic common reed (*Phragmites australis*) in North American treatment wetlands. *Ecol. Eng.* **2015**, *74*, 364–370. [CrossRef]

92. Lee, B.H.; Scholz, M. What is the role of *Phragmites australis* in experimental constructed wetland filters treating urban runoff? *Ecol. Eng.* **2007**, *29*, 87–95. [CrossRef]

93. Zhao, H.; Guan, Y.; Qu, B. PFCA uptake and translocation in dominant wheat species (*Triticum aestivum* L.). *Int. J. Phytoremediat.* **2018**, *20*, 68–74. [CrossRef]

94. Gobelius, L.; Lewis, J.; Ahrens, L. Plant Uptake of Per- and Polyfluoroalkyl Substances at a Contaminated Fire Training Facility to Evaluate the Phytoremediation Potential of Various Plant Species. *Environ. Sci. Technol.* **2017**, *51*, 12602–12610. [CrossRef]
95. Mulkeen, C.J.; Williams, C.D.; Gormally, M.J.; Healy, M.G. Seasonal patterns of metals and nutrients in *Phragmites australis* (Cav.) Trin. ex Steudel in a constructed wetland in the west of Ireland. *Ecol. Eng.* **2017**, *107*, 192–197. [CrossRef]
96. Saeed, T.; Sun, G. A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: Dependency on environmental parameters, operating conditions and supporting media. *J. Environ. Manag.* **2012**, *112*, 429–448. [CrossRef]