



Occurrence of per- and polyfluorinated alkyl substances in wastewater treatment plants in Northern Italy



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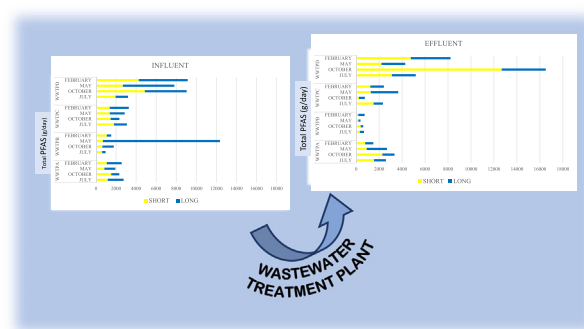
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HIGHLIGHTS

- PFAS have been monitored in wastewater and sludge samples in Northern Italy.
- Perfluoropentanoic acid (PFPeA) dominated influent wastewater.
- Shorter chain PFAS including perfluorobutane sulfonic acid dominated effluent wastewater.
- Secondary biological treatment were not effective in removing PFAS from wastewater.
- Larger plant showed lower removal efficiency.

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewater treatment plants are known to be relevant input sources of per- and polyfluoroalkyl substances (PFAS) in the aquatic environment. This study aimed to investigate the occurrence, fate, and seasonal variability of twenty-five PFAS in four municipal wastewater treatment plants (WWTP A, B, C, and D) surrounding the city of Milan (Northern, Italy). Composite 24-h wastewater samples were collected in July and October 2021 and May and February 2022 from influents and effluents of the four WWTPs. PFAS were detected at concentrations ranging between 24.1 and 66.9 $\mu\text{g L}^{-1}$ for influent and 13.4 and 107 $\mu\text{g L}^{-1}$ for effluent wastewater samples. Perfluoropentanoic acid was the most abundant (1.91–30.0 $\mu\text{g L}^{-1}$) in influent samples, whereas perfluorobutane sulfonic acid predominated (0.80–66.1 $\mu\text{g L}^{-1}$) in effluent samples. In sludge, PFOA was detected in plant A at concentrations in the range of 96.6–165 ng kg^{-1} dw in primary sludge samples and 98.6–440 ng kg^{-1} dw in secondary treatment sludge samples. The removal efficiency of total PFAS varied between 6 % and 96 %. However, an increase of PFAS concentrations was observed from influents to effluents for plant D (during July and October), plant A (during October and May), and plant C (during May) indicating that biotransformation of PFAS precursors can occur during biological treatments. This was supported by the observed increase in concentrations of PFOA from primary to secondary treatment sludge samples in plant A. Moreover, the plant operating at shorter hydraulic retention times (plant D) showed lower removal efficiency (<45 %). Seasonal variation of PFAS in influent and effluent appears rather low and more likely due to pulse release instead of seasonal factors.

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1. Introduction

Poly- and perfluoroalkyl substances (PFAS) are a group of manufactured chemicals having a carbon chain in which hydrogen atoms are totally (perfluoroalkyl substances) or partly (polyfluoroalkyl substances) replaced by fluorine atoms and connected with different functional groups (e.g. carboxylic and sulphonic acid groups). PFAS also include polymers such as fluoropolymers, perfluoropolyethers, and side-chain fluorinated polymers. PFAS are categorized into short-chain (i.e., with a number of perfluorinated carbons <6 and 7 for perfluorosulfonic and perfluorocarboxylic acids, respectively), and long-chain PFAS (OECD, 2011). PFAS have been produced since the 1940s and used globally for many industrial and domestic applications owing to their water and oil-repellent properties. For example, they have been used in firefighting foams, semiconductors, lubricants, textiles, surfactants, and paints, as well as in food-packing materials, commercial household products (such as stain, waterproofing, cleansers), and cosmetics (Oliaei et al., 2013; Sunderland et al., 2019, Vo et al., 2020, Gaines, 2022).

Numerous studies have been published over the last 15 years on the toxicity effects of PFAS on aquatic organisms, which comprise repercussions on growth, development, and reproduction (Fabbri et al., 2014; Lu et al., 2015; Ji et al., 2008; Lee et al., 2019; USEPA, 2017). Moreover, toxicological effects of PFAS on human health have been evaluated to include immune system alteration, thyroid function alteration, liver disease, lipid and insulin dysregulation, kidney disease, adverse effects on growth and reproduction, and cancer (Genser et al., 2015; Lee and Choi, 2017; Attanasio, 2019; Steenland et al., 2009; Blake et al., 2018; Waterfield et al., 2020; EFSA Panel on Contaminants in the Food Chain, 2020). These studies mainly concerned the toxicity of long-chain PFAS such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). Because of their potential health and environmental impacts, the production of long-chain PFAS decreased since the 2000s leading to a progressive increase in the usage of short-chain PFAS in many applications. In fact, long-chain PFAS were first voluntarily phased out by many industries (Kraff and Riess, 2015) and then they were subject to national (EU, 2019/1021) and international (e.g. UNEP, 2019a) restrictions. PFOS and PFOA are listed under Annex A of the Stockholm Convention on persistent organic pollutants (POPs), to progressively eliminate the use of these substances globally (UNEP, 2019a), whereas perfluorohexane sulfonic acid (PFHxS), its salts, and PFHxS-related compounds have been proposed for listing under the Stockholm Convention (UNEP, 2019b). At European Union (EU) level, PFOS and PFOA are restricted under the EU POP Regulation (EU, 2019; EU, 2020).

Wastewater treatment plants (WWTPs) are relevant input sources of PFAS in the aquatic environment as they receive waste from residential, commercial, and industrial facilities (Paul et al., 2008; Gago-Ferrero et al., 2017). Some potential PFAS sources in wastewater are for example the industrial facilities where PFAS are produced, the textiles and leather factory, the semiconductor industry, industrial surfactants, resins, plastics manufacturing, domestic use and disposal of consumer products, and household applications (Clara et al., 2008; Ahrens, 2011, Buck et al., 2011). In the last ten years, PFAS have been monitored in influent and effluent wastewater in many parts of the world (Castiglioni et al., 2015; Gago-Ferrero et al., 2017; Lenka et al., 2021; Kim et al., 2022; Jiang et al., 2023, Szabo et al., 2023). Most of the monitoring studies on wastewater have been carried out in China (31 %), Europe (30 %), and North America (16 %) (Lenka et al., 2021; Cookson and Detwiler, 2022). Many studies reported that conventional WWTPs are not effective in removing PFAS from wastewater and higher concentrations of some PFAS are often found in effluent than in influent, suggesting the formation of shorter chain PFAS during wastewater treatments from polyfluorinated precursors (Arvaniti and Stasinakis, 2015; Thompson et al., 2022). In 2010, an EU-wide monitoring survey investigated 156 polar organic contaminants, including PFAS in effluents from 90 European WWTPs. Among PFAS, PFOA was the most detected compound (detection frequency of 99 %) followed by perfluoroheptanoic acid (PFHpA, 94 %) and PFOS (93 %). Perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluorohexanoic acid (PFHxA), and PFHxS were also frequently

detected (detection frequency range of 70–89 %). Italy took part in the European monitoring survey by analysing two wastewater samples. Later in 2015, Castiglioni et al. provided data on PFAS monitoring in municipal and industrial WWTPs serving the city of Milan (Northern Italy) in three-year monitoring campaigns (2011–2013) (Castiglioni et al., 2015). PFOA and PFOS were the dominant PFAS in effluent wastewater (Castiglioni et al., 2015). Currently, the number of PFAS monitoring studies in Italian WWTPs is limited to only 3 % of the global geographical distribution (Lenka et al., 2021), and no seasonal variability of PFAS occurrence has been previously conducted. The types and concentrations of PFAS globally change over time because of the regulatory and voluntary actions as pointed out in many recently published studies where short-chain PFAS predominate both influent and effluent wastewater (Lenka et al., 2021). In addition, most of the studies have been focused on aqueous matrix as the determination of PFAS in sludge is difficult due to the demanding efforts required by the analysis in this complex matrix. Knowing the occurrence and fate of PFAS in WWTPs distributed in different parts of the world allows an understanding of their global production, usage, and transport.

This study aimed to determine the occurrence, fate, and removal of PFAS in WWTPs located in an urbanized region with low manufacturing output in North of Italy. The effect of different wastewater treatments and the treatment capacity of the plant on the removal efficiencies are outlined to better understand the fate of PFAS in WWTPs. The seasonal variation of PFAS contamination in wastewater influent and effluent was also investigated to define sampling frequency in future PFAS monitoring campaigns.

2. Sampling

Composite 24-h wastewater samples (32 samples) were collected on the same day and hour in July and October 2021 and May and February 2022 from influent and effluent of four WWTPs (A, B, C, and D) located in the surrounding of Milan, Italy. Information on the WWTPs (location, type of treatment, processing capacity, equivalent person, average daily flow rate, and hydraulic retention time) is summarized in Table S1. The source of wastewater was primarily residential, with a contribution from local industries lower than 20 %. Many types of industries discharge their wastewaters into these plants such as food industries, chemical industries, fuel distribution, treatment, and coating of metals. Moreover, plant D also receives wastewaters from textile dyeing, plastic, and rubber manufacturing industries. A minimum period of three rain-free days was observed prior to sample collection. Influent and effluent samples were collected with a MAXX SP5 autosampler (LabUnlimited Carl Stuart Group, UK) in polyethylene bottles (Sigma-Aldrich, Italy) pre-rinsed with methanol and pure water. At the moment of collection, the temperature of the wastewater samples was 14 °C during February and 22 °C during the warmer months. After collection, samples were stored at –20 °C until analysis. A field blank was prepared with every kit, transferred to a clean bottle on-site, and then extracted concurrently with samples. Field blanks were extracted within the same batches as samples and matched with the corresponding WWTPs.

Sludge samples were collected from the sludge tanks using pre-cleaned high-density polyethylene bottles. An aliquot of approximately 1 kg of each sludge sample was dried at 40 °C, ground and homogenized with a mortar and pestle and stored at –20 °C until extraction. The dry matter of sludge samples was determined by drying 10 g of fresh sludge at 105 °C. Sludge samples were collected during February 2022 from the four WWTPs. During July and October 2021 and May 2022, samples were collected only from plant A (as unlike other plants, it employed membrane bioreactor) and plant D (because of the highest treatment capacity).

3. Materials and methods

3.1. Standards and reagents

Analytical standards and isotopically labeled analogues PFAS were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). They consist a stock solution of: i) native standards (PFAC30PAR) containing

perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorobutane sulfonamide (FBSA), perfluorobutane sulfonic acid (PFBS), PFHxA, 4:2 fluorotelomer sulfonic acid (4:2 FTS), perfluoropentane sulfonic acid (PFPeS), PFHpA, fluorohexane sulphonamide (FHxSA), PFHxS, PFOA, 6:2 fluorotelomer sulfonic acid (6:2 FTS), perfluoroheptane sulfonic acid (PFHpS), PFNA, perfluorooctane sulfonamide (PFOSA), PFOS, PFDA, 8:2 fluorotelomer sulfonic acid (8:2 FTS), perfluoroundecanoic acid (PFUnDA), N-methyl-perfluoro-1-octanesulfonamidoacetic acid (N-MeFOSAA), N-ethyl-perfluoro-1-octanesulfonamide (NEtFOSA), perfluorodecane sulfonic acid (PFDS), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), and perfluorotetradecanoic acid (PFTeDA); ii) isotopically labeled standards (MPFAC-24ES) containing $^{13}\text{C}_4$ PFBA, $^{13}\text{C}_5$ PFPeA, $^{13}\text{C}_5$ PFHxA, $^{13}\text{C}_4$ PFHpA, $^{13}\text{C}_8$ PFOA, $^{13}\text{C}_9$ PFNA, $^{13}\text{C}_6$ PFDA, $^{13}\text{C}_7$ PFUnA, $^{13}\text{C}_2$ PFDoA, $^{13}\text{C}_2$ PFTrDA, $^{13}\text{C}_3$ PFBS, $^{13}\text{C}_3$ PFxS, $^{13}\text{C}_8$ PFOS, $^{13}\text{C}_8$ PFOSA, d_5 -NetFOSAA, d_3 -N-Me-FOSAA, $^{13}\text{C}_2$ 4:2 FTS, $^{13}\text{C}_2$ 6:2 FTS, and $^{13}\text{C}_2$ 8:2 FTS. LC/MS grade solvents and all other reagents used for sample preparation such as methanol (MeOH), ammonium acetate, glacial acetic acid, and ammonium hydroxide (28 % ammonia in water $\geq 99.99\%$) were provided by VWR International (Milan, Italy).

3.2. Sample preparation

The water samples (100 mL) were filtered with Munktell filter paper discs, Grade 388, 12–15 μm (Munktell Filter AB, Falun, Sweden). Then, wastewater samples were adjusted to pH 3 with acetic acid and spiked with the isotopically labeled standard solution to a concentration of 80 ng L^{-1} .

Analytes were extracted by solid-phase extraction (SPE, Lee Sun New et al., 2021) using Agilent SampliQ Weak Anion Exchange (6 mL, 150 mg; CPS analitica for Chemistry, Agilent Technologies). In particular, the cartridges were conditioned sequentially with 4 mL of methanol 0.5 % (v/v) ammonia, 4 mL of methanol, 4 mL of water, and 3 mL of water 1 % acetic acid. Then, the samples were loaded onto the cartridges with a flow rate of ca. 2 mL min^{-1} . The cartridges were washed with 4 mL of acetate buffer 25 mmol L^{-1} pH 4 and 4 mL of ultrapure water; after that, they were vacuum dried for 10 min. The elution was carried out with 4 mL methanol and 4 mL of methanol 0.5 % (v/v) ammonia.

The eluates were evaporated under a gentle nitrogen stream at 25 °C till 500 μL . Aliquots of 80 μL were collected and reconstituted with 20 μL of ultrapure water, obtaining a solution MeOH:H₂O (80:20, v/v) before injection. Samples were analyzed in duplicate.

For sludge samples, approximately 0.25 g of dried sludge was extracted with ultrasonication for 30 min using methanol with 0.1 % of ammonia as extraction solution according to EPA 3550C 2007 and EPA 8327 2021. After extraction, the pH of the extract was adjusted to pH 3 with acetic acid.

3.3. Instrumental analysis

Samples were analyzed by an Ultimate 3000 ultra-HPLC (UHPLC) system connected via a heated ESI source to a TSQ Vantage™ triple-stage quadrupole mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The UHPLC system consisted of a binary pump equipped with a degasser, a thermostated microwell plate autosampler, set at 14 °C, and a thermostated column oven. The chromatographic column was an Agilent Zorbax Eclipse Plus C18 (2.1 \times 100 mm, 1.8 μm); the Agilent Infinity Lab PFC (4.6 \times 30 mm) delay column was added after the pump but before the injector to overcome the background PFAS contamination issue that could occur in a single analytical run. The column was thermostated at 35 °C, and mobile phases were: (A) H₂O:MeOH (95:5, v/v) 2 mmol L^{-1} ammonium acetate, and (B) MeOH 2 mmol L^{-1} ammonium acetate. The flow rate was set at 300 $\mu\text{L min}^{-1}$; the injection volume was 40 μL . The gradient elution of 35 min was optimized as follows: after an isocratic step at 10 % B for 4 min, B was linearly increased to 90 % in 13 min and kept constant for 14.5 min, and then it was brought to the starting 10 % in 0.5 min, and the column let to equilibrate for 3 min. The UHPLC-MS/MS system was managed by Xcalibur software (v.2.1, Thermo Fisher Scientific). The ESI source parameters were: spray

voltage 3.2 kV, capillary temperature 220 °C, sheath gas pressure, ion sweep gas pressure, and auxiliary gas pressures 50, 0, and 25 (arbitrary units), respectively. Multiple reaction monitoring (MRM) parameters for each standard compound were optimized in negative ion mode with direct injection of standard solutions at a concentration of 10 ng mL^{-1} . The selected MRM conditions are reported in Table S2. Quantification was done using the isotopic dilution method with labeled PFAS analogues; the most intense precursor/product ion transition was the quantifier ion, whereas the second one was the qualifier ion. Standard solutions were prepared in MeOH to check the linearity of the method. These solutions were prepared at five different concentrations ranging between 0.01 ng mL^{-1} and 25 ng mL^{-1} with the relative isotopically labeled standard at a concentration of 50 ng mL^{-1} . The linearity ranges of calibration curves are reported in Table S2.

3.4. Quality control

To evaluate the method developed for water and sludge samples, different quality parameters such as recovery, reproducibility, and sensitivity were studied. The results are presented in Table S2. Recovery tests for method evaluation were carried out by spiking pure water samples at levels of 25 ng L^{-1} , 250 ng L^{-1} , and 500 ng L^{-1} for each PFAS. The same recovery tests were conducted on a wastewater pool showing results comparable to those obtained with pure water that was chosen for method validation due to the presence of endogenous quantities of analytes of interest in wastewater. Recoveries (RE (%)) for individual analytes were calculated by the ratio between the samples fortified before the extraction (set 1) and the ones spiked after the extraction (set 2). The evaluation was done according to Eq. (1) normalizing the obtained natives' areas with the relative Internal Standard (IS) peaks areas (spiked at a concentration of 80 ng L^{-1}). The endogenous amount was considered by subtracting Area(C₀) that is referred to a not spiked sample.

$$RE(\%) = \frac{Area(set1) - Area(C_0)}{Area(set2) - Area(C_0)} \times 100 \quad (1)$$

For the sludge sample the spike was at 100 ng kg^{-1} dw. Five replicates were done to evaluate the reproducibility of the method. Precision was evaluated by carrying out intra-day, and inter-day precision calculations in extracts spiked at fortification values of 250 ng L^{-1} and 100 ng kg^{-1} dw for water and sludge samples, respectively. Recoveries were >70 % with relative standard deviations (RSDs) in the range of 2–9 % and <20 % for wastewater and sludge samples, respectively. PFAS concentrations measured in the sludge sample used for recovery calculation were lower than limit of detection (LOD) defined as the minimum amount of analyte providing a peak with a signal-to-noise ratio (S/N) = 3. Method limit of quantification (MLOQ), defined as the minimum amount of analyte providing a peak with a signal-to-noise ratio (S/N) = 10 was estimated for each PFAS. MLOQs ranged between 0.35–3.15 ng L^{-1} , and 0.07–0.15 ng kg^{-1} dw for wastewater and sludge samples, respectively.

3.5. Calculation of PFAS removal and mass load

For each WWTP, the removal efficiency was calculated knowing the total PFAS concentration in influent wastewater samples (C_{inf}) and effluent wastewater samples (C_{eff}) according to Eq. (2):

$$Removal(\%) = (C_{inf} - C_{eff}/C_{inf}) \times 100 \quad (2)$$

Moreover, daily mass load (g/day) of individual PFAS was calculated by multiplying influent and effluent concentrations of single PFAS at each WWTP by the corresponding daily flow rate (Table S1).

3.6. Statistical analysis

Data analysis was conducted using R statistical software (v4.3.0, R Core Team, 2023). Asymptotic two-sample Kolmogorov-Smirnov test was used

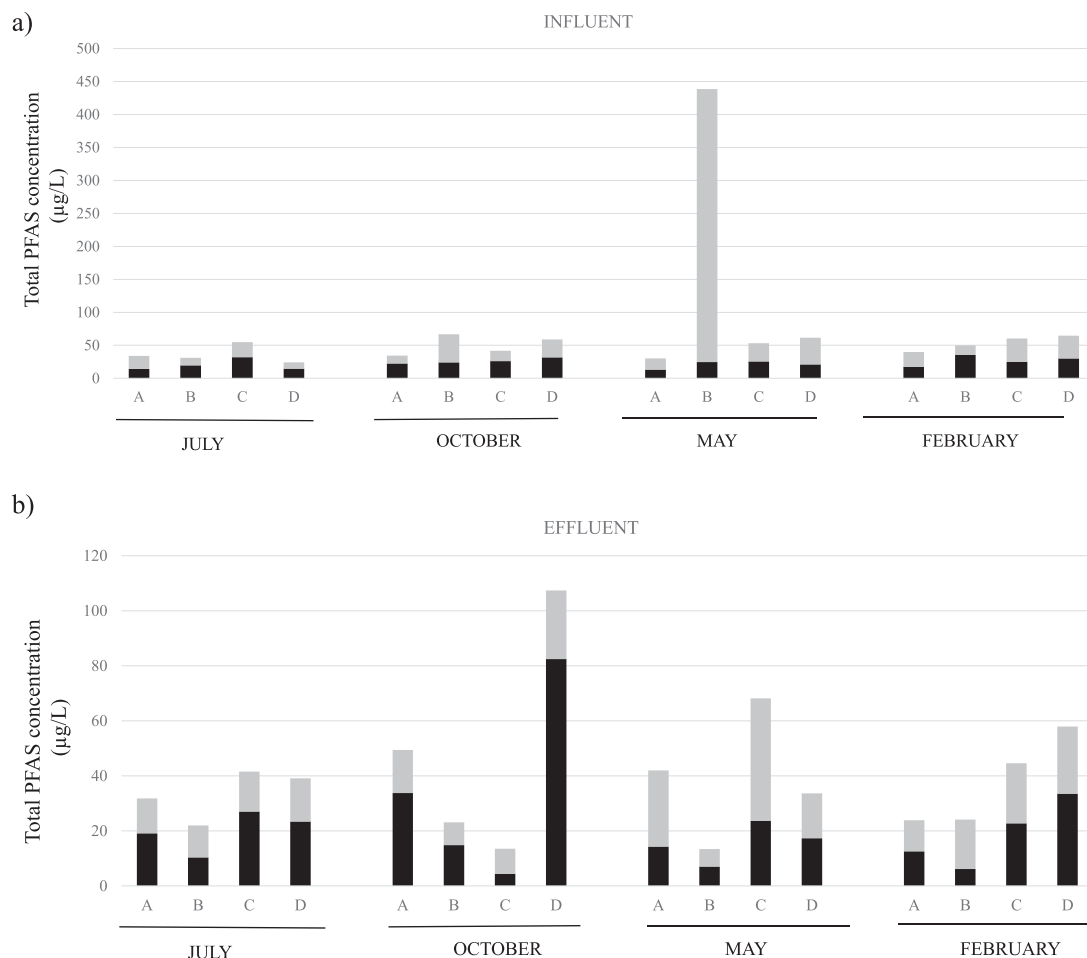


Fig. 1. Concentrations of total short chain and long chain PFASs in influent (a) and effluent (b) wastewater samples in July and October 2021, May and February 2022.

to assess for relationships between the concentration of individual PFAS at all WWTP during the dry season (July, October, and May) and wet season (February).

4. Results and discussions

Twenty-five PFAS from five classes (perfluorocarboxylic acids, perfluorosulfonic acids, fluorotelomer sulfonates, perfluorosulfonamides, perfluorosulfonamidoacetic acids) were detected in wastewater samples from influents and effluents of four Italian WWTPs during the sampling campaigns in July and October 2021, February, and May 2022. Concentrations of PFAS in influent and effluent wastewater samples are summarized in Tables S3 and S4, respectively.

4.1. PFAS concentrations in influent wastewater

Total PFAS concentrations in influent ranged from 24.1 to 66.9 $\mu\text{g L}^{-1}$. Plant D showed the highest influent PFAS concentrations (24.1–64.5 $\mu\text{g L}^{-1}$) followed by Plant C (41.8–60.4 $\mu\text{g L}^{-1}$) as expected because these two plants treat the highest wastewater daily volume (Table S1). Plant B was less contaminated than plants C and D. However, a spike in concentration was found during May in plant B (the total PFAS amount was 438 $\mu\text{g L}^{-1}$) due to the high level of PFHxS detected (393 $\mu\text{g L}^{-1}$). This could be attributed to local industries as reported in previous studies, which linked maximal values of PFAS concentrations to industrial wastewater (Vo et al., 2020; Lenka et al., 2022). Total long-chain PFAS concentrations were slightly higher (range 11.4–42.6 $\mu\text{g L}^{-1}$, whereas including the spike in concentration the range was 11.4–414 $\mu\text{g L}^{-1}$) than those of short-chain PFAS concentration (13.1–35.2 $\mu\text{g L}^{-1}$) (Fig. 1a). Influent wastewater was mainly

dominated by short-chain PFPeA (1.91–30.0 $\mu\text{g L}^{-1}$) followed by NMeFOSAA (MLOQ–20.3 $\mu\text{g L}^{-1}$), PFOS (MLOQ–13.1 $\mu\text{g L}^{-1}$), PFBS (MLOQ–11.7 $\mu\text{g L}^{-1}$), PFHxS (MLOQ–11.6 $\mu\text{g L}^{-1}$ excluding the spike in concentration), PFHxA (0.85–7.87 $\mu\text{g L}^{-1}$), PFOA (1.04–7.55 $\mu\text{g L}^{-1}$), PFBA (0.76–5.56 $\mu\text{g L}^{-1}$), PFHpA (0.71–4.06 $\mu\text{g L}^{-1}$) and PFTrDA (MLOQ–3.27 $\mu\text{g L}^{-1}$) in all four investigated WWTPs.

Fig. 2a shows the percentage composition of total PFAS in influent water samples from the four investigated WWTPs. Among short-chain PFAS, PFPeA accounted for a range of 4–60 % (mean value 23 %) of the total PFAS concentration. Other short-chain dominant homologues were PFHxA (0.25–26 %, mean value 8 %) PFBS (0.02–18 %, mean value 7 %), PFBA (0.7–11 %, mean value 5 %), and PFHpA (0.13–12, mean value 4 %). PFPeA, PFBA, PFHpA, and PFHxA were detected in all samples, whereas PFBS was detected in 94 % of the samples. Among long-chain PFAS, PFHxS and NMeFOSAA dominated accounting for a range of 0.009–90 % (mean value 14 %) and 0.008–34 % (mean value 13 %), respectively, of the total PFAS concentration. Other long-chain dominant PFAS were PFOS (0.004–19 %, mean value 7 %), PFOA (0.2–11 %, mean value 6 %), and PFTrDA (0.002–25 %, mean value 5 %). PFOA was detected in all the wastewater samples. NMeFOSAA, PFTrDA and PFOS were also frequently detected (>80 % of the total samples), whereas PFHxS showed lower detection frequency (56 %). Regarding FTS, 4:2 FTS was less detected (<6 % of the samples) than 6:2 FTS and 8:2 FTS which were present above the MLOQ in <50 % of the investigated WWTP influent samples. PFPeS and PFDS were present above the MLOQ in <12 % and 19 % of WWTP samples, respectively. PFOSA and NeFOSA were present above the MLOQ in <19 % and 25 % of the influent samples, respectively. FBSA was above MLOQ in 62 % of the investigated samples at a concentration < 0.91 $\mu\text{g L}^{-1}$. PFNA and PFDA were above MLOQ in <94 % and 62 % of the samples, respectively at concentration lower

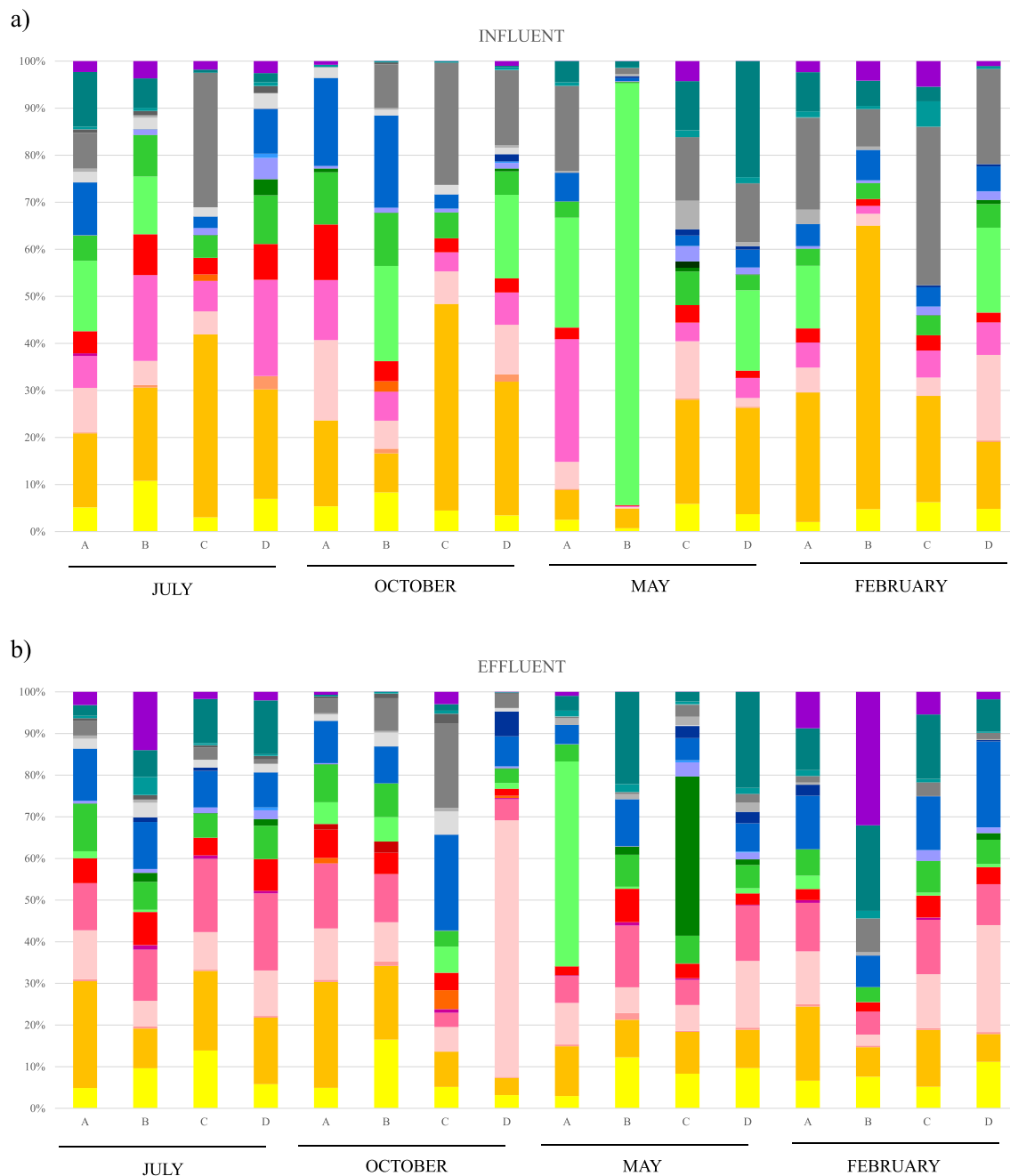


Fig. 2. Percentage compositions of PFASs in influent (a) and effluent (b) wastewater samples collected from the four investigated WWTPs (A, B, C and D) in July and October 2021, May and February 2022.

than 1.83 ngL^{-1} . PFDoA, PFUnDA and PFTeDA were frequently detected (>60 %) at concentration lower than $3.27 \mu\text{g L}^{-1}$. PFHpS were present above the MLOQ in <6 % of the samples, and FHxSA, was under the MLOQ in all the influent samples. The presence of short-chain PFAS in influent wastewater have been already reported in previous studies (Lenka et al., 2021) indicating the prevalent production and use of shorter chain as substitute of longer chain PFAS in many consumer products and industrial applications (Wang et al., 2013a, 2013b; Lenka et al., 2021;). For example, PFHxA, PFBS, and PFBA have been used as alternative to PFOA and PFOS for surface treatment of textile, leather, and carpet as well as for treatment of food contact material, in production of fluoropolymer, in decorative metal plating and as fire-fighting foams (Wang et al., 2013a, 2013b). PFBS is also a by-product of PFBS-precursors or PFBS-related substances, which are mainly used as surfactants and repellent protection of textiles, leather, carpets, and hard surfaces, as these last degrade during use in the waste stage or in the environment

(Nielsen, 2017; ECHA, 2019). Since PFHpA is not commercially produced in EU, its presence in wastewater can be the result of the environmental transformation of longer chain PFAS (ECHA, 2022). However, PFHpA and PFPeA have been found in food contact material such as microwave popcorn bag (Zafeiraki et al., 2014; Schaider et al., 2017). In addition, PFBA, PFBS, PFHxA, PFHpA, and PFPeA have been found in several consumer products which include carpets, gloves, impregnation sprays, leather, ski wax, and outdoor textiles (Kotthoff et al., 2015). The presence of PFOA and PFOS in wastewater samples suggests that although they have been restricted in consumer products (EU Regulation, 2019/1021; Regulation (EU) 2020/784), they have still not been completely phased out. PFHxS has replaced PFOS as surfactant and protective coating in applications such as aqueous firefighting foams, textile coating, metal plating and in polishing agents (Wang et al., 2013a, 2013b; Boucher et al., 2018). N-MeFOSAA is a degradation product of the volatile *N*-methyl perfluorooctanesulfonamido ethanol (N-MeFOSE) used in

protective surface coatings (3M, 1999; Boulanger et al., 2005; Buck et al., 2011). PFTrDA derived from a mixture of fluorotelomer-based precursors (which include mainly PFNA) marketed for use as fluorinated surfactant (Buck et al., 2011). The contamination of PFAS found in this study could be attributed to domestic waste (use and disposal of consumer products containing additives or impurities) and to industrial discharges because, although the contribution of local industries was a minority (<20 %), the levels of PFAS in wastewater influents were very high (in the order of $\mu\text{g L}^{-1}$) compared to those reported in urban wastewater in previous studies (which reported PFAS concentrations in the order of ng L^{-1}) (Castiglione et al., Rotander et al., 2015; Campo et al., 2014; Lenka et al., 2021). In addition, previous studies showed that PFAS concentrations in wastewater from intensive industry were higher than $1 \mu\text{g L}^{-1}$ (Vo et al., 2020; Gagliano et al., 2020; Wang et al., 2013a, 2013b; Rotander et al., 2015). Water solubility also plays a role in distribution of PFAS in wastewater. Short-chain PFAS are generally more soluble in water than longer PFAS due to the smaller number of carbon atoms in their chain linked to the carboxylate and sulfonate groups, which are hydrophilic (USEPA, 2019; Wang et al., 2011). However, the concentration of some longer PFAS such as N-MeFOSAA and PFOS were higher than shorter PFAS (PFBA and PFBS) although their water solubility was lower (water solubility, N-MeFOSAA, $0.00052 \text{ mg L}^{-1}$; PFOS, 0.049 mg L^{-1} ; PFBS, 8859.8 mg L^{-1} and PFBA, 344 mg L^{-1}) (USEPA, 2012). This could suggest that there were constant sources of these substances flowing through WWTPs.

4.2. PFAS concentrations in effluent wastewaters

Total PFAS concentrations in effluent ranged from 13.4 to $107 \mu\text{g L}^{-1}$. Effluents from plant D had the highest PFAS concentrations (33.6 – $107 \mu\text{g L}^{-1}$) followed by plant C (41.8 – $60.4 \mu\text{g L}^{-1}$), whereas effluents from plant B were the least contaminated (13.4 – $24.1 \mu\text{g L}^{-1}$). Unlike in influent wastewater, total long-chain PFAS concentrations were lower (range 6.33 – $44.5 \mu\text{g L}^{-1}$) than short-chain PFAS concentrations (4.41 – $82.4 \mu\text{g L}^{-1}$) (Fig. 1b). Moreover, effluent wastewater was mainly dominated by PFBS (0.80 – $66.1 \mu\text{g L}^{-1}$) followed by PFHxS (<MLOQ– $20.7 \mu\text{g L}^{-1}$), PFPeA (1.15 – $12.6 \mu\text{g L}^{-1}$), PFOS (1.50 – $12.0 \mu\text{g L}^{-1}$), PFHxA (0.46 – $7.72 \mu\text{g L}^{-1}$), PFTrDA (<MLOQ– $7.75 \mu\text{g L}^{-1}$), PFBA (0.70 – $6.46 \mu\text{g L}^{-1}$), PFOA (0.51 – $4.49 \mu\text{g L}^{-1}$), NMeFOSAA (<MLOQ– $3.67 \mu\text{g L}^{-1}$) and PFHpA (0.53 – $3.33 \mu\text{g L}^{-1}$).

Fig. 2b shows the percentage composition of total PFAS in effluent water samples from the four investigated WWTPs. PFBS accounted for a range 6–61 % (mean value 26 %) of total PFAS concentration. Other short-chain dominant homologues were PFHxA (3–18 %, mean value 10 %), PFBA (3–16 %, mean value 7 %), PFPeA, (4–26 %, mean value 7 %) and PFHpA (2–8 %, mean value 4 %). PFBS, PFHxA, and PFPeA and PFHpA were detected in all effluent wastewater samples. Among long-chain PFAS, PFOS (4–23 %, mean value 21 %), NMeFOSAA (0.0005 %–20 %, mean value 8 %) and PFOA (3–11 %, mean value 6 %) were dominant. PFHxS ranged between 0.01 and 49 % (mean value 0.7 %) of the total effluent samples. PFOS, PFOA and PFDoDA were detected in all the effluent samples, whereas NMeFOSAA and PFHxS were detected in 94 % and 81 % of the total effluent wastewater samples, respectively. FTS, namely 4:2 FTS, 6:2 FTS and 8:2 FTS were present above the MLOQ in <69 %, 50 % and 56 % of the investigated WWTP effluent samples, respectively. PFPeS and PFOSA were present above the MLOQ in less 19 % of the effluent samples. FHxSA and PFDS were above the MLOQ in <12 % and 19 %, respectively. FBSA was above the MLOQ in 94 % of the samples at concentration < $0.10 \mu\text{g L}^{-1}$. PFDA and NETFOSA were present above MLOQ in <50 and 56 % of the samples, respectively. PFNA, PFUnDA, PFTrDA and PFTeDA were frequently detected (>75 %) at concentration lower than $7.73 \mu\text{g L}^{-1}$. PFHpS was below the MLOQ in all the effluent samples. Many studies reported that domestic effluents have lower concentrations of PFAS than industrial effluents and are mainly dominated by PFOA, PFOS, PFHxS, PFBA, and PFHxA (Castiglioni et al., 2015; O'Connor et al., 2022; Lenka et al., 2021). Moreover, FTS have been found to be predominant in industrial effluents (O'Connor et al., 2022). In our study, FTS were detected occasionally, and high concentrations of PFOA, PFOS, PFHxS, PFBA, and PFHxA were measured in effluent samples. Thus, the

results found in effluent wastewater from this study suggest that the contamination of PFAS in the four WWTPs was due to both domestic and industrial waste.

4.3. PFAS seasonal variability

Seasonal variations of PFAS pollution in the four Italian WWTPs was investigated by monitoring wastewater during cold and wet season (February) and comparing the mass load values of PFAS to those calculated during the dry season (April, May, and October). In influent wastewater samples, total PFAS mass load ranged between 963 and 3196 g/day in July 1773 and 9044 g/day in October 1933 and 12,366 g/day in May 2021, 1519 and 9151 g/day in February 2022 (Fig. 3a; Table S5). The higher mass load estimated during May 2022 was due to the spike in concentration of PFHxS ($393 \mu\text{g L}^{-1}$) measured at plant B, which was probably linked to local industrial wastewater discharge. The concentrations of total PFAS in the cold and wet season were slightly higher than those in the dry season. The discharge of PFAS into wastewater mainly comes from industrial and manufacturing facilities and products consumption (Hamid and Li, 2016). The higher influent PFAS concentrations in winter could be attributed to human consumption of products containing PFAS such as winter clothes made of waterproof materials rich in PFAS (Zhou et al., 2017). PFPeA was the most dominant PFAS found in July (191 – 1198 g/day), October (147 – 2571 g/day) and February (686 – 1300 g/day). PFHxS was dominant in May ($11,089 \text{ g/day}$) because of the spike in concentration at Plant B. However, PFPeA was the second most abundant PFAS found in May (517 – 1766 g/day) in plant B, C and D. NMeFOSAA showed lower detection frequency (50 %) and mass load (0 – 0.88 kg/day) during July. The concentrations in October (0.14 – 1445 g/day) and May (168 – 977 g/day) were lower than those measured in February (120 – 1850 g/day). The dominance of PFPeA and NMeFOSAA in influent samples, which reached the highest concentration during the cold season (February), suggests a direct source from consumption products. For other PFAS, differences in concentrations and detection frequency due to seasonality were not observed.

In effluent samples, total PFAS mass load ranged between 684 and 5192 g/day in July; 614 and 16,514 g/day in October; 378 and 4276 g/day in May 2021; 735 and 8217 g/day in February 2022 (Fig. 3b; Table S6). The highest mass load value of total PFAS ($16,514 \text{ g/day}$) was measured at plant D in October 2021, whereas the lowest value was measured at plant B (378 g/day) in May 2022. PFPeA was the most dominant PFAS in effluent samples in July, reaching a value of 831 g/day , whereas PFBS was dominant in October ($10,166 \text{ g/day}$) and in February (2108 g/day) in plant D.

Asymptotic two-sample Kolmogorov-Smirnov test indicated no significant differences between the concentration of individual PFAS during wet and dry season in both influents ($D = 0.08$, p -value = 0.7232 , alternative hypothesis: two-sided) and effluents ($D = 0.12333$, p -value = 0.204 , alternative hypothesis: two-sided) showing p -value greater than the significance level of 0.05.

As a whole, seasonal variation of PFAS in influent and effluent appears rather low and could be attributed more likely to pulse release instead of seasonal factors.

4.4. Removal efficiency

Removal efficiency of PFAS in the different WWTPs were calculated using Eq. (2) and are shown in Fig. S1. The overall concentration of total PFAS decreased after treatment with a few exceptions: at plant D an increase of total PFAS concentrations from influent to effluent was observed during July and October; in plant A during October and May; in plant C during May (Fig. S1). In a previous work, Guerra et al. reported that different condition during the treatment processes, such as hydraulic retention time, aeration time, and microbial abundance, may affect PFAS removal (Guerra et al., 2014). Plant B showed the highest removal of PFAS (29–97 %) probably because it operated at longer hydraulic retention time (HRT, 12–14 h) compared to the other WWTPs. Plant B also received a lower load of wastewater (80,000 equivalent person, p.e.) with lower

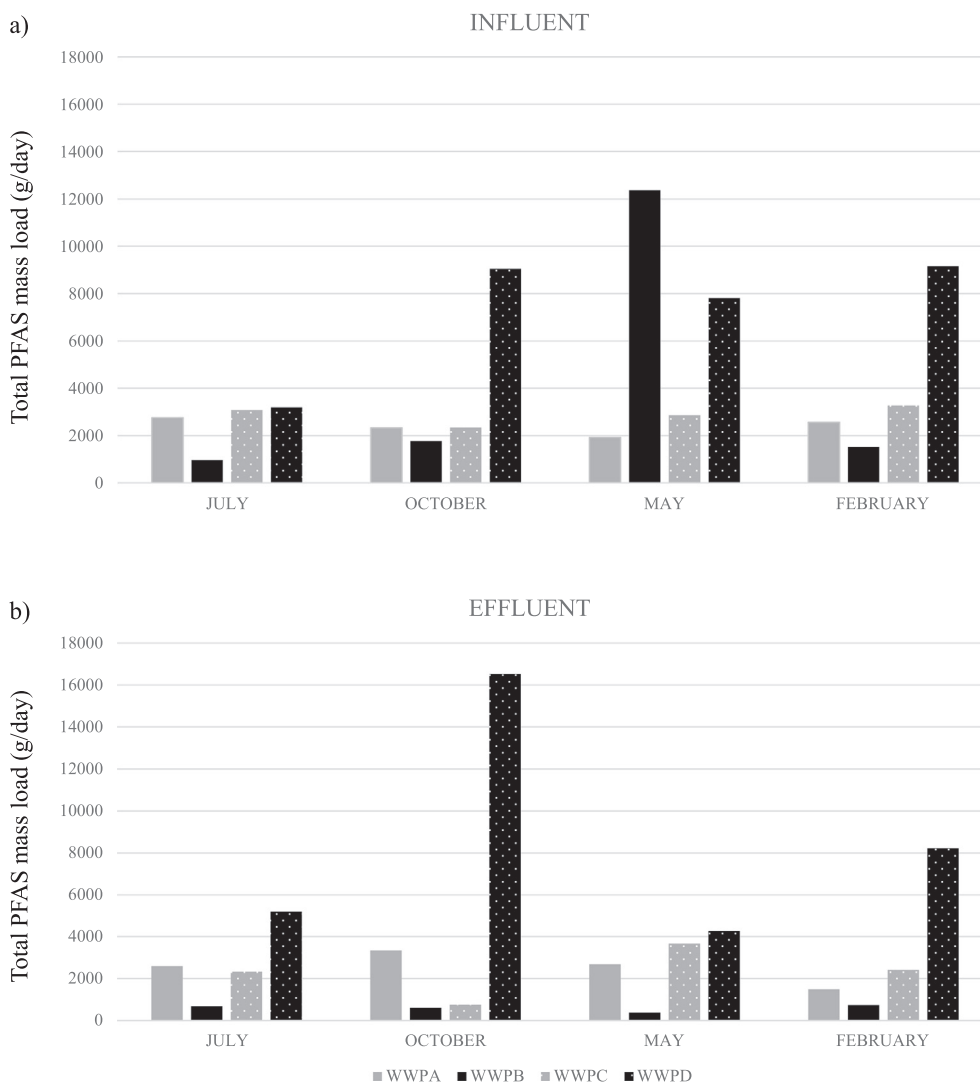


Fig. 3. Total PFAS mass load estimated in influent (a) and effluent (b) from the four studied WWTPs (A, B, C and D) during months of July and October 2021, May and February 2022.

contribution deriving from factories (10 % p.e.). On the other hand, plant D which operated at shorter hydraulic retention times (5–6 h) showed the lower removal efficiency (–82–45 %). Plant D received a greater load of industrial waste besides serving a larger population. All four wastewater treatment processes considered in this study utilize the activated sludge procedure after the denitrification. In addition, plant A employed membrane biological reactor (MBS) system after biological oxidation.

Negative values of total PFAS percentage indicate an increase of total PFAS concentrations from influent to effluent. The increased concentrations of PFAS concentrations in effluent have been already reported in most WWTPs worldwide and it is mainly attributed to biotransformation of PFAS precursors in WWTPs applying biological treatments, which determine an increase of concentrations of PFAS (Arvaniti and Stasinakis, 2015). The percentage of total PFAS removal across the plants were –62 % and 29 % during July; –82 % and 67 % during October; –39 % and 96 % during May; 10–51 % during February (Fig. S1). During the wintertime (February), an increased concentration of PFAS in the effluents of the four WWTPs was not observed, and this could be attributed to the lower temperature (14 °C) of the wastewater that does not promote the degradation of longer chain PFAS to shorter chain PFAS (Arvaniti and Stasinakis, 2015; Thompson et al., 2022).

Tables S7–S10 show the percentage differences between influent and effluent concentrations of single PFAS for the four WWTPs. Negative percent

differences indicate that concentrations in the effluent are higher than influent and positive indicate higher concentrations in the influent than effluent. Focusing on the most dominant PFAS found in this study, PFOA showed an influent-to-effluent removal efficiency ranging between –99 % and 78 %. In plant A, the concentration of PFOA increased >50 % after treatment. Plant B and C showed the best removal for PFOA (75 % and 78 %, respectively). Lower percentages of removal were observed for PFOS (–173,778–22 %). Plant C and D showed an increase in PFOS concentration after treatment. Plant B showed the highest removal efficiency (42–84 %) except during July (–173,778 %). PFHxA showed removal efficiency ranging between –107 % and 73 %. Plant B gave positive values of removal efficiency only during May and October (35 % and 53 %, respectively). Plant D showed the worst removal efficiency for PFHxA. PFPeA showed satisfactory removal efficiency (26–94 %) in three out of four WWTPs. Plant A showed the worst removal (–162–62 %) for PFPeA, whereas PFBA removal efficiency was –249–47 %. These last two PFAS reached the best removal efficiency in Plant B. PFHpA removal efficiency was of –80–58 %. The major variability of removal efficiency across the plant was observed for PFBS (–86,546 % to 72 %), NMeFOSAA (–81,979–99 %) and PFTeDA (–57,296–99 %). Plant A showed the worst NMeFOSAA removal. PFBS showed a greater increase in concentration from influent to effluent in plant A and D. The observed increase in concentrations of several PFAS in effluent wastewater suggests that further source of PFAS in wastewater plant is the degradation

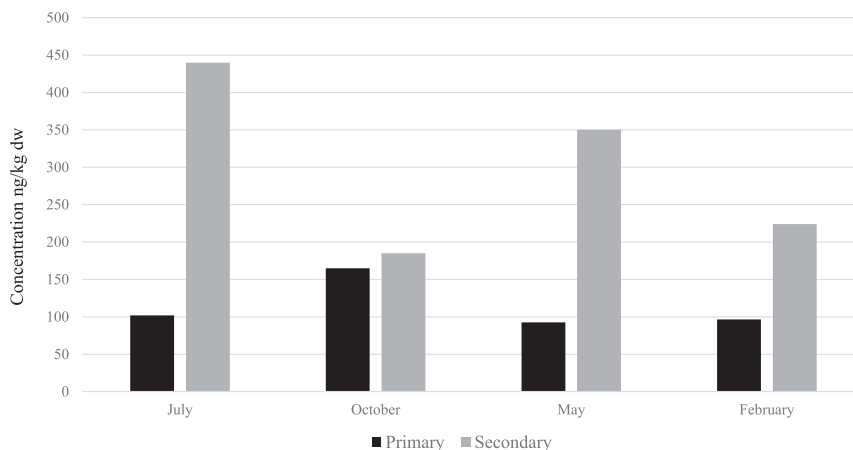


Fig. 4. Concentration of PFOA (ng kg⁻¹ dry weight) in primary and secondary sludge collected from WWTP A.

of PFAS precursors during treatments. This could be supported by the observation that plant A, which employed the MBR system, showed an increase in concentrations of many shorter PFASs from influent to effluent. Many studies reported that in biological treatment polyfluoroalkyl precursor including FOSA, FOSAA, FTOH, FTSA and FTS can degrade to shorter chain PFAS such as PFOA, PFOS and PFHxA, leading to an increase of total PFAS in effluent (Lenka et al., 2021; Zhao et al., 2013). PFPeA and PFHxA are found to be biodegradation products of 6:2 FTS and 6:2 FTOH (Wang et al., 2011b; Zhang et al., 2013); PFHxA, PFPeA, PFOA, PFHpA are degradation products of 6:2 and 8:2 FTOH (Yu et al., 2018). However, in our study we monitored only FOSA (present in three influent samples at concentration lower than 0.35 μg L⁻¹) and FTS (present in <8 samples at influent concentration < 1.06 μg L⁻¹) as precursors. PFHxA and PFOA are reported to be degradation products of FOSA in WWTPs (Yi et al., 2018); PFBA is reported to be a by-product of PFPeA, PFHxA (Hamid et al., 2020). Our results showed that the treatment capacity and treatment processes were the largest contributors to removal efficiency. In addition, microbial degradation processes (such as activated sludge and MBR) led to an increase of PFAS in effluents due to the formation of biotransformation products.

4.5. PFAS in wastewater sludge

Concentrations of PFAS measured in sludge samples after primary and secondary treatments are reported in Table S11. Most of PFAS monitored in wastewater treatment sludge were present at concentrations <MLOQs. However, PFOA was detected in sludge from plant A and its concentrations in primary sludge samples were lower (96.6–165 ng kg⁻¹ dw) than those in secondary treatment sludge samples (98.6–440 ng kg⁻¹ dw) (Fig. 4). Furthermore, a limited number of PFAS were detected occasionally in secondary sludges: PFOS was detected in plant D in October (142 ng kg⁻¹) and in plant B in February (250 ng kg⁻¹); PFBS was detected in plant A in May (96.6 ng kg⁻¹); PFHpA was detected in plant A in July (108 ng kg⁻¹ dw), and PFHxA was detected in plant A in October (239 ng kg⁻¹ dw). This confirms that secondary wastewater treatments are further source of PFAS pollution since they lead to biodegradation of PFAS precursors, determining an increase in concentrations of some PFAS from influent to effluent and from primary to secondary sludge.

Solid-water partition coefficient (K_d) is an important parameter for understanding the transport of hydrophobic contaminants in the aqueous environment, being these in contact with both the aqueous and solid phase. K_d was calculated for PFOA from the concentrations in the influent samples (C_w , expressed in ng L⁻¹) and in the primary sludge samples (C_s , expressed in ng kg⁻¹) according to $K_d = C_s/C_w$ (L kg⁻¹) (3). K_d values for PFOA ranged between 0.04 and 0.09 L kg⁻¹. These values were much lower than those found in previous studies such as in sludge samples collected from sewage treatment plants located in Valencia and Castilla-La-Mancha, Spain (25.4 10³ L kg⁻¹, mean value) (Campo et al., 2014), in

industrial areas located in Catalonia, Spain (30–54 L kg⁻¹) (Milinovic et al., 2016), and in Singapore (188–597 L kg⁻¹) (Yu et al., 2009).

4.6. Comparison with previous studies

PFAS concentrations measured in wastewater samples in our study were compared to those previously reported in selected studies worldwide (Table S12). In particular, data from WWTPs using conventional wastewater treatment plants with secondary biological treatment (activated sludge) are reported. The number and type of PFAS analyzed for each study are also included in the table. In general, we found PFAS concentrations much higher than those reported in previous studies. For example, PFAS concentrations in effluent wastewater samples measured in our study were much higher (13.4–107 μg L⁻¹) than those found by Castiglioni et al. in municipal (15–27 ng L⁻¹) and industrial (205–1128 ng L⁻¹) serving the city of Milan (Italy) in three-year monitoring campaigns (2011–2013) (Castiglioni et al., 2015). Castiglioni et al. found PFOA and PFOS dominant among PFAS and poorly removed in all the WWTPs (removal rates were of 13–50 %) (Castiglioni et al., 2015). In our study we often found concentrations of PFOA and PFOS increasing from influent-to-effluent. EU-wide monitoring survey carried out in 2010 detected seven PFAS (mostly long-chain PFAS) in effluents from 90 European WWTPs at concentrations lower than those found in our study with PFHxA (304 ng L⁻¹) and PFOA (255 ng L⁻¹) the most abundant (Loos et al., 2013). PFAS measured in sewage treatment plants from Spain (Campo et al., 2014), Bavaria, Germany (Becker et al., 2010), Belgium (Jeong et al., 2022), and Baltic Sea region (Undeman et al., 2019; Lenka et al., 2021) showed lower concentrations in influent and effluent compared to those reported in our study. However, industrial wastewater from firefighting exercises located in France showed higher concentrations of PFAS (5.3 × 10⁶ to 1.2 × 10⁸ ng L⁻¹) than those reported in our study with fluorotelomers the most abundant followed by PFOS (Dauchy et al., 2019).

Chinese municipal WWTPs showed PFAS concentrations much lower than those found in our study in both influent and effluent, with PFBA often predominant (Jiang et al., 2023; Mu et al., 2022). In our study, we found PFPeA dominant in influent and PFBS and PFOS mainly dominant in effluents. The levels of PFAS in wastewater from 77 different industrial plants in Korea were also lower in both influent and effluent than those found in our study (Kim et al., 2021).

PFAS contamination in African WWTPs was much lower than those found in our study, with PFBA and PFHxS the most abundant in influent and PFBA the most abundant in effluent (Jiang et al., 2023). In Australian wastewater, PFAS levels were lower with PFPeA, PFHxA, PFHpA, PFOA, PFNA, and PFDA increasing significantly between influent and final effluent (Coggan et al., 2022). PFAS contamination was lower than those found in WWTPs in Michigan (Helmer et al., 2022) and in Southeast United States (Kim et al., 2022), showing in both cases an increase of concentration across WWTPs after biological treatments (Helmer et al., 2022; Kim et al., 2022).

For sludge, the concentration of PFOA found in our study was much lower than those reported in previous studies (see Fig. S2) carried out in Spain through 2010 (Campo et al., 2014), in Australia throughout 2017 (Coggan et al., 2022) in Sweden through 2004–2016 (Fredriksson et al., 2022), in Southeast United State during 2020–2021 (Kim et al., 2022), and in China during 2015–2016 (Jiang et al., 2023). Comparable values of PFOA contamination were found in sludge from African WWTPs (South Sudan, Tanzania, and Kenya) during 2015–2016 (Jiang et al., 2023).

5. Conclusion

In this study, twenty-five target PFAS were monitored in influent and effluent wastewater from four municipal WWTPs located in the North of Italy (Milan). Influent wastewater was mainly dominated by PFPeA (23 %) followed by PFHxS (14 %) and NMeFOSAA (13 %). PFOA and PFOS and other short-chain PFAS (PFHxA, PFBS, PFBA and PFHpA) were <8 %. Effluent wastewater was dominated by PFBS (26 %) and PFOS (21 %). PFHxA, PFBA, PFPeA, PFHpA, NMeFOSAA, and PFOS dominated by <10 %. PFAS concentrations were higher than those in urban wastewater and of the same order of magnitude in industrial wastewater previously reported. Shorter chain PFAS concentrations were higher than long-chain concentrations in effluents, and for some PFAS, higher concentrations have been monitored in effluents than in influent indicating that biotransformation of PFAS precursors occurs during biological treatments. Secondary biological treatment seems to be not effective in removing PFAS from wastewater, and MBR are even less effective. WWTP with shorter hydraulic retention times showed lower removal efficiency (–82–45 %). Seasonal variation of PFAS in influent and effluent appears rather low and more likely due to pulse release instead of seasonal factors.

CRedit authorship contribution statement

Benedetta Giannelli Moneta: Methodology, Investigation, Writing – original draft. **Maria Luisa Feo:** Writing – original draft, Investigation. **Marco Torre:** Data curation, Visualization. **Patrizio Tratzi:** Formal analysis. **Sara Elsa Aita:** Investigation, Methodology, Validation. **Carmela Maria Montone:** Investigation, Methodology, Validation. **Enrico Taglioni:** Investigation, Methodology. **Silvia Mosca:** Data curation. **Catia Balducci:** Writing – review & editing. **Marina Cerasa:** Writing – review & editing. **Ettore Guerriero:** Resources. **Francesco Petracchini:** Project administration, Funding acquisition. **Chiara Cavaliere:** Methodology, Validation, Writing – review & editing. **Aldo Laganà:** Supervision. **Valerio Paolini:** Conceptualization, Writing – review & editing, Supervision.

Data availability

Data will be made available on request.

Declaration of competing 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.165089>.

References

- 3M, 1999. Fluorochemical Use, Distribution and Release Overview; U.S. EPA Docket AR-226-0550; St. Paul, MN.
- Ahrens, L., 2011. Polyfluoroalkyl compounds in the aquatic environment: a review of their occurrence and fate. *J. Environ. Monit.* 13, 20–31. <https://doi.org/10.1039/C0EM000373E>.
- Arvaniti, O.S., Stasinakis, A.S., 2015. Review on the occurrence, fate and removal of perfluorinated compounds during wastewater treatment. *Sci. Total Environ.* 524, 81–92. <https://doi.org/10.1016/j.scitotenv.2015.04.023>.
- Attanasio, R., 2019. Sex differences in the association between perfluoroalkyl acids and liverfunction in US adolescents: analyses of NHANES 2013–2016. *Environ. Pollut.* 254, 113061. <https://doi.org/10.1016/j.envpol.2019.113061>.
- Becker, A.M., Suchan, M., Gerstmann, S., Frank, H., 2010. Perfluorooctanoic acid and perfluorooctane sulfonate released from a waste water treatment plant in Bavaria, Germany. *Environ. Sci. Pollut. Res.* 17, 1502–1507. <https://doi.org/10.1007/s11356-010-0335-x>.
- Blake, B.E., Pinney, S.M., Hines, E.P., Fenton, S.E., Ferguson, K.K., 2018. Associations between longitudinal serum perfluoroalkyl substance (PFAS) levels and measures of thyroid hormone, kidney function, and body mass index in the Fernald Community Cohort. *Environ. Pollut.* 242, 894–904.
- Boucher, J.M., Cousins, I.T., Scheringer, M., Hungerbühler, K., Wang, Z., 2018. Toward a comprehensive global emission inventory of C4–C10 perfluoroalkanesulfonic acids (PFASs) and related precursors: focus on the life cycle of C6- and C10-based products. *Environ. Sci. Technol. Lett.* 6, 1–7. <https://doi.org/10.1021/acs.estlett.8b00531>.
- Boulanger, B., Vargo, J.D., Schnoor, J.L., Hornbuckle, K.C., 2005. Evaluation of perfluorooctane surfactants in a wastewater treatment system and in a commercial surface protection product. *Environ. Sci. Technol.* 39, 5524–5530. <https://doi.org/10.1021/es050213u>.
- 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 Leeuwenk, S.P.J., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. *Integr. Environ. Assess. Manag.* 7, 513–541. <https://doi.org/10.1002/ieam.258>. *Critical Review*.
- Campo, J., Masiá, A., Picó, Y., Farré, M., Barceló, D., 2014. Distribution and fate of perfluoroalkyl substances in Mediterranean Spanish sewage treatment plants. *Sci. Total Environ.* 472, 912–922. <https://doi.org/10.1016/j.scitotenv.2013.11.056>.
- Castiglioni, S., Valsecchi, S., Polesello, S., Rusconi, M., Melis, M., Palmiotti, M., Manenti, A., Davoli, E., Zuccato, E., 2015. 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.* 282, 51–60. <https://doi.org/10.1016/j.jhazmat.2014.06.007>.
- Clara, M., Scharf, S., Weiss, S., Gans, O., Scheffknecht, C., 2008. Emissions of perfluorinated alkylated substances (PFAS) from point sources - identification of relevant branches. *Water Sci. Technol.* 58, 59–66. <https://doi.org/10.1016/wst.2008.641> *Open access article*.
- Coggan, T.L., Moodie, D., Kolobaric, A., Szabo, D., Shimeta, J., Crosbie, N.D., Lee, E., Fernandes, M., Clarke, B.O., 2022. An investigation into per- and polyfluoroalkyl substances (PFAS) in nineteen Australian wastewater treatment plants (WWTPs). *Heliyon* 5, e02316. <https://doi.org/10.1016/j.heliyon.2019.e02316>.
- Cookson, E.S., Detwiler, R.L., 2022. Global patterns and temporal trends of perfluoroalkyl substances in municipal wastewater: a meta-analysis. *Water Res.* 221, 118784. <https://doi.org/10.1016/j.watres.2022.118784>.
- Dauchy, X., Boiteux, V., Colin, A., Bach, C., Rosin, C., Muno, J.F., 2019. Poly- and perfluoroalkyl substances in runoff water and wastewater sampled at a firefighter training area. *Arch. Environ. Contam. Toxicol.* 76, 206–221. <https://doi.org/10.1007/s00244-018-0585-z>.
- ECHA, 2019. Member State Committee support document for identification of perfluorobutane sulfonic acid and its salts as substances of very high concern according to Articles 57 and 59 of Regulation (EC) 1907/2006. <https://echa.europa.eu/documents/10162/891ab33d-d263-cc4b-0f2d-d84cfb7f424a>.
- ECHA, 2022. Member State Committee support document for identification of perfluoroheptanoic acid and its salts as a substance of very high concern under Articles 57 and 59 of Regulation (EC) 1907/2006. <https://echa.europa.eu/documents/10162/0eb7e7f6-a1a1-831e-4d0d-e13c1b13c28a>.
- EFSA Panel on Contaminants in the Food Chain, 2020. Public consultation on the draft scientific opinion on the risks to human health related to the presence of perfluoroalkyl substances in food. European Food Safety Authority, Parma, Italy <https://www.efsa.europa.eu/en/consultations/call/public-consultation-draft-scientific-opinion-risks-human-health>.
- Fabbi, R., Montagna, M., Balbi, T., Raffo, E., Palumbo, F., Canesi, L., 2014. Adaptation of the bivalve embryotoxicity assay for the high throughput screening of emerging contaminants in *Mytilus galloprovincialis*. *Mar. Environ. Res.* 99, 1–8. <https://doi.org/10.1016/j.marenvres.2014.05.007>.
- Fredriksson, F., Eriksson, U., Kärman, A., Yeung, L.W.Y., 2022. Per- and polyfluoroalkyl substances (PFAS) in sludge from wastewater treatment plants in Sweden — first findings of novel fluorinated copolymers in Europe including temporal analysis. *Sci. Total Environ.* 846, 157406. <https://doi.org/10.1016/j.scitotenv.2022.157406>.
- Gagliano, E., Falciglia, P.P., Vagliasindi, F.G.A., Roccaro, P., 2020. Removal of poly- and perfluoroalkyl substances (PFAS) from water by adsorption: role of PFAS chain length, effect of organic matter and challenges in adsorbent regeneration. *Water Res.* 171, 115381.
- Gago-Ferrero, P., Gros, M., Ahrens, L., Wiberg, K., 2017. Impact of on-site, small and large scale wastewater treatment facilities on levels and fate of pharmaceuticals, personal care products, artificial sweeteners, pesticides, and perfluoroalkyl substances in recipient waters. *Sci. Total Environ.* 601, 1289–1297. <https://doi.org/10.1016/j.scitotenv.2017.05.258>.
- Gaines, L.G.T., 2022. Historical and current usage of per- and polyfluoroalkyl substances (PFAS): a literature review. *Am. J. Ind. Med.*, 1–26 <https://doi.org/10.1002/ajim.23362>.

- Genser, B., Teles, C.A., Barreto, M.L., Fischer, J.E., 2015. Within- and between-group regression for improving the robustness of causal claims in cross-sectional analysis. *Environ. Health* 14, 60. <https://doi.org/10.1186/s12940-015-0047-2>.
- Guerra, P., Kim, M., Kinsman, L., Ng, T., Aace, M., Smyth, S.A., 2014. Parameters affecting the formation of perfluoroalkyl acids during wastewater treatment. *J. Hazard. Mater.* 272, 148–154.
- Hamid, H., Li, L., 2016. Role of wastewater treatment plant (WWTP) in environmental cycling of poly- and perfluoroalkyl (PFAS) compounds. *Ecocycles* 2, 43–53. <https://doi.org/10.19040/ecocycles.v2i2.62>.
- Hamid, H., Li, L.Y., Grace, J.R., 2020. Aerobic biotransformation of fluorotelomer compounds in landfill leachate-sediment. *Sci. Total Environ.* 713, 136547. <https://doi.org/10.1016/j.scitotenv.2020.136547>.
- Helmer, R.W., Reeves, D.M., Cassidy, D.P., 2022. Per- and polyfluorinated alkyl substances (PFAS) cycling within Michigan: contaminated sites, landfills and wastewater treatment plants. *Water Res.* 210, 117983. <https://doi.org/10.1016/j.watres.2021.117983>.
- Jeong, Y., Da Silva, K.M., Iturraspe, E., Fujii, Y., Boogaerts, T., van Nuijs, A.L.N., Koelmel, J., Covaci, A., 2022. Occurrence and contamination profile of legacy and emerging per- and polyfluoroalkyl substances (PFAS) in Belgian wastewater using target, suspect and non-target screening approaches. *J. Hazard. Mater.* 437, 129378. <https://doi.org/10.1016/j.jhazmat.2022.129378>.
- Ji, K., Kim, Y., Oh, S., Ahn, B., Jo, H., Choi, K., 2008. Toxicity of perfluorooctane sulfonic acid and perfluorooctanoic acid on freshwater macroinvertebrates (*Daphnia magna* and *Moina macropora*) and fish (*Oryzias latipes*). *Environ. Toxicol. Chem.* 27, 2159–2168. <https://doi.org/10.1897/07-523.1>.
- Jiang, L., Yao, J., Sheng, N., Guo, Y., Dai, J., Pan, Y., 2023. Comprehensive profiles of per- and polyfluoroalkyl substances in Chinese and African municipal wastewater treatment plants: new implications for removal efficiency. *Sci. Total Environ.* 857, 159638. <https://doi.org/10.1016/j.scitotenv.2022.159638>.
- Kim, K.Y., Ndabambi, M., Choi, S., Oh, J.E., 2021. Legacy and novel perfluoroalkyl and polyfluoroalkyl substances in industrial wastewater and the receiving river water: temporal changes in relative abundances of regulated compounds and alternatives. *Water Res.* 191, 116830.
- Kim, J., Xin, X., Mamo, B.T., Hawkins, G.L., Li, K., Chen, Y., Huang, Q., Huanga, C.-H., 2022. Occurrence and fate of ultrashort-chain and other per- and polyfluoroalkyl substances (PFAS) in wastewater treatment plants. *Environ. Sci. Technol. Water* 2, 1380–1390.
- Kotthoff, M., Müller, J., Jüriling, H., Schlummer, M., Fiedler, D., 2015. Perfluoroalkyl and polyfluoroalkyl substances in consumer products. *Environ. Sci. Pollut. Res. Int.* 22, 14546–14549. <https://doi.org/10.1007/s11356-015-4202-7>.
- Kraff, M.P., Riess, J.G., 2015. Per- and polyfluorinated substances (PFASs): environmental challenges. *Curr. Opin. Colloid Interface Sci.* 20, 192–2012. <https://doi.org/10.1016/j.cocis.2015.07.004>.
- Lee, J.E., Choi, K., 2017. Perfluoroalkyl substances exposure and thyroid hormones in humans: epidemiological observations and implications. *Ann. Pediatr. Endocrinol. Metab.* 22, 6–14. <https://doi.org/10.6065/apem.2017.22.1.6>.
- Lee, J.W., Choi, K., Park, K., Seong, C., Yu, S.D., Kim, P., 2019. Adverse effects of perfluoroalkyl acids on fish and other aquatic organisms: a review. *Sci. Total Environ.* 707, 135334. <https://doi.org/10.1016/j.scitotenv.2019.135334>.
- Lenka, S.P., Kah, M., Padhye, L.P., 2021. A review of the occurrence, transformation, and removal of poly- and perfluoroalkyl substances (PFAS) in wastewater treatment plants. *Water Res.* 199, 117187. <https://doi.org/10.1016/j.watres.2021.117187>.
- Lenka, S.P., Kah, M., Padhye, L.P., 2022. Occurrence and fate of poly- and perfluoroalkyl substances (PFAS) in urban waters of New Zealand. *J. Hazard. Mater.* 428, 128257. <https://doi.org/10.1016/j.jhazmat.2022.128257>.
- Loos, R., Carvalho, R., Antó nio, D.C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Sveraes, K., Haglund, P., Fick, J., Lindberg, R.H., Schwesig, D., Gawlik, B.M., 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Res.* 47, 6475–6487. <https://doi.org/10.1016/j.watres.2013.08.024>.
- Lu, G.H., Liu, J.C., Sun, L.S., Yuan, L.J., 2015. Toxicity of perfluorononanoic acid and perfluorooctane sulfonate to *Daphnia magna*. *Water Sci. Eng.* 8, 40–48. <https://doi.org/10.1016/j.wse.2015.01.001>.
- Milinic, J., Lacorte, S., Rigol, A., Vidal, M., 2016. Sorption of perfluoroalkyl substances in sewage sludge. *Environ. Sci. Pollut. Res.* 23, 8339–8348. <https://doi.org/10.1007/s11356-015-6019-9>.
- Mu, H., Li, J., Chen, L., Hu, H., Wang, J., Gu, C., Zhang, X.X., Ren, H.Q., Wu, B., 2022. Distribution, source and ecological risk of per- and polyfluoroalkyl substances in Chinese municipal wastewater treatment plants. *Environ. Int.* 167, 107477. <https://doi.org/10.1016/j.envint.2022.107477>.
- New, L.S., Giardina, M., Anumol, T., Mavandadi, F., Gan, C.S., 2021. Targeted Quantitation of Legacy and Emerging Per- and Polyfluoroalkyl Substances (PFAS) in Water Using the Agilent 6470 Triple Quadrupole LC/MS System. *Agilent Application Note*. 25 Jun <https://www.agilent.com/cs/library/applications/an-targeted-quantitation-of-pfas-in-water-6470-lcmsms-5994-3600en-agilent.pdf>.
- Nielsen, C.J., 2017. Potential PFBS and PFHxS Precursors - literature study and theoretical assessment of abiotic degradation pathways leading to PFBS and PFHxS. <https://www.miljodirektoratet.no/globalassets/publikasjoner/M792/M792.pdf> University of Oslo.
- O'Connor, J., Bolan, N.S., Kumar, M., Nitai, A.S., Ahmed, M.B., Bolan, S.S., Vithanage, M., Rinklebe, J., Mukhopadhyay, R., Srivastava, P.B., Bhatnagar, A., Wang, H., Siddique, H.M., Kirkham, M.B., 2022. Distribution, transformation and remediation of poly- and per-fluoroalkyl substances (PFAS) in wastewater sources. *Process. Saf. Environ. Prot.* 164, 91–108. <https://doi.org/10.1016/j.psep.2022.06.002>.
- OECD, Organisation for Economic Co-operation and Development, 2011. OECD portal on perfluorinated chemicals. <https://www.oecd.org/chemicalsafety/portal-perfluorinated-chemicals/>.
- Oliaei, F., Kriens, D., Weber, R., Watson, A., 2013. PFOS and PFC releases and associated pollution from a PFC production plant in Minnesota (USA). *Environ. Sci. Pollut. Res.* 204 (20), 1977–1992. <https://doi.org/10.1007/S11356-012-1275-4> 2012.
- Paul, A.G., Jones, K.C., Sweetman, A.J., 2008. A first global production, emission, and environmental inventory for perfluorooctane sulfonate. *Environ. Sci. Technol.* 43, 386–392. <https://doi.org/10.1021/es802216n>.
- R Core Team, 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria URL <https://www.R-project.org>.
- Regulation (EU), 2019. 2019/1021 of the European Parliament and of the Council of 20 June 2019 on persistent organic pollutants. *Off. J. L* 169, 45–77 25.6. <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX:32019R1021>.
- Regulation (EU), 2020. 2020/784. Commission Delegated Regulation (EU) 2020/784 of 8 April 2020 amending Annex I to Regulation (EU) 2019/1021 of the European Parliament and of the Council as regards the listing of perfluorooctanoic acid (PFOA), its salts and PFOA-related compounds. *Off. J. L* 188, 1–3 15.6. <https://eur-lex.europa.eu/eli/reg/del/2020/784/oj>.
- Rotander, A., Kärman, A., Toms, L.M.L., Kay, M., Mueller, J.F., Gómez Ramos, M.J., 2015. Novel fluorinated surfactants tentatively identified in firefighters using liquid chromatography quadrupole time-of-flight tandem mass spectrometry and a casecontrol approach. *Environ. Sci. Technol.* 49, 2434–2442.
- Schaider, L.A., Balan, S.A., Blum, A., Andrews, D.Q., Strynar, M.J., Dickinson, M.E., Lunderberg, D.M., Lang, J.R., Peaslee, G.F., 2017. Fluorinated compounds in U.S. fast food packaging. *Environ. Sci. Technol. Lett.* 4, 105–111. <https://doi.org/10.1021/acs.estlett.6b00435>.
- Steenland, K., Tinker, S., Frisbee, S., Ducatman, A., Vaccarino, V., 2009. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. *Am. J. Epidemiol.* 170, 1268–1278. <https://doi.org/10.1093/aje/kwp279>.
- Sunderland, E.M., Hu, X.C., Dassuncao, C., Tokranov, A.K., Wagner, C.C., Allen, J.G., 2019. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. *J. Expo. Sci. Environ. Epidemiol.* 29, 131–147. <https://doi.org/10.1038/s41370-018-0094-1>.
- Szabo, D., Marchiandi, J., Samandra, S., Johnston, J.M., Mulder, R.A., Green, M.P., Clarke, B.O., 2023. High-resolution temporal wastewater treatment plant investigating to understand influent mass flux of per- and polyfluoroalkyl substances (PFAS). *J. Hazard. Mater.* 447, 130854. <https://doi.org/10.1016/j.jhazmat.2023.130854>.
- Thompson, K.A., Mortazavian, S., Gonzalez, D.J., Bott, C., Hooper, J., Schaefer, C.E., Dickenson, R.V., 2022. Poly-a and perfluoroalkyl substances in municipal wastewater treatment plants in the United States: seasonal patterns and meta-analysis of long-term trends and average concentrations. *ACS EST Water* 2, 690–700. <https://doi.org/10.1021/acsestwater.1c00377>.
- Udemann, E., Rasmussen, K., Kokorite, I., Leppanen, M.T., Larsen, M.M., Pazzro, K., Siedlewicz, G., 2019. Micropollutants in urban wastewater: large-scale emission estimates and analysis of measured concentrations in the Baltic Sea catchment. *Mar. Pollut. Bull.* 178, 113559. <https://doi.org/10.1016/j.marpolbul.2022.113559>.
- UNEP, 2019a. All POPs listed in the Stockholm Convention. <http://chm.pops.int/TheConvention/ThePOPs/AllPOPs/tabid/2509/Default.aspx>.
- UNEP, 2019b. Chemicals proposed for listing under the Convention. <http://chm.pops.int/TheConvention/ThePOPs/ChemicalsProposedforListing/tabid/2510/Default.aspx>.
- USEPA, 2012. Estimation Program Interface (EPI) Suite. Ver. 4.11. Nov. Available from, as of Dec 21, 2015 <https://www2.epa.gov/tsca-screening-tools>.
- USEPA, 2017. Basic Information about Per- and Polyfluoroalkyl Substances (PFASs). <https://www.epa.gov/pfas/basic-information-about-and-polyfluoroalkyl-substances-pfas#tab-1>.
- USEPA, 2019. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. United States Environmental Protection Agency, Washington, DC, USA.
- Vo, H.N.P., Ngo, H.H., Guo, W., Nguyen, T.M.H., Li, J., Liang, H., Deng, L., Chen, Z., Nguyen, T.A.H., 2020. Poly- and perfluoroalkyl substances in water and wastewater: a comprehensive review from sources to remediation. *J. Water Process. Eng.* 36, 101393. <https://doi.org/10.1016/j.jwpe.2020.101393>.
- Wang, Z.Y., MacLeod, M., Cousins, I.T., Scheringer, M., Hungerbühler, K., 2011. Using COSMOtherm to predict physicochemical properties of poly- and perfluorinated alkyl substances (PFASs). *Environ. Chem.* 8, 389–398. <https://doi.org/10.1071/EN10143>.
- Wang, N., Liu, J., Buck, R.C., Korzeniowski, S.H., Wolstenholme, B.W., Folsom, P.W., Sulecki, L.M., 2011b. 6:2 fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. *Chemosphere* 82, 853–858. <https://doi.org/10.1016/j.chemosphere.2010.11.003>.
- Wang, Z., Cousins, I.T., Scheringer, M., Hungerbühler, K., 2013a. Fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFASs) and their potential precursors. *Environ. Int.* 60, 242–248. <https://doi.org/10.1016/j.envint.2013.08.02>.
- Wang, S., Huang, Y., Yang, Y., Hui, Y., Ge, Y., Larssen, T., Yu, G., Deng, S., Wang, B., Harman, C., 2013b. First report of a chinese PFOS alternative overlooked for 30 years: its toxicity, persistence, and presence in the environment. *Environ. Sci. Technol.* 47, 10163–10170.
- Waterfield, G., Rogers, M., Grandjean, P., Auffhammer, M., Sunding, D., 2020. Reducing exposure to high levels of perfluorinated compounds in drinking water improves reproductive outcomes: evidence from an intervention in Minnesota. *Environ. Health* 19, 42. <https://doi.org/10.1186/s12940-020-00591-0>.
- Yi, S., Harding-Marjanovic, K.C., Houtz, E.F., Gao, Y., Lawrence, J.E., Nichiporuk, R.V., Iavarone, A.T., Zhuang, W.-Q., Hansen, M., Field, J.A., Sedlak, D.L., Alvarez-Cohen, L., 2018. Biotransformation of AFFF component 6:2 fluorotelomer thioether amido sulfonate generates 6:2 fluorotelomer thioether carboxylate under sulfate-reducing conditions. *Environ. Sci. Technol. Lett.* 5 (2018), 283–288. <https://doi.org/10.1021/acs.estlett.8b00148>.
- Yu, J., Hua, J., Tanakab, S., Fujiib, B., 2009. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in sewage treatment plants. *Water Res.* 43, 2399–2408. <https://doi.org/10.1016/j.watres.2009.03.009>.
- Yu, X., Nishimura, F., Hidaka, T., 2018. Effects of microbial activity on perfluorinated carboxylic acids (PFCAs) generation during aerobic biotransformation of fluorotelomer alcohols in activated sludge. *Sci. Total Environ.* 610–611, 776–785. <https://doi.org/10.1016/j.scitotenv.2017.08.075>.

- Zafeiraki, E., Costopoulou, D., Vassiliadou, I., Bakeas, E., Leondiadis, L., 2014. Determination of perfluorinated compounds (PFCs) in various foodstuff packaging materials used in the Greek market. *Chemosphere* 94, 169–176. <https://doi.org/10.1016/j.chemosphere.2013.09.092>.
- Zhang, C., Yan, H., Li, F., Hu, X., Zhou, Q., 2013. Sorption of short- and long-chain perfluoroalkyl surfactants on sewage sludges. *J. Hazard. Mater.* 260, 689–699. <https://doi.org/10.1016/j.jhazmat.2013.06.022>.
- Zhao, L., McCausland, P.K., Folsom, P.W., Wolstenholme, B.W., Sun, H., Wang, N., Buck, R.C., 2013. 6:2 Fluorotelomer alcohol aerobic biotransformation in activated sludge from two domestic wastewater treatment plants. *Chemosphere* 92, 464–470. <https://doi.org/10.1016/j.chemosphere.2013.02.032>.
- Zhou, Y., Wang, T., Jiang, Z., Kong, X., Li, Q., Sun, Y., Wang, P., Liu, Z., 2017. Ecological effect and risk towards aquatic plants induced by perfluoroalkyl substances: bridging natural to culturing flora. *Chemosphere* 167, 98–106. <https://doi.org/10.1016/j.chemosphere.2016.09.146>.