



## Estimation of *per*- and poly-fluoroalkyl substances mass loads in the Danube River using passive sampling



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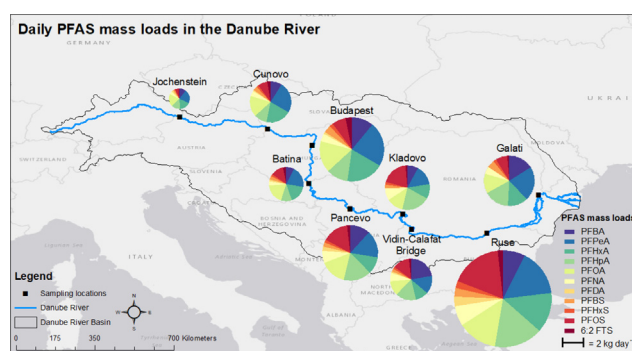
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### HIGHLIGHTS

- PFAS were measured in surface waters of the Danube River by LC-MS/MS.
- 11 PFAS were detected in the Danube River.  $\Sigma_{11}$ PFAS concentrations ranged 8–30 ng L<sup>-1</sup>.
- 4.9 kg of  $\Sigma_{11}$ PFAS were delivered to the Black Sea daily during Summer 2019.
- PFOS concentrations exceed the environmental quality standard at all sampling locations.
- Passive sampling can be used for ongoing monitoring of PFAS for which calibration data is known.

### GRAPHICAL ABSTRACT



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### ABSTRACT

As Europe's second longest river, the Danube is an important water source for drinking water and irrigation for many countries, before discharging into the Black Sea in the East. *Per*- and poly-fluoroalkyl substances (PFAS) have been observed over the last two decades in concentrations exceeding the European Union's drinking water guidelines for total sum of 20 select PFAS of 0.1 µg L<sup>-1</sup>. Their presence is a result of current and historical use and high environmental persistence, necessitating their monitoring for human risk assessments. The aim of this study is to use recently developed passive sampling technology to calculate time-integrated water concentrations and mass loads of 11 select PFAS at 9 sites along the Danube River. Results indicate  $\Sigma_{11}$  PFAS concentrations in the range of 9.3–29.6 ng L<sup>-1</sup> were not in exceedance of EU drinking water guidelines, but perfluorooctanesulfonic acid (PFOS) was in exceedance of the environmental quality standard (0.65 ng L<sup>-1</sup>) at all sampling locations. The highest  $\Sigma_{11}$  PFAS mass loads were observed at Ruse (9.5 kg day<sup>-1</sup>) and Budapest (6.3 kg day<sup>-1</sup>), believed to be driven by proximity to industrial facilities and large populations (urban runoff). Finally, we estimate 4.9 kg of total PFAS ( $\Sigma_{11}$  PFAS) were delivered to the Black Sea daily over Summer 2019.

### 1. Introduction

*Per*- and polyfluoroalkyl substances (PFASs) are a group of man-made chemicals that have been produced and used in a wide range of consumer

products and industrial applications since the 1950s (Buck et al., 2011). The toxicity and persistence of some PFAS compounds has led regulatory bodies to ban or highly regulate the production and use of several PFAS. Of the many thousands of PFAS compounds, perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexanesulfonic acid (PFHxS) have gained the most attention through addition to the Stockholm Convention on persistent organic pollutants (POPs), and subsequent heavy

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restrictions imposed by the European Union (EU) (EU, 2019; EU, 2020a; UNEP, 2021). Due to the chemical and thermal stability of the perfluoroalkyl moiety, PFAS are highly persistent in the environment and are ubiquitous in aquatic environments (Buck et al., 2011). Known point sources include industrial/manufacturing facilities, grounds where aerial firefighting foams (AFFFs) have historically been used, wastewater treatment plants (WWTPs), and landfills. Diffuse non-point sources are often an accumulation of multiple sources such as atmospheric deposition of volatile PFAS, degradation of precursor compounds and surface runoff (Kurwadkar et al., 2022).

The Danube River Basin (DRB) covers over 800,000 km<sup>2</sup> of land, and is an important source of drinking water and irrigation for a population of 83 million people (Liška et al., 2021). Implemented by the EU, one of the major aims of the European Water Framework Directive (WFD) is to achieve good water quality status for a list of 45 priority substances and an additional 15 watch list substances (EU, 2013; EU, 2018). Of these 60 compounds, PFOS is the only PFAS compound to make the list, with an environmental quality standard (EQS) set at <0.65 ng L<sup>-1</sup> to be considered “good” (EU, 2018). For other PFAS not mentioned in the WFD, in 2020 the European Commission introduced a maximum allowable sum of 20 select PFAS for drinking water of 0.1 µg L<sup>-1</sup> (EU, 2020b).

The emission and subsequent long-term contamination of PFAS across Europe has been widely reported in recent years (McLachlan et al., 2007; Loos et al., 2010; Clara et al., 2009; Loos et al., 2017; Ng et al., 2022; Valsecchi et al., 2015). With breakthroughs in analytical and sampling methodology our capabilities for providing an accurate assessment of the extent of these contaminants has rapidly increased. Environmental processes can influence the fate of organic contaminants such as PFAS including upstream and tributary inflows, tidal action, point and diffuse source emissions, atmospheric deposition, volatilization, removal to sediment, and chemical transformation (Álvarez-Ruiz et al., 2021). High PFOA concentrations have long been observed in European rivers, which has generally been attributed to fluoropolymer manufacturing and ammonium perfluorooctanoate (APFO) production. McLachlan et al. (2007) first observed PFOA concentrations of 16.4 ng L<sup>-1</sup> in the Danube River, compared to 200 ng L<sup>-1</sup> in Italy's Po River in the same study (McLachlan et al., 2007). This study of 14 major European rivers investigated water concentrations and estimated annual mass loads for four perfluoroalkyl carboxylic acids (PFCAs) across Europe. Total emissions were estimated at 18.22 t year<sup>-1</sup>; and the Danube was typically the second or third largest mass load contributor for each PFAS studied. McLachlan et al. highlights the limitations of calculating contaminant mass loads in rivers by single time point samples, however these limitations are typically outweighed by the insight into the relationship of environmental concentrations and emissions that can be achieved by assessing mass loads (McLachlan et al., 2007). More recent studies have detected PFOA and PFOS in the Danube and its tributaries at concentrations of up to 40 and 101 ng L<sup>-1</sup>, respectively (Loos et al., 2010; Loos et al., 2017). Most recently, Ng et al. (2022) published their findings on target and suspect screening of 4777 PFAS in surface waters of the Danube River. Though surface water concentrations were not directly reported, the study found the number of PFAS detected in river water decreased between upstream and downstream sites. Furthermore, Ng et al. (2022) identified PFAS are present in multiple environmental compartments (i.e., WWTP effluents, surface and groundwaters and biota) at threatening levels. Ng et al. concluded four PFAS compounds; PFOS, PFOA, PFHxS and (Perfluorododecyl)methyl oxirane, currently represent a high risk in river water (Ng et al., 2022).

Despite some restriction of use of certain PFAS, the legacy use of consumer products containing PFAS, their environmental persistence, and diffuse sources are likely to contribute to ongoing PFAS contamination of surface waters for years to come, highlighting the need for ongoing environmental monitoring for human and environmental risk assessment. The investigation of riverine mass loads can help to understand the relationship between the observed environmental concentrations and emissions (McLachlan et al., 2007). Episodic events such as accidental spills, industrial release, and runoff driven by rainfall are challenging to capture and may unknowingly influence active grab sampling (Kaserzon et al.,

2019a). For example, Müller et al. (2011) suggest the variation of PFAS concentration can increase by a factor of 2–4 following rain events (Müller et al., 2011). Passive sampling is a means of collecting a time-integrated sample which allows for a more representative assessment of chemical concentrations, particularly for chemicals of high variability over time and regardless of episodic weather events (Lai et al., 2019). Furthermore, the time-weighted average water concentrations derived from the use of passive sampling can alleviate some of the limitations highlighted by McLachlan et al. (2007) of calculating riverine mass loads. A microporous polyethylene tube (MPT) passive sampler has been successfully calibrated and validated in surface and groundwater for a broad range of PFAS with long-term deployment capability and sensitivity for a broad range of PFAS (Kaserzon et al., 2019b; Gardiner et al., 2022). The MPT sampler design has the benefit of a reduced effect of the water boundary layer between the sampler and sorbent medium. This leads to minimal impact on actual flow rate as the rate limiting step is the transfer of PFAS through the wall of the MPT sampler (Gardiner et al., 2022).

The aim of the present study was to use the recently developed MPT passive sampler calibrated to surface waters to calculate time-integrated water concentrations and mass loads of PFAS along the Danube River, to better understand the relationship between environmental concentrations and emissions. We report the concentrations and daily mass loads of 11 select PFAS at nine sampling sites by using passive sampling devices deployed in the Danube River during Summer 2019.

## 2. Materials and methods

### 2.1. Study area description

Duplicate MPT passive sampling devices were deployed in the Danube River surface water for a period of approx. 100 days between mid-June to late-August in 2019. Sites selected for this study were the same sampling sites used for passive sampling in the Joint Danube Survey 4 of 2019 (JDS4) shown in Fig. 1 and described in Table 1. MPT passive samplers were deployed alongside other passive sampling devices mentioned in the JDS4 survey scientific report (Vrana et al., 2021). Conductivity measurements across the sampling sites ranged from 351 µS cm<sup>-1</sup> (Jochenstein) to 415 µS cm<sup>-1</sup> (Pančevo) with percent coefficient of variation between sites of 6 % (Table S9).

### 2.2. Standards and reagents

All laboratory equipment and materials (e.g., glassware, scissors, tweezers) were thoroughly rinsed with acetone and methanol (Merck, Darmstadt, Germany; purity 99.8 %) and allowed to dry prior to use. Ammonium acetate (>97 %, C<sub>2</sub>H<sub>7</sub>NO<sub>2</sub>) was obtained from ChemSupply (Gillman, SA, Australia). Ammonium hydroxide solution (28–30 %, NH<sub>4</sub>OH) was obtained from Sigma Aldrich (Missouri, United States). Water with resistivity >18.2 MΩ cm (298 K) (MQ) was obtained from a Millipore system. All analytical standards were purchased from Wellington Laboratories (Guelph, Canada). A native standard mix was made up containing 47 PFAS parent compounds. Details of the PFAS standards including their acronyms and neutral molecular formulae together with relevant isotopically labelled internal standards and labelled instrument performance recovery standards are presented in Table S1.

### 2.3. Sampling and extraction using MPT passive sampling devices

MPT passive sampling devices were prepared and extracted using the same protocol described in Kaserzon et al. (2019b). Briefly, microporous polyethylene tubes of 2 mm thickness with pore size of 2.5 µm and 35 % porosity (Pall Corp, Germany) were cut to 4 cm lengths. Tubes were filled with 400 ± 10 mg Strata X-AW loose sorbent material (Phenomenex, Australia) and capped at both ends with 10 mm round plastic tubing inserts (STOCKCAP, Australia). At each site, samplers were deployed in surface water using open wire frame holders at a depth of approximately 1 m



**Fig. 1.** Deployment locations (black squares) of microporous polyethylene tube (MPT) sampling devices within the Danube River Basin. The main Danube channel is indicated by thick blue lines, tributaries indicated by thin blue lines.

below the water surface. Samplers were deployed from bridges, buoys or jetties hanging on ropes using buoys to keep the sampler holders afloat. Following exposure, samplers were transported in a cooled container to a storage facility at Masaryk University in Brno, Czech Republic, and stored in a freezer at  $-20\text{ }^{\circ}\text{C}$  until shipment by a fast courier service to the processing laboratory at the University of Queensland in Brisbane, Australia.

For extraction, MPT samplers and a laboratory blank were transferred to 15 mL centrifuge tubes and spiked with 10  $\mu\text{L}$  of labelled PFAS internal standard mix ( $0.2\text{ mg L}^{-1}$  solution) onto the external surface of each MPT. 4 mL of 0.2 % ammonia: methanol was added to each centrifuge tube with the sampler, then sonicated for 10 min. Methanol extracts were then transferred to a clean, pre-labelled centrifuge tube. The process of adding 4 mL 0.2 % ammonia: methanol to the original tube and sonication for 10 min was repeated two more times. The combined extracts were reduced to 1 mL under a stream of nitrogen then centrifuged (2580 RCF for 15 min,  $22\text{ }^{\circ}\text{C}$ ). Supernatant was transferred into a 2 mL polypropylene vial (Agilent Technologies, Australia) further evaporated to 200  $\mu\text{L}$ , reconstituted to 500  $\mu\text{L}$  using MQ water and spiked with 10  $\mu\text{L}$  of PFAS labelled instrument performance recovery standard mix ( $0.2\text{ mg L}^{-1}$  solution).

#### 2.4. Data modelling

By assuming the sampler operates in kinetic mode, water concentrations are derived from passive sampler concentrations using the formula:

$$C_w = \frac{C_s \times m_s}{R_s t} \quad (1)$$

**Table 1**

Sampling site locations as distance from the mouth of the river (km), the daily average discharge ( $\text{m}^3\text{ s}^{-1}$ ) and the period of deployment for MPT samplers (no. of days), reported in the Joint Danube Survey 4: Scientific Report (Liška et al., 2021).

Site	Jochenstein	Cunovo	Budapest	Batina	Pancevo	Kladovo	Vidin-Calafat Bridge	Ruse	Galati
Latitude and longitude	48° 31.240'N 13° 42.122'E	48° 31.240'N 13° 42.122'E	48° 31.240' 13° 42.122'E	48° 31.240'N 13° 42.122'E	48° 31.240'N 13° 42.122'E	48° 31.240'N 13° 42.122'E	48° 31.240'N 13° 42.122'E	48° 31.240'N 13° 42.122'E	48° 31.240'N 13° 42.122'E
Distance from mouth (km)	2205	1855	1632	1434	1154	926	796	490	152
Daily average discharge ( $\text{m}^3\text{ s}^{-1}$ )	1402	2171	2436	2276	3670	3643	3690	4270	6243
Deployment period (days)	101	103	105	104	104	104	104	104	102

where  $C_w$  is the concentration of the compound in water ( $\text{ng L}^{-1}$ ),  $C_s$  is the concentration of the compound in the MPT sampler ( $\text{ng g}^{-1}$ ),  $m_s$  is the mass of sorbent (g) in the sampler,  $R_s$  is the sampling rate ( $\text{L day}^{-1}$ ) and  $t$  is the time deployed (days). Sampling rates ( $R_s$ ) used in this study were derived from calibration studies in surface waters using the methods described in Gardiner et al. (2022) and are presented in Table S2. Not all PFAS included in the analytical method have  $R_s$  and as such, surface water concentrations and mass loads were only calculated for those 11 PFAS with available  $R_s$ .

Mean daily mass loads of PFAS at all sampling sites were calculated using passive sampler time-weighted average PFAS concentrations ( $\text{ng L}^{-1}$ ) and the average daily discharge, derived from the  $\text{m}^3\text{ s}^{-1}$  water flow rate reported in the JDS4 report (Liška et al., 2021) using the formula:

$$ML_d = C_w \times flow_d \quad (2)$$

where  $ML_d$  is the mean daily mass loads ( $\text{g day}^{-1}$ ),  $C_w$  is the concentration of the compound in water ( $\text{ng L}^{-1}$ ),  $flow_d$  is the average daily flow rate ( $\text{L day}^{-1}$ ). Due to the fluctuation in flow regime experienced over the year mass loads were calculated and expressed as an average daily rate. Hence, these results represent the average daily mass loads present in the Danube River over summer 2019.

All data analysis were performed using R v 4.1.0. For statistical analysis, missing values were replaced with the value equal to half of the limit of detection ( $0.05\text{ ng L}^{-1}$  in all cases). Shapiro-Wilk test confirmed the data are normally distributed ( $p > 0.05$ ), so Pearson Correlation was performed. A principal components analysis of mean PFAS concentrations scaled to unit variance was conducted to aid data interpretation (Fig. S1).

## 2.5. LC-MS/MS analysis

Samples were analysed by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) using a Nexera HPLC (Shimadzu Corp., Kyoto, Japan) coupled to 6500 QQQ mass spectrometer (Sciex, Melbourne, Australia) with an electrospray ionization (ESI) interface operating in negative ion mode. Chromatographic separation was performed with a Kinetex EVO C18 (2.6  $\mu\text{m}$  particle size,  $100 \times 2.1$  mm; Phenomenex) HPLC column at 50 °C. A pre-injection column (Kinetex EVO C18, 5  $\mu\text{m}$  particle size,  $30 \times 2.1$  mm) was used after the mobile phase mixing chamber to delay elution of any solvent-derived background PFAS contamination. Mass spectrometry parameters and chromatographic conditions are summarised in Tables S3 and S4. Using isotope dilution, an eight-point calibration curve was made ranging from 0.1 to 100 ng mL<sup>-1</sup>. Data acquisition and processing was carried out using MultiQuant™ software (Sciex).

## 2.6. Quality control

Laboratory blank and field blank MPT samplers were prepared, extracted, and analysed in parallel with the deployed samplers. The laboratory blank was kept stored in the laboratory where MPTs were prepared and extracted, the field blank was carried in the field during deployment and retrieval but was kept in MQ water. The instrument limit of detection (LODs) was calculated by multiplying 3 times the standard deviation obtained from injecting the lowest calibrant standard 8 times, limit of quantification (LOQ) was calculated by multiplying 10 times the standard deviation obtained from injecting the lowest calibrant standard 8 times. Instrument LODs and LOQs are summarised in Table S5. Extraction recovery of target analytes were calculated by using PFAS labelled internal standards (IS) that were spiked prior to extraction. A non-extracted side spike (NESS) referring to a vial that is spiked with the same volume of internal standards and has the same solvent: water ratio was prepared in parallel to spiking and extracting deployed samplers. The NESS was used to provide information on the concentration of internal standards expected (i.e., representing 100 % of IS spiked) without matrix influences to better account for matrix effect and signal suppression. Extraction recoveries were mostly within the range of 30–120 % (Table S6), samples with recoveries outside of this range are considered semi-quantitative (Taverniers et al., 2004). One duplicate from the Kladovo site, was excluded due to unacceptable extraction recoveries (<5 %) and large coefficient of variation (>100 %) between replicates.

## 3. Results and discussion

### 3.1. PFAS water concentrations

Time averaged water concentrations ( $C_w$ ) of 11 PFAS compounds over approx. 100-day deployment period were calculated using Eq. (1) (Table 2). Two PFAS other than the 11 reported PFAS were detected in some duplicate samplers >LOD. These were PFTeDA ( $2.4 \pm 1.3$  and  $1.9 \pm 0.1$  ng sampler<sup>-1</sup> detected at Budapest and Batina, respectively) and FOA ( $2.2 \pm 0.14$  ng sampler<sup>-1</sup> detected at Batina). Because of the low detection rates these compounds will not be discussed any further. Ten PFAS (i.e., PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFBS, PFHxS, PFOS and 6:2 FTS) were detected at all nine sampling sites, whereas PFDA was only detected above the LOD at 7 sites. The highest  $\Sigma_{11}$ PFAS concentrations were observed at Budapest and Ruse at 29.6 and 25.5 ng L<sup>-1</sup>. These sites also had the highest sum of PFAS listed on the Stockholm Convention (i.e., PFOA, PFHxS and PFOS) at 7.4 and 8.4 ng L<sup>-1</sup>, accounting for 25 and 33 % of the observed  $\Sigma_{11}$ PFAS concentrations, respectively. In comparison to previous Joint Danube Survey campaigns in 2007 (JDS2) and 2013 (JDS3), a reduction in PFOA and PFOS concentrations were observed along the course of the river, most noticeably at Budapest where a maximum of 4.8 ng L<sup>-1</sup> PFOA was observed (in comparison to 30–40 ng L<sup>-1</sup> previously reported) (Loos et al., 2010; Loos et al., 2017). In these same studies, authors reported considerably higher maximum

**Table 2**  
Time weighted average PFAS concentrations (ng L<sup>-1</sup>) of 11 select PFAS, with totals for Stockholm Convention listed PFAS and Total PFAS. Values are mean water concentration  $\pm$  standard deviation. Where microporous polyethylene tube (MPT) sampler extracts were <limit of quantification (LOQ), the water concentration equivalent of the LOQ was calculated and is indicated as <C<sub>w</sub> LOQ.

Site	PFAS										Totals		
	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFBS	PFHxS	PFOS	6:2 FTS	$\Sigma$ PFOA, PFHxS and PFOS	$\Sigma_{11}$ PFAS
Jochenstein	1.7 $\pm$ 0.1	3.6 $\pm$ 0.4	2.8 $\pm$ 0.7	2.4 $\pm$ 0.6	3.2 $\pm$ 1.0	0.8 $\pm$ 0.2	0.5	0.4 $\pm$ 0.1	0.2 $\pm$ 0.1	0.9 $\pm$ 0.4	0.2 $\pm$ 0.1	4.3	16.7
Cunovo	1.9 $\pm$ 0.5	5.2 $\pm$ 1.5	4.2 $\pm$ 1.2	2.2 $\pm$ 1.0	3.5 $\pm$ 1.1	0.9 $\pm$ 0.3	0.2 $\pm$ 0.1	0.7 $\pm$ 0.2	0.3 $\pm$ 0.1	1.6 $\pm$ 0.1	0.5 $\pm$ 0.1	5.4	21.2
Budapest	3.2 $\pm$ 0.3	6.7 $\pm$ 1.0	5.6 $\pm$ 1.1	3.2 $\pm$ 0.3	4.8 $\pm$ 1.5	1.2 $\pm$ 0.1	0.3 $\pm$ 0.2	1.1 $\pm$ 0.2	0.5 $\pm$ 0.2	2.1 $\pm$ 0.9	0.9 $\pm$ 0.2	7.4	29.6
Batina	1.3 $\pm$ 0.2	3.4 $\pm$ 0.7	2.9 $\pm$ 0.8	1.7 $\pm$ 0.5	3.2 $\pm$ 1.1	0.7 $\pm$ 0.3	0.3 $\pm$ 0.1	0.6 $\pm$ 0.2	0.3 $\pm$ 0.1	2.1 $\pm$ 0.5	0.5 $\pm$ 0.1	5.6	17
Pancevo	2.0 $\pm$ 0.3	2.7 $\pm$ 1.0	1.7 $\pm$ 0.9	2.8 $\pm$ 1.2	2.6 $\pm$ 1.7	1.2 $\pm$ 0.7	0.4 $\pm$ 0.3	0.4 $\pm$ 0.2	0.3 $\pm$ 0.2	2.6 $\pm$ 2.0	0.3 $\pm$ 0.2	5.5	17
Kladovo	1.1	2.0	1.5	2.8	1.7	1.2	<0.1	0.4	0.3	2.7	0.2	4.7	13.9
Vidin-Calafat Bridge	2.8 $\pm$ 0.9	1.8 $\pm$ 0.8	1.3 $\pm$ 0.6	2.0 $\pm$ 0.9	1.4 $\pm$ 0.6	0.8 $\pm$ 0.4	0.2	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	1.7 $\pm$ 0.8	0.2 $\pm$ 0.1	3.3	12.7
Ruse	1.9 $\pm$ 0.8	4.0 $\pm$ 0.9	3.5 $\pm$ 0.8	4.1 $\pm$ 0.7	3.4 $\pm$ 1.1	1.7 $\pm$ 0.4	0.8 $\pm$ 0.1	0.7 $\pm$ 0.2	0.6 $\pm$ 0.2	4.4 $\pm$ 1.1	0.4 $\pm$ 0.2	8.4	25.5
Galati	1.5 $\pm$ 0.7	2.0 $\pm$ 0.1	1.2 $\pm$ 0.1	1.5 $\pm$ 0.1	1.2 $\pm$ 0.1	0.4 $\pm$ 0.1	<0.1	0.5 $\pm$ 0.1	0.2 $\pm$ 0.1	0.7 $\pm$ 0.1	0.1 $\pm$ 0.1	2.1	9.3

Due to higher variability between replicate samplers and poorer analytical recoveries observed at sites Jochenstein and Pancevo, these data should be treated with caution.

PFOS concentrations in Danube tributaries including the Jantra (57 ng L<sup>-1</sup>) and Arges (101 ng L<sup>-1</sup>) in the 2007 study (Loos et al., 2010). However, in 2013 these values decreased to approx. 8 and 6 ng L<sup>-1</sup> (Loos et al., 2017), indicating the levels of PFOA and PFOS in the Danube and its tributaries may have been decreasing for some time now. Although PFOS concentrations appear to be decreasing, the observed PFOS concentrations in the range of 0.7–4.4 ng L<sup>-1</sup> in the current study were in exceedance of the EQS for PFOS of 0.65 ng L<sup>-1</sup> at all nine sites.

Within the 11 selected PFAS, a high correlation (*Pearson R* > 0.8) across two groups was observed; the first group includes PFPeA, PFHxA, PFOA, PFBS and 6:2 FTS, the second group includes PFNA, PFHpA and PFOS (Fig. 2). These same groupings are present in a PCA, where PC1 and PC2 explain 81 % of the variance (Fig. S1). These groupings may indicate these PFAS are contributed to the Danube River by similar point or diffuse sources, or identify PFAS which are degradation products of precursors. For example, PFPeA and PFHxA are known biodegradation products of 6:2 FTS which could partially explain their grouping together (Méndez et al., 2022). Regarding Stockholm Convention listed PFAS, a moderate correlation exists between PFHxS and PFOS (*Pearson R* = 0.75), and PFHxS and PFOA (*Pearson R* = 0.65), which may indicate that there is an overlap between contributing sources of PFOS and PFHxS, and PFOA and PFHxS, but no significant correlation exists between PFOS and PFOA (*p* < 0.05) which indicates that there is little to no overlap in the sources of PFOA and PFOS contribution. To understand which point or diffuse sources these groups are associated with would require further targeted studies.

Presence of PFAS in surface waters often correlates to population size (McLachlan et al., 2007; Pistocchi and Loos, 2009; Gallen et al., 2014). Sites with the highest observed PFAS concentrations were all located downstream of populous cities; Budapest - 1.7 million inhabitants; Cunovo is downstream of Vienna and Bratislava – with a combined 2.4 million inhabitants, and Ruse - 166 thousand inhabitants. Due to the size of these populations and proximity to sampling locations, cities are likely to have contributed to the increased PFAS concentrations observed at these locations. In a previous study on PFAS emissions in the Danube River, Lindim et al. (2015) found human population alone cannot solely account for the observed PFAS. More accurate estimations for PFOA and PFOS emissions were obtained by modelling the combined contributions of population, local gross domestic product and WWTPs (Lindim et al., 2015).

Other common point sources for PFAS include WWTPs; industrial facilities for chemical manufacturing, paper and cardboard, textile mills and metal finishing; and locations where historical AFFFs have been used, particularly around military and commercial airports (Kurwadkar et al., 2022). Typical WWTP processes are unable to remove many PFAS compounds and commonly observe an increase in PFCAs and perfluoroalkane sulfonates (PFASs) between the influent and effluent, attributed to degradation of PFAS precursors such as fluorotelomer sulfonates (FTSs) and fluorotelomer alcohols (FTOHs) (Coggan et al., 2019; Eriksson et al., 2017). Alygizakis et al. (2019) characterized wastewater effluents in the Danube Basin for organic contaminants including nine PFAS (i.e., PFOS, PFHxS, PFOA, PFHxA, PFBS, PFDA, PFHpA, PFNA and PFHpS), observing several PFCAs and PFASs above 20 ng L<sup>-1</sup>, some nearing 50 ng L<sup>-1</sup> with direct flow into the Danube or its tributaries; indicating WWTPs remain a point source of PFAS in the Danube Basin (Alygizakis et al., 2019). Due to ongoing transformation of PFAS precursors to the stable PFCAs and PFASs in WWTPs, it is likely that low to mid-range concentrations of PFAS will continue to be observed in the Danube River as direct input from WWTPs.

### 3.2. PFAS mass loads

Daily mass loads for the study period were estimated based on the average concentration of PFAS and the mean flow rate at each of the nine sampling sites (Fig. 3, and Table S7). Similar to the observed trends in concentration at each site, the highest  $\Sigma_{11}$ PFAS mass loads were observed at Ruse, Budapest and Pancevo with 9.5, 6.3 and 5.4 kg day<sup>-1</sup>, respectively. The  $\Sigma_{11}$ PFAS mass loads observed at Ruse were more than double the median  $\Sigma_{11}$ PFAS mass loads of all sites, and a 2- to 3-fold increase for most PFAS from the previous site at Vidin-Calafat Bridge. This is likely due to industrial inputs from the industrial park on the bank of the Danube River at Ruse. PFBA was the only compound to decrease between these two sites possibly due to lack of contribution of this particular chemical from local sources.

The Sava River is the largest tributary to the Danube River, increasing the Danube's average flow rate by over 40 % at the confluence. Located 16 km downstream of the Sava confluence and adjacent to the city of Pancevo, a 2-fold increase in  $\Sigma_{11}$ PFAS mass load compared to the previous site Batina was observed, driven mainly by PFOS and PFHpA (Fig. 3). Characterization by Alygizakis et al. (2019) indicates some WWTPs discharge a significant amount of PFOS into the Sava (e.g., 29.4 ng L<sup>-1</sup> at Ljubljana) (Alygizakis et al., 2019), though this cannot account for all PFAS. Other likely contributors to the observed increase are the high number of industrial facilities located near Pancevo. >2-fold increase in  $\Sigma_{11}$ PFAS mass load between Ruse and the preceding site Vidin-Calafat Bridge, and near 2-fold decrease between the following site Galati suggests close proximity to point sources in Ruse, which is adjacent to an Industrial Park on the bank of the Danube River (Fig. 3).

Located 152 km upstream from the river mouth, sampling at Galati gives the most accurate representation of PFAS mass loads contributed to the Black Sea, as the Danube begins to branch into three distributaries 120 km from the mouth. Despite having the lowest observed PFAS concentrations of all nine sites, the fourth highest mean daily  $\Sigma_{11}$ PFAS mass load of 4.2 kg day<sup>-1</sup> was observed at Galati. These emissions are contributed mostly by PFCAs; PFPeA (26 %), PFBA (19 %), PFHxA (15 %) and PFOA (15 %); and 26 % of the  $\Sigma_{11}$ PFAS mass load (1.1 kg day<sup>-1</sup>) is contributed by Stockholm Convention listed PFAS (i.e., PFOA, PFHxS and PFOS) (Table S7). The decrease in observed mass loads for several PFAS between Ruse and Galati by nearly 2-fold is likely due to fewer sources of PFAS within the region which is mostly agricultural land. However, PFBA and PFBS maintain a similar load between the two sites. These PFAS may have been contributed from the common practice of biosolid application to agricultural lands, as had been observed to cause large scale PFAS plumes in agricultural fields in Germany (Röhler et al., 2021). Alternatively, Busch et al. (2010) found PFBA and PFBS represent approximately 50 % of PFAS present in landfill leachates collected in Germany, which may explain their presence (Busch et al., 2010). The Siret and Prut Rivers have confluence with the Danube after the site at Galati. However, this is unlikely to have

	PFDA																		
PFDA	1.00																		
PFHxS		1.00																	
PFOS			0.73	1.00															
PFHpA	0.69	0.81	0.85	1.00															
PFNA					1.00														
PFBA						1.00													
PFOA							1.00												
PFHxA								1.00											
PFPeA									1.00										
6:2 FTS										1.00									
PFBS											1.00								

Fig. 2. Pearson correlation matrix for time weighted average water concentrations across all 9 sites. Blank squares indicate the Pearson correlation was not significant (*p* < 0.05).

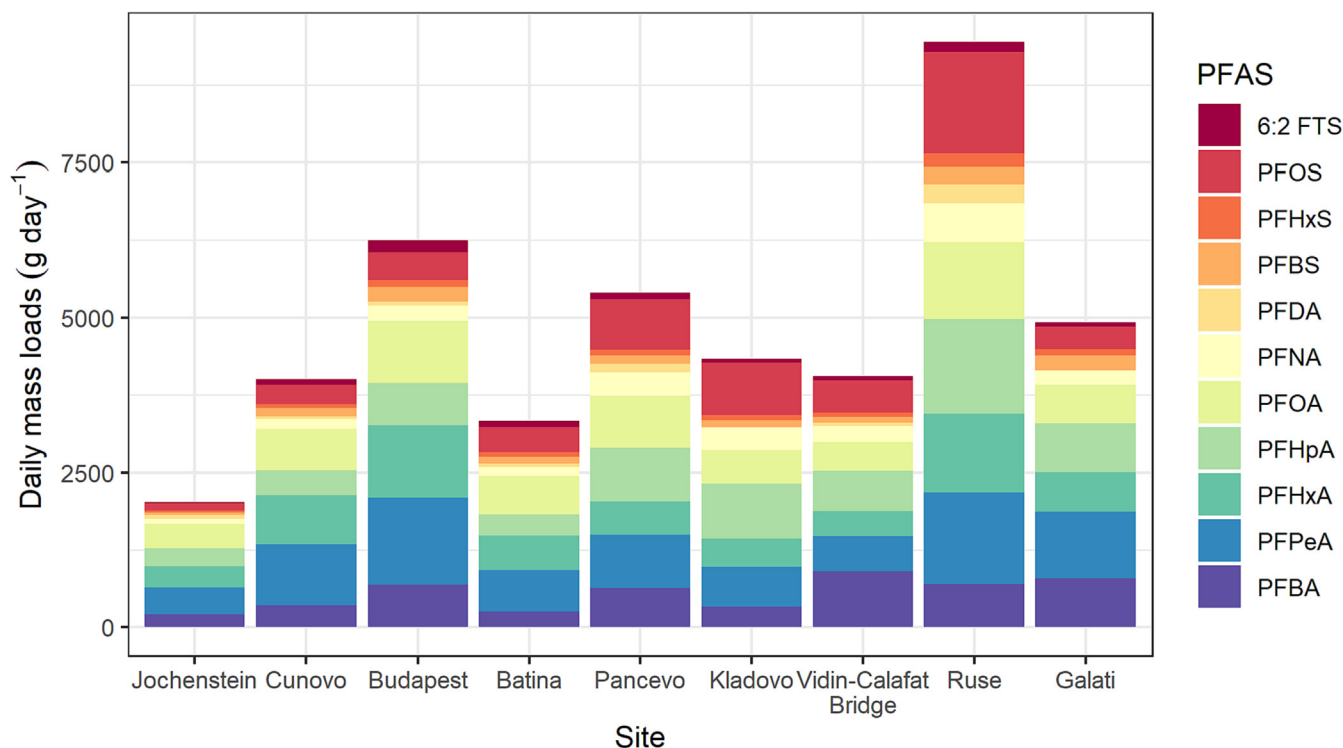


Fig. 3. Daily mass loads of 11 PFAS in the Danube River at nine sampling sites. Bars indicate the makeup of PFAS at each site, values are grams per day.

a strong influence on the observed PFAS concentrations or mass loads due to the comparatively low levels of PFOS and PFOA observed in these rivers in the previous JDS2 and JDS3 campaigns (Loos et al., 2017).

Mass loads of PFAS in the Danube River were first investigated by McLachlan et al. (2007) in a Europe-wide study that included 14 major rivers. The study estimated an annual mass load of 18.22 t year<sup>-1</sup> of 4 select PFCAs (i.e., PFHxA, PFHpA, PFOA and PFNA) per year were being discharged into receiving waters, with the Danube River responsible for approximately one quarter of this (approx. 4.5 t year<sup>-1</sup>) (McLachlan et al., 2007). Comparatively, the mass loads observed in the current study for  $\Sigma_{11}$ PFAS ranged from 0.7 t year<sup>-1</sup> at Jochenstein to 3.5 t year<sup>-1</sup> at Ruse. Clara et al. (2009) observed mass loads within a similar range as the current study for PFHxA (660 g day<sup>-1</sup>) and PFHpA (230–270 g day<sup>-1</sup>) in the Austrian stretch of the Danube. However, a 2 to 5-fold decrease for PFOS and a 4 to 8-fold decrease for PFOA were observed in the current study compared to Clara et al. (2009) (where 510–570 g day<sup>-1</sup> PFOS and 2900–3200 g day<sup>-1</sup> PFOA were observed) (Clara et al., 2009). Finally, Loos et al. (2017) reported much higher mass loads from the JDS3 campaign (2013) sampled near Galati of 0.47 and 0.54 t year<sup>-1</sup> of PFOA and PFOS, respectively. This was compared to the equivalent 0.23 and 0.14 t year<sup>-1</sup> of PFOA and PFOS observed in the current study (Loos et al., 2017). To our knowledge, no other studies have investigated riverine mass loads of PFAS in the Danube River.

While the focus of this study was on the presence of 11 select PFAS in the Danube River, environmental monitoring using passive sampling devices such as the MPT sampler could be useful to examine additional contaminants of emerging concern such as pharmaceuticals, personal care products and pesticides (Clokey et al., 2023), as well as in combination with non-target analytical approaches for a more comprehensive risk evaluation of environmental contaminants, similar to analysis of surface waters by Ng et al. (2022).

### 3.3. Limitations and uncertainties of passive sampling

Passive sampling offers added benefit to long term monitoring with lower detection limits achievable due to sorbent enrichment over time;

and ability to provide a time-weighted average concentration over the deployment period to negate any surge effects that may unknowingly influence grab sampling (Kaserzon et al., 2019a). Furthermore, the chosen MPT sampler is not strongly influenced by flow rate of surface waters, as the rate limiting factor is the diffusion of PFAS through the microporous polyethylene wall of the sampler. This reduces the effect of the water boundary layer between the wall of the sampler and the water (Gardiner et al., 2022). The MPT passive sampler employs a weak anion exchange sorbent, which is the equivalent sorbent used in standard methods for extraction of PFAS from water using SPE (Rosenblum and Wendelken, 2019). The sampler has proven to be effective for target PFAS with linear uptake over several weeks (Kaserzon et al., 2019a; Gardiner et al., 2022). The use of 400 mg of sorbent phase allows increased capacity for PFAS and linear uptake over several weeks to months including for short chain PFAS (i.e., PFBA, PFBS) (Kaserzon et al., 2019a).

However, it is also important to understand the limitations of passive sampling strategies. Major limitations can include representativeness of the model used to derive water concentration estimates from the passive sampler (i.e., in the kinetic or equilibrium sampling modes), as well as the placement of passive sampling devices in sub-optimal locations not necessarily representative of a well-mixed system. Site selection may not fully represent the entire river if sites chosen are not within a well-mixed homogeneous part of the river or are located directly within or near a point source. Though this is true for both active and passive sampling techniques, the misrepresentation is exacerbated by passive sampling due to the time enrichment factor. In this study, samplers were deployed at locations that were considered well mixed by local expert opinion.

To give confidence to the results obtained in the current study, comparison was made to grab water samples from the same sites in the JDS4 survey, reported to the NORMAN EMPODAT database (Network, 2023) and Ng et al. (2022). Where PFAS were present >LOD, the time integrated concentrations overlapped with the concentration ranges reported by both NORMAN and Ng et al. (2022) (Table S8). We quantified several PFAS above the LOD that NORMAN or Ng et al. (2022) did not. This can be attributed to the enrichment (>2 L of water) of the river water over prolonged period, making lower LODs achievable. Furthermore, the passive samplers

provide a consistent measuring technique in the Danube over the same period, allowing for comparative spatial results to be assessed.

#### 4. Conclusions

Despite the heavy restriction and subsequent reduction of some PFAS manufacturing and use, the low-level contamination of PFAS in surface waters is likely to continue to years to come. Two groups of strongly correlated PFAS concentrations in the Danube River indicate at least two different types of sources are responsible for PFAS pollution in the DRB. However, moderate correlation in concentrations observed between PFHxS and PFOS, and PFHxS and PFOA; but no correlation between PFOS and PFOA, may indicate that multiple and/or overlapping sources could be driving occurrences for Stockholm Convention listed PFAS. A decline in environmental PFOS, PFOA and PFHxS concentrations in the Danube have been observed over the last decade, which may be attributed to reduced use of these PFAS and their inclusion on the Stockholm Convention (Muir and Miaz, 2021). However, concentrations of PFOS exceeded the EQS of 0.65 ng L<sup>-1</sup> at all nine sites. Over the course of sampling in Summer 2019, an average of 4.9 kg day<sup>-1</sup> Σ<sub>11</sub>PFAS were contributed to the Black Sea from the Danube River. The continued use and improvement of passive sampling techniques for PFAS in environmental monitoring is a means of ongoing assessment of such contamination.

The use of passive sampling for deriving time-weighted average water concentrations is a means of collecting a representative sample for calculating mass load contributions of contaminants to water courses. Episodic events such as abnormal weather, accidental spills and industrial release are time-integrated to provide information on the “average” conditions. However, some challenges remain. Sub-optimal placement of passive sampling devices may lead to over- or under-estimation of true water concentrations. The later can be overcome by careful consideration of sampling sites. While the focus of this study was PFAS, this sampling approach using the MPT sampler or other passive sampler design can be a powerful tool in the assessment of contaminants of known or emerging concern.

#### CRedit authorship contribution statement

**Carly Beggs:** Writing – original draft, Visualization, Formal analysis, Conceptualization. **Rachel Mackie:** Validation, Writing – review & editing. **Branislav Vrana:** Methodology, Investigation, Writing – review & editing. **Roman Prokeš:** Methodology, Investigation. **Sara Ghorbani Gorji:** Writing – review & editing. **Bastian Schulze:** Writing – review & editing. **Kevin V. Thomas:** Writing – review & editing. **Jochen F. Mueller:** Conceptualization, Resources. **Sarit L. Kaserzon:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing.

#### 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.2023.164458>.

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