# Perfluoroalkyl acids (PFAAs): Distribution, trends and aquatic ecological risk assessment in surface water from Tagus River basin (Spain)[☆]

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## ABSTRACT

Rivers can receive the input of treated or untreated sewage effluents from wastewater treatment plants, urban and industrial discharges and agricultural run-off, becoming an important pathway for the transport and mobilization of pollutants to the oceans. In the present study, the occurrence of 20 PFAAs was determined in the water of Tagus River basin (Spain). PFAAs were detected in 76 out of 92 water samples collected during 5 years (2013−2018), being perfluorooctanesulfonic acid (PFOS) the predominant compound (<0.01−34 ng/L). The annual average PFOS concentrations (2.9−11 ng/L) detected in Tagus River were above the annual average environmental quality standards (AA-EQS) established in the Directive, 2013/39/EU (0.65 ng/L for inland surface waters) but below the maximum allowable concentration (MAC-EQS; 36000 ng/L). The levels of PFAAs detected in urban and industrial areas were statistically higher ($p < 0.01$) than those at background or remote areas. The mass flow rates amounted to <0.01−46 kg/y for PFOS and <0.01−22 kg/y for perfluorooctanoic acid (PFOA). A quantitative ecotoxicological risk assessment was conducted to evaluate the environmental potential risk related to PFAAs in the aquatic ecosystem. Risk characterization ratios ($RCR_{water}$, $RCR_{sed}$ and $RCR_{oral,\,fish}$) were below 1 in all cases.

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## 1. Introduction

Perfluoroalkyl acids (PFAAs) belong to a large group of synthetic amphiphilic compounds with unique properties that have been used in several industrial and commercial applications (Kissa, 2001). These emerging organic contaminants present persistence, toxicity, potential for bioaccumulation and remarkable ubiquity in the environment (Eriksson et al., 2016; Lam et al., 2017; Wang et al., 2017; Pan et al., 2018; De la Torre et al., 2019; Guisi et al., 2019). Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been included to Annex B (May 2009) and Annex A (May 2019), respectively, as persistent organic pollutants in the Stockholm Convention (POP, 2018, 2019). PFOS is also listed as substance of priority concern in the European Water FrameWork Directive (Directive, 2013/39/EU) due to its persistence, toxicity and widespread use and detection in rivers, lakes, transitional and coastal waters. This EU Directive controls the chemical quality of surface waters to protect the aquatic environment and human health and defines environmental quality standards (EQSs) for inland surface waters (rivers, lakes, related artificial or heavily modified water bodies), other surface waters and biota (crustaceans, molluscs or fishes).

Rivers can receive the input of treated or untreated sewage effluents from wastewater treatment plants, urban and industrial discharges and agricultural run-off, becoming an important pathway for the transport and mobilization of pollutants to the oceans (Martínez et al., 2010; Sun et al., 2011). Because of the high water solubilities of PFAAs, surface water is a significant medium for their long-range transport and widespread global distribution (Prevedouros et al., 2006; Niisoe et al., 2015; Zhao et al., 2015; Nguyen et al., 2017; Pan et al., 2018). The presence of PFAAs in surface waters could pose a risk to aquatic organisms, but also to humans through the consumption of contaminated freshwater fish (Hung et al., 2018; Fair et al., 2019) and tap water (Kaboré et al., 2018; Boone et al., 2019).

Under the leadership of the Spanish Ministry for the Ecological Transition, a Spanish monitoring program framed in the Stockholm

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Convention National Implementation Plan has been developed to evaluate the current status and temporal trends of PFOS in river water (PNA, 2019). Thus, a study area of high representativeness was selected and evaluated to reflect the ecological status of one of the main European watersheds: the Tagus River watershed in its Spanish section. The Tagus River is the longest river in the Iberian Peninsula and its watershed area includes some special sites that have been selected to ensure the long-term survival of Europe's most valuable and threatened species and habitats (Natura, 2000). Therefore the monitoring of the presence of emerging pollutants is mandatory to preserve the ecological quality of its different ecosystems.

Since surface water levels can exhibit some variations in PFAA levels (Liu et al., 2015; Munoz et al., 2019; Wang et al., 2019), sampling campaigns should be ideally repeated at the surveyed sites to account for temporal variability. Resampling can also allow to tentatively conclude on the compliance to annual EQS, but this can be difficult to implement due to logistics constraints. The present study aims to contribute to addressing the knowledge gaps, taking the Tagus River watershed as a case-study. Since PFAA concentrations can also vary spatially in water bodies (Loos et al., 2017; Gobelius et al., 2018) several sampling locations were targeted in the present survey along a 300-km tract of the river.

In this work, a characterization of the presence of 20 PFAAs during five years (23 different sampling campaigns) in Tagus River was performed, examining the compliance with EQS. Besides, a quantitative ecotoxicological risk assessment was conducted to establish the risk characterization ratios (RCRs) in the aquatic compartment and for secondary poisoning via the aquatic food chain, and then estimate the environmental potential risk related to the presence of PFAAs in river water.

## 2. Materials and methods

### 2.1. Study area

The Tagus River is the longest river (1007 km) in the Iberian Peninsula from its source in Montes Universales (Sierra de Albarracín, Teruel, Spain) until its mouth in the Atlantic Ocean (Mar da Palha, Lisboa, Portugal). Its watershed, with a total surface of 80600 km$^2$ serves more than 10 million inhabitants (124 hab/km$^2$). Its uses and demands include the supply of populations (domestic, public and commercial use, as well as small consumer industries connected to the network), the irrigation of crops and the use of water in livestock production and fish farms. The river flows through ecosystems with a rich flora and fauna considered as natural and conservation reserves, such as the Alto Tagus Natural Park (Guadalajara), the Monfragüe National Park (Cáceres), the International Tagus Natural Park (Cáceres -Spain- and Portugal) and the Tagus Estuary Natural Reserve (Portugal).

### 2.2. Sample collection

A total of 92 river water samples were collected in 23 different monitoring campaigns performed during five years (from February 2013 to August 2018). Details with regard to the sampling campaigns and locations are shown in Table S1 and Fig. 1 respectively. Four sampling points were selected to represent different typologies of the Tagus River watershed in its Spanish section: P1 is located in a remote area near the source (in the Alto Tagus Natural Park), P2 and P3 are located in urban and industrial areas (P2: metropolitan area of Madrid and P3: metropolitan area of Toledo) and P4 is located in a background reservoir next to the Portuguese border. The collection was carried out in clean, acetonitrile-water-methanol rinsed polypropylene (PP) bottles. The samples were

kept at 4 °C until their immediate extraction.

### 2.3. Chemical analysis

A total of 20 PFAAs, including perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), PFOS, perfluorodecanesulfonic acid (PFDS), perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA), perfluorooctanesulfonamide (FOSA), *N*-methyl perfluorooctane sulfonamide (*N*-MeFOSA) and *N*-ethyl perfluorooctanesulfonamide (*N*-EtFOSA), were the target analytes selected in the present study (Table S2).

The sample treatment was based on methodology reported previously (Navarro et al., 2011, 2018a). Briefly, water samples (2 L) spiked with MPFAC-MXA ($^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, $^{13}C_4$-PFBA, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUdA and $^{13}C_2$-PFDoA), *N*-d3-MeFOSA and *N*-d5-EtFOSA (Wellington Laboratories Inc.,Guelph, Canada) were extracted with Oasis WAX (500 mg, 6 mL; Waters, Milford, MA, USA) and purified with EnviCarb (500 mg, 6 mL; Sigma-Aldrich, St. Louis, MO, USA) cartridges. The final extracts spiked with $^{13}C_9$-PFNA solution (Wellington Laboratories Inc.,Guelph, Canada) were analyzed on a high performance liquid chromatography system (Varian HPLC 212) connected to a triple quadrupole mass spectrometer (Varian 320 MS-TQ) (see Supplementary material).

### 2.4. Quality assurance

Isotopic dilution method was used for identification and quantification if proper standards were available. Mean recoveries of isotopically labeled surrogate standards ranged between 64% and 88% (Table S3). Limits of quantification (LOQs), defined as the concentration giving a signal to noise ratio greater than 10 were in the range of 0.01–0.10 ng/L (Table S4). Blanks were conducted with each sampling site at each sampling campaign and extracted under the same conditions than samples. The most recurrent analytes in blank samples were PFHxA (0.81 ± 0.05 ng/L, mean ± SD, 73% of detection frequency), PFDA (0.04 ± 0.02 ng/L, 10%) and PFUdA (0.02 ± 0.01 ng/L, 8%) (Table S4). Blank correction was applied where blank levels were above LOQ. In addition, instrumental blanks consisting of methanol were run before each sample injection to check the possibility of cross-contamination from HPLC-MS/MS system.

### 2.5. Calculations and statistical evaluation

#### 2.5.1. Calculation of mass flow rate
Mass flow rates (F) of individual compounds (kg/y) were calculated according to:

$$F = C \ x \ Q \tag{1}$$

where C is the PFAA concentration detected in surface water (in kg/m$^3$) and Q is the mean daily flow (in m$^3$/y) measured at the time of sampling campaigns (Table S1).

#### 2.5.2. Calculation of the environmental exposure assessment parameters
The predicted environmental concentration (PEC) in surface water (PEC$_{water}$), the PEC for the sediment compartment (PEC$_{sed}$),

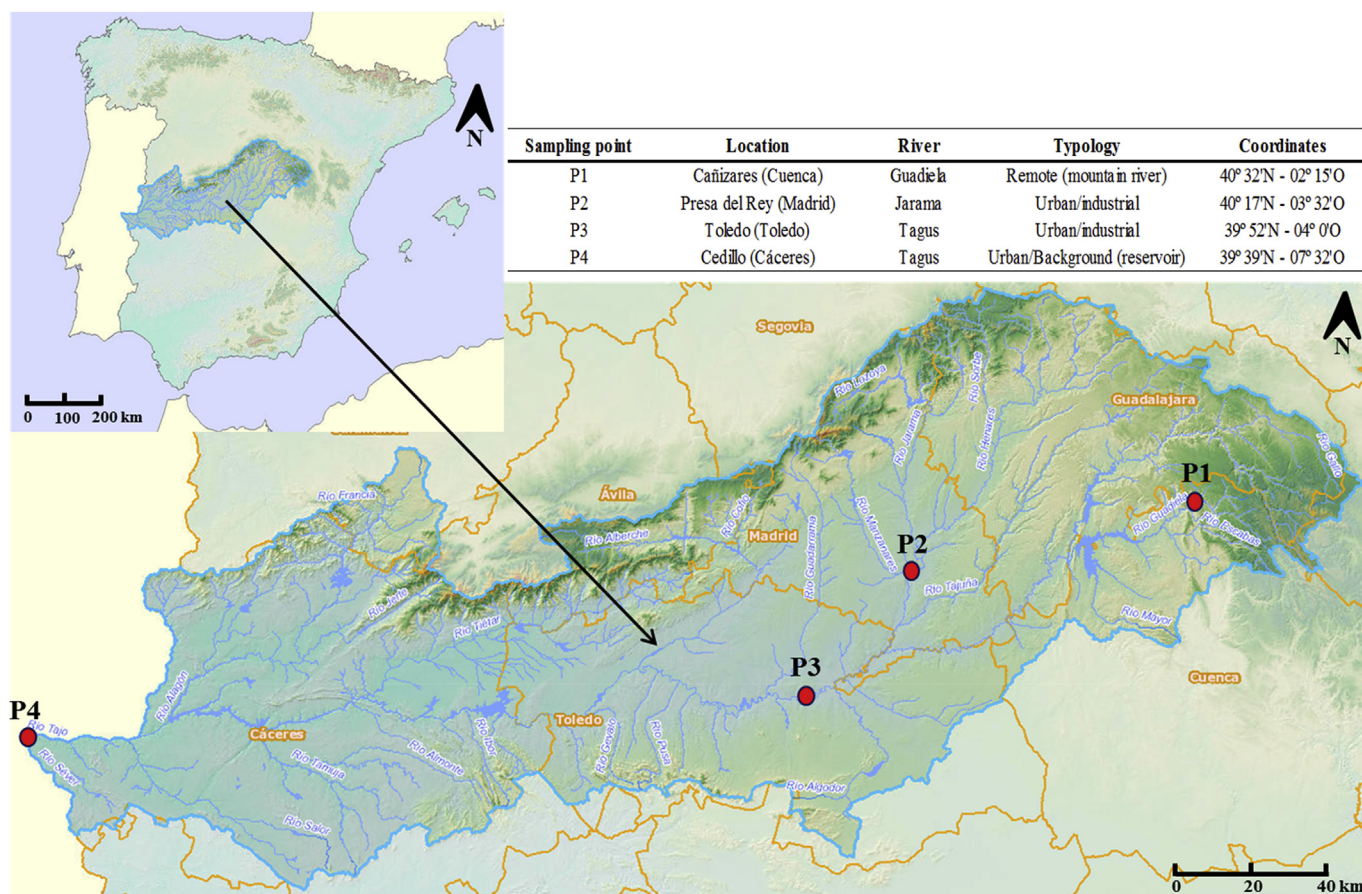| Sampling point | Location | River | Typology | Coordinates |
|---|---|---|---|---|
| P1 | Cañizares (Cuenca) | Guadiela | Remote (mountain river) | 40º 32'N - 02º 15'O |
| P2 | Presa del Rey (Madrid) | Jarama | Urban/industrial | 40º 17'N - 03º 32'O |
| P3 | Toledo (Toledo) | Tagus | Urban/industrial | 39º 52'N - 04º 0'O |
| P4 | Cedillo (Cáceres) | Tagus | Urban/Background (reservoir) | 39º 39'N - 07º 32'O |

**Fig. 1.** Geographical distribution of the sampling points along the Tagus River watershed.

the assessment of secondary poisoning via the aquatic food chain ($PEC_{oral,predator\,(Aq)}$), the predicted no effect concentration (PNEC) and the risk characterization ratio (RCR) were derived following the equations detailed in Supplementary material and previously described elsewhere (Navarro et al., 2018b). The parameters used in these calculations are detailed in Tables S9 and S10.

### 2.5.3. Statistical calculations

Statistical analyses were performed with the software SPSS 14.0 for Windows. Kruskal-Wallis Test was performed to evaluate differences between sampling points and campaigns. Relationships between compounds were assessed by Spearman Rho correlations.

## 3. Results and discussion

### 3.1. Distribution of PFAAs in Tagus River basin

PFAAs were detected in 76 out of 92 water samples. The mean PFAA levels found in the Tagus River basin were $1.7 \pm 1.2$ ng/L (mean ± SD) for PFBS, $4.4 \pm 2.9$ ng/L for PFHxS, $5.9 \pm 5.8$ ng/L for PFOS, $1.8 \pm 1.2$ ng/L for PFPeA, $2.0 \pm 2.5$ ng/L for PFHxA, $1.3 \pm 0.7$ ng/L for PFHpA, $3.6 \pm 2.6$ ng/L for PFOA, $0.7 \pm 0.5$ ng/L for PFNA, $0.8 \pm 0.6$ ng/L for PFDA and $15 \pm 13$ ng/L (Table 1, Fig. 2). Complete details of PFAA concentrations obtained at each location and sampling campaign are listed in Table S1, showing important differences between sampling points. From the 23 sampling campaigns conducted, only in 10 cases samples collected in P1 offered values > LOD, which is expected considering that P1 was located in a remote area near the source of the river, without pollution

contribution. Quantification frequency increased at P4 (83%) and reached maximum values for P2 and P3 (100%). Considering all data, PFOS was the most frequently quantified compound (71%), followed in decreasing order by PFOA (64%), PFHxA (50%), PFHxS (45%), PFPeA (45%), PFBS (43%), PFHpA (39%), PFDA (35%), PFNA (34%), PFBA (8%) and PFUdA (7%).

In general, contamination levels in the Tagus River basin were in agreement with others reported for European rivers (Table S5). The highest measured concentrations were for PFOS (<0.01–34 ng/L, min-max, Table 1) and PFHxS (<0.03–12 ng/L), being the PFOS levels (4.3 ng/L; median, Table 1) statistically higher ($p < 0.01$, Kruskal-Wallis Test) than PFBS values (1.5 ng/L). PFOS was also the predominant compound in river waters from France (9.9–39.7 ng/L, Labadie and Chevreuil, 2011b), Spain (0.94–58.1 ng/L, Picó et al., 2012) and Germany (<0.01–10.5 ng/L, Heydebreck et al., 2015). No statistically significant differences ($p > 0.05$, Kruskal-Wallis Test) were observed between PFOA (<0.01–11 ng/L) and shorter chain perfluoroalkyl carboxylic acids, PFCAs, (<0.02–13 ng/L). Long-chain PFCAs (C > 8) were detected with less frequency (34%, 35%, 7%, PFNA, PFDA and PFUdA, respectively) and at lower concentrations (<0.01–2.6 ng/L; Table 1). Besides, PFDS, PFDoA, PFTrDA, PFTeDA, PFHxDA and PFODA were not detected in any sample. This tendency has been previously observed in river water (Sun et al., 2011; Lorenzo et al., 2016; Pignotti et al., 2017). Samples obtained from P2, P3 and P4 sites showed a similar profile composition with a higher contribution of PFOS ($31 \pm 1\%$, mean ± SD for P2, P3 and P4) followed by PFOA ($22 \pm 4\%$), PFHxS ($19 \pm 5\%$), PFHxA ($16 \pm 4\%$), PFPeA ($11 \pm 2\%$), PFBS ($11 \pm 1\%$), PFHpA ($9 \pm 4\%$) (Figure S1). This PFAA composition profile clearly differs from that obtained at P1

**Table 1**
Descriptive statistics (mean ± SD; (median); min-max) of PFAA water concentration (ng/L) in each sampling point.

| | PFBS | PFHxS | PFOS | PFBA | PFPeA | PFHxA | PFHpA | PFOA | PFNA | PFDA | PFUdA | ΣPFAAs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P1 | – | – | – | – | – | 0.2 ± 0.3 | – | – | – | – | – | 0.7 ± 1.2 |
| | – | – | – | – | – | (0.1) | – | – | – | – | – | (0.1) |
| | – | – | <0.01−1.8 | – | – | 0.02−0.9 | – | – | – | – | – | <0.37−3.4 |
| P2 | 1.9 ± 1.0 | 5.7 ± 2.5 | 8.4 ± 7.2 | – | 2.5 ± 1.2 | 5.0 ± 3.7 | 1.5 ± 0.6 | 5.0 ± 2.8 | 0.8 ± 0.5 | 1.0 ± 0.6 | 0.3 ± 0.1 | 24 ± 12 |
| | (1.7) | (5.0) | (6.0) | – | (2.3) | (4.3) | (1.5) | (4.0) | (0.6) | (0.8) | (0.3) | (22) |
| | <0.06−3.8 | <0.03−12 | 2.5−34 | <0.02−5.3 | <0.10−4.7 | <0.02−13 | <0.05−2.9 | <0.01−11 | <0.02−2.1 | <0.01−2.6 | <0.04−0.4 | 7.9−47 |
| P3 | 2.2 ± 1.3 | 4.5 ± 2.7 | 7.2 ± 4.6 | – | 2.1 ± 0.8 | 2.4 ± 1.3 | 1.4 ± 0.6 | 4.2 ± 1.9 | 0.6 ± 0.4 | 0.6 ± 0.5 | – | 21 ± 9.5 |
| | (1.8) | (5.1) | (5.8) | – | (2.0) | (2.5) | (1.3) | (4.1) | (0.5) | (0.4) | – | (18) |
| | <0.06−5.3 | <0.03−11 | 2.7−18 | <0.02−4.0 | <0.10−4.3 | <0.02−5.0 | <0.05−2.7 | <0.01−8.8 | <0.02−1.6 | <0.01−2.1 | – | 5.1−43 |
| P4 | 0.4 ± 0.2 | 0.8 ± 0.2 | 1.3 ± 0.6 | – | 0.5 ± 0.3 | 0.9 ± 0.7 | 0.6 ± 0.2 | 1.1 ± 0.5 | – | – | – | 4.1 ± 2.4 |
| | (0.3) | (0.9) | (1.4) | – | (0.5) | (0.8) | (0.6) | (1.1) | – | – | – | (4.8) |
| | <0.06−0.9 | <0.03−1.0 | <0.01−3.1 | <0.02−2.7 | <0.10−1.0 | <0.02−2.6 | <0.05−0.9 | <0.01−2.0 | <0.02−0.3 | <0.01−0.4 | <0.04−0.4 | <0.37−8.9 |
| Total | 1.7 ± 1.2 | 4.4 ± 2.9 | 5.9 ± 5.8 | 3.0 ± 1.5 | 1.8 ± 1.2 | 2.0 ± 2.5 | 1.3 ± 0.7 | 3.6 ± 2.6 | 0.7 ± 0.5 | 0.8 ± 0.6 | 0.3 ± 0.3 | 15 ± 13 |
| | (1.5) | (4.5) | (4.3) | (2.7) | (1.7) | (1.3) | (1.3) | (2.7) | (0.5) | (0.6) | (0.3) | (13) |
| | <0.06−5.3 | <0.03−12 | <0.01−34 | <0.02−5.3 | <0.10−4.7 | <0.02−13 | <0.05−2.9 | <0.01−11 | <0.02−2.1 | <0.01−2.6 | <0.04−0.4 | <0.37−47 |

PFDS, PFDoA, PFTrDA, PFTeDA, PFHxDA, PFODA, FOSA, N-MeFOSA and N-EtFOSA were not detected in any sample. Mean ± standard deviation, (median), min-max.

(Figure S1), mainly due to the low PFAA frequency of detection (43%, P1) in this site.

Geographical differences between sampling points were evaluated. Results revealed that the total PFAA (ΣPFAA) concentrations detected at sampling points located in urban and industrial areas were statistically higher (P2: 24 ± 12 ng/L, mean ± SD; P3: 21 ± 9.5 ng/L; $p < 0.01$, Kruskal-Wallis Test) than those at background or remote areas (P1: 0.7 ± 1.2 ng/L; P4: 4.1 ± 2.4 ng/L; Table 1). The levels of the individual compounds detected in P2 and P3 were also statistically higher ($p < 0.01$, Kruskal-Wallis Test) than those in P4, pointing out the cities of Madrid and Toledo as potentially important pollution sources. These findings were in accordance with a previous study conducted in surface waters in France, in which the most polluted sites were found near urban areas or industrial sites (Munoz et al., 2015).

Seasonal and temporal trends of PFAA water concentrations were also evaluated (Table S6, Fig. 3). The levels of PFAAs obtained during the winter campaigns (9 ng/L, median for ΣPFAA) seemed to offer lower values compared to spring (14 ng/L), summer (18 ng/L) and autumn (17 ng/L), but this result lacked statistical significance ($p > 0.05$, Kruskal-Wallis Test) for the ΣPFAA and each of the quantified PFAAs. Regarding temporal trends, a statistically significant ($r > -0.829$, $p < 0.05$) decrease of some PFAA such as PFHxS, PFOS, PFOA, PFDA and PFUdA had been observed (Table S6). Some studies displayed temporal trends in which shorter chain PFAAs tended to increase in river water (Möller et al., 2010; Llorca et al., 2012; Heydebreck et al., 2015; Zhao et al., 2015; Lorenzo et al., 2016; Gebbink et al., 2017), nevertheless the predominance of PFOS and PFOA has been also reported (Labadie and Chevreuil, 2011a; Labadie and Chevreuil, 2011b; Llorca et al., 2012; Heydebreck et al., 2015; Zhao et al., 2015; Lam et al., 2017; Pignotti et al., 2017).

In general, positive correlations ($r > 0.408$, $p < 0.05$; Table S6), were found among the PFAAs studied in water samples, suggesting that they may be from similar sources. PFOS was strongly correlated with PFOA ($r = 0.911$, $p < 0.01$) and significantly with the rest of PFCAs (C4−C10; $r > 0.679$, $p < 0.01$) and perfluoroalkyl sulfonic acids, PFSAs, (C4 and C6; $r > 0.578$, $p < 0.01$). Similarly, PFOA correlated significantly with the rest of PFCAs (C4−C10; $r > 0.734$, $p < 0.01$) and PFSAs (C4 and C6; $r > 0.636$, $p < 0.01$). In order to identify possible PFAA sources in the Tagus River basin, PFOA/PFOS and PFHpA/PFOA ratios were evaluated. Median PFOA to PFOS ratio in our study (0.7, 0.2−1.6; median, min-max) was two orders of magnitude lower than those determined in water samples for urban areas from Japan (12, 0.1−7041; Saito et al., 2004; Kim and

Kannan, 2007) or WWTPs effluents from New York (52, 7−166; Sinclair and Kannan, 2006). However, the values observed were in accordance with ratios obtained in another Spanish river (Júcar River; 0.4−2.5; Campo et al., 2016) and rural areas from Japan (0.1−18; median 1.8; Saito et al., 2004; Kim and Kannan, 2007) or urban lake water from New York (2.5; Kim and Kannan, 2007), pointing out that the influence of municipal wastewater to contamination in Tagus River could be considered low. The PFHpA/PFOA ratio has been used as an indicator of urban (ratio < 1) or atmospheric deposition (ratio > 1) source of PFAA in water (Simcik and Dorweiler, 2005; McLachlan et al., 2007). The ratios obtained in the present study (0.2−0.9; min-max) suggest that atmospheric deposition may not be a major source of PFAAs in this watershed, being similar to other values reported for urban areas from the United States (0.53−0.90; Simcik and Dorweiler, 2005).

### 3.2. Compliance with EQS

The annual average environmental quality standards (AA-EQS) for PFOS established in the Directive, 2013/39/EU is 0.65 ng/L for inland surface waters. The annual average PFOS concentrations (9.2, 11, 4.5, 4.8, 3.1 and 2.9 ng/L for 2013, 2014, 2015, 2016, 2017 and 2018, respectively) were above this AA-EQS but below the maximum allowable concentration (MAC-EQS; 36 μg/L) (Directive, 2013/39/EU). The sampling points where annual average PFOS concentrations more often complied with the AA-EQS were P1 and P4, while in P2 and P3 levels surpassed it more frequently, reaching values up to 20 times over. Surface water concentrations higher than the AA-EQS for PFOS have been detected in other European rivers (Zhao et al., 2015; Campo et al., 2016; Gebbink et al., 2017; Nguyen et al., 2017; Wilkinson et al., 2017; Gobelius et al., 2018), however, lower levels have also been found (Pignotti et al., 2017). Additionally, AA-EQS for inland surface waters have been fixed in Italy for other PFAAs: PFBA (7 μg/L), PFBS and PFPeA (3 μg/L), PFHxA (1 μg/L) and PFOA (0.1 μg/L) (Decreto Legislativo n. 172, 2015). In our study the annual average concentrations for these PFAAs (PFBS: 1.0−3.9 ng/L; PFBA: 2.0−4.0 ng/L; PFPeA: 1.0−2.6 ng/L; PFHxA: 1.4−2.7 ng/L; PFOA: 2.2−6.5 ng/L) were below the Italian AA-EQS established.

The EQS should not be exceeded in order to protect human health and the environment. In the case of PFOS, which has been identified as priority hazardous substance, the EQS should be applied with effect from 22 December 2018. Although the present water monitoring was carried out previous to the quoted date, the results point out the necessity for improving the water quality to
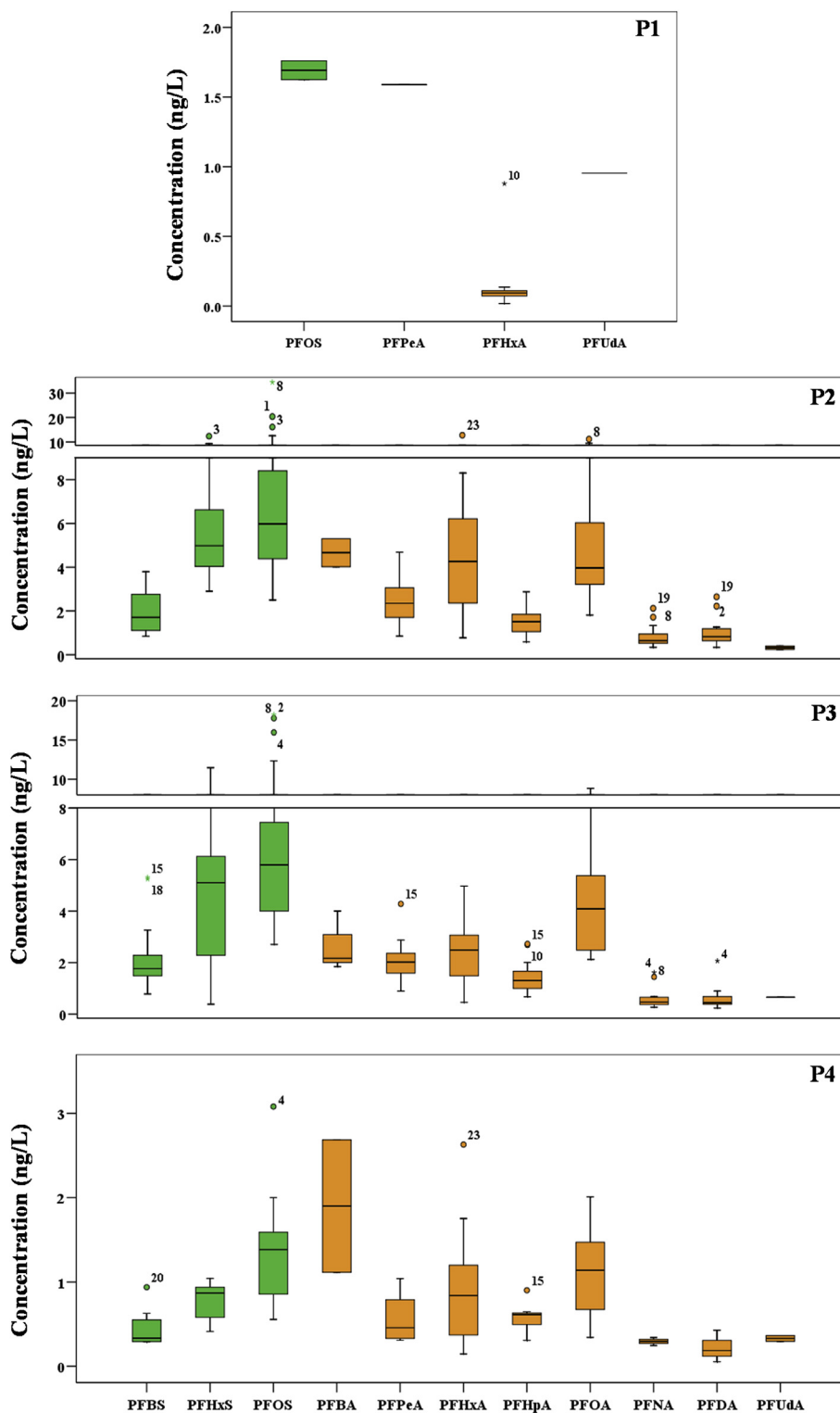
**Fig. 2.** Box and whisker plots of PFAA water concentration (ng/L) obtained in each sampling point: P1-Cañizares (Cuenca), P2-Presa del Rey (Madrid), P3-Toledo (Toledo) and P4-Cedillo (Cáceres). Upper edge of the box, line within the box and lower edge of the box, represents the 75th, 50th, and 25th percentiles. Vertical lines extend from the minimum to the maximum value, excluding outliers (circles) and extreme (asterisks) values which were labeled with sampling campaign code.

protect aquatic ecosystems and contribute to the progressive reduction of emissions of hazardous substances to water.

### 3.3. Mass flow rate of PFAAs in Tagus River basin

The mean mass flow rates calculated in the Tagus River basin were 1.4 ± 1.3 kg/y for PFBS, 3.6 ± 4.2 kg/y for PFHxS, 5.9 ± 8 kg/y for PFOS, 1.3 ± 1.4 kg/y for PFPeA, 2.4 ± 4.5 kg/y for PFHxA, 1.4 ± 1.6 kg/y for PFHpA, 3.6 ± 4 kg/y for PFOA, 0.5 ± 0.5 kg/y for PFNA and PFDA and 14 ± 16 kg/y for ΣPFAAs (Table S7), being PFOS, PFHxS and PFOA the compounds with higher contribution. The estimated mass flow rates of ΣPFAAs was 0.1 ± 0.1 kg/y at P1, 7.0 ± 5.8 kg/y at P2,
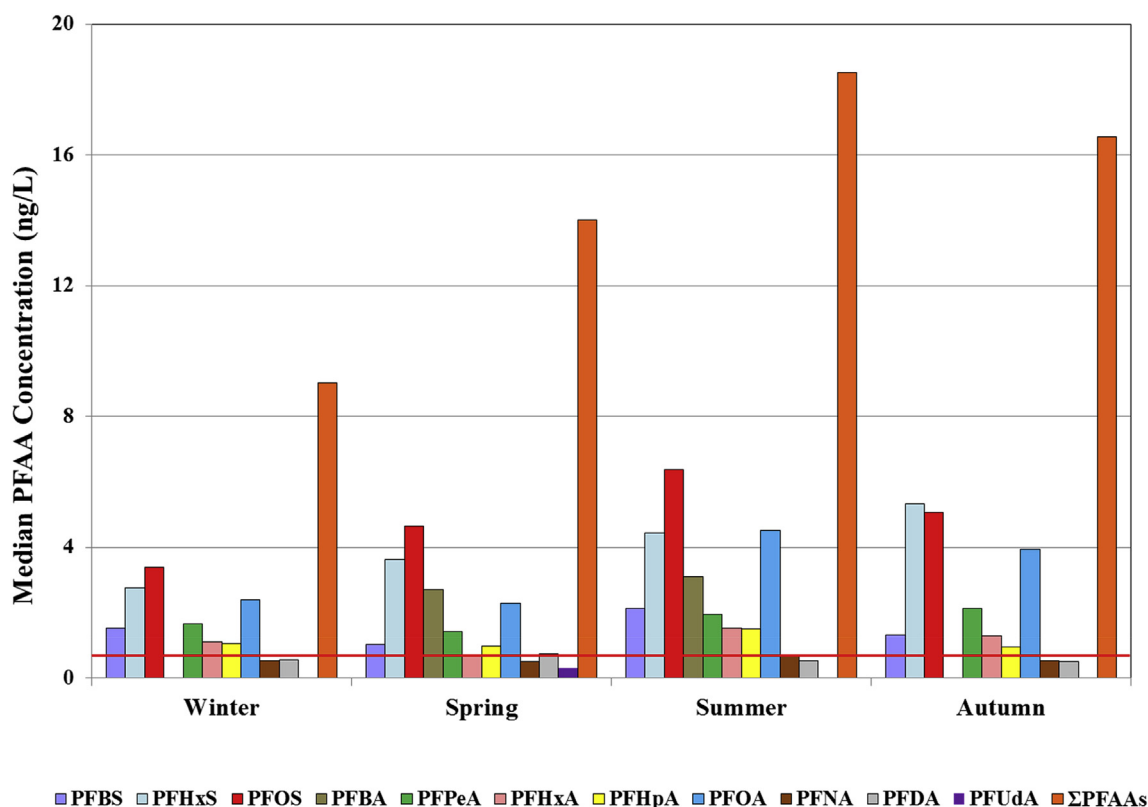
**Fig. 3.** Median PFAA concentration (ng/L) obtained in the different seasons. The horizontal red line represents the AA-EQS established for PFOS in the Directive, 2013/39/EU (0.65 ng/L for inland surface waters). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

$21 \pm 13$ kg/y at P3 and $21 \pm 23$ kg/y at P4. As expected, mass flow rates for ΣPFAAs at P1 were significantly lower ($p < 0.01$, Kruskal-Wallis Test) than those at the rest of locations. In the case of the urban and industrial areas, P3 (located in the metropolitan area of Toledo) showed significantly higher ($p < 0.01$, Kruskal-Wallis Test) values than P2 (located in the metropolitan area of Madrid). The mass flow rates of ΣPFAAs at P4 (located in a background reservoir next to the Portuguese border) did not show statistically significant differences ($p > 0.05$, Kruskal-Wallis Test) with urban/industrial areas, being the values at P4 comparable to those obtained at P3. Although ΣPFAA concentrations at P4 were significantly lower ($p < 0.01$, Kruskal-Wallis Test) than concentrations at P2 and P3, the flow rates at this point were higher (significantly higher than P2, $p < 0.01$, Kruskal-Wallis Test), suggesting a possible dilution effect in the concentrations at P4 and pointing out a possible constant PFAA emission through the basin. The mass flows might vary due to seasonal trends, but mean daily flow rates were obtained at the time of sampling campaigns and no statistically significant differences ($p > 0.05$, Kruskal-Wallis Test) were observed. The results indicated that the PFAA discharge was more closely related to industrial or urban sources and pointed out a great influence of the human impact in the pollution of the river course.

Compared to PFAA mass flows from other rivers, the mass flows from Tagus River (0.002–88 kg/y for ΣPFAAs, Table S8) were in accordance with values recently found in other European rivers such as Baltic Proper Basin (0.54–78 kg/y for ΣPFAAs) or Kattegat Basin (1.5–82 kg/y for ΣPFAAs) in Sweden (Nguyen et al., 2017) and Weser River ($102 \pm 22$ kg/y for ΣPFAAs) in Germany (Zhao et al., 2015). Higher values have also been observed for ΣPFAA flows in France (485 kg/y, Labadie and Chevreuil et al., 2011b), Spain (5672 kg/y, Picó et al., 2012), Sweden (6.1–418 kg/y, Nguyen et al., 2017) or Germany ($335 \pm 100$ kg/y, Zhao et al., 2015).

### 3.4. Environmental exposure assessment in the aquatic compartment

The safe water concentrations protective of the avian (fish-eating birds) wildlife have been determined to be 43–50 ng/L (So et al., 2004; Rostkowski et al., 2006; Giesy et al., 2010) for PFOS. Lower values (<0.01–34 ng/L) were detected for PFOS in Tagus River basin (Table 1), suggesting a low ecological risk to aquatic organisms related to the exposure to PFOS. To corroborate that, an environmental exposure assessment in the aquatic compartment was performed (Tables S11-S13). The aquatic food chain considered was water/aquatic organism/fish/fish-eating bird or mammal, then the concentration in fish is a result of uptake from the aqueous phase and intake of contaminated food (aquatic organisms). The parameters used in the calculations are indicated in Tables S9 and S10. $PEC_{water}$ values ranged from $4.71 \times 10^{-10}$ mg/L to $1.66 \times 10^{-6}$ mg/L, $PEC_{sed}$ values ranged from $1.08 \times 10^{-8}$ mg/L to $1.72 \times 10^{-6}$ mg/L and $PEC_{oral, \; predator \; (Aq)}$ values from $4.33 \times 10^{-8}$ mg/L to $5.26 \times 10^{-6}$ mg/L (Table S11). The $PNEC_{water}$ for each substance were obtained from laboratory toxicity tests, and except for PFOS, $PNEC_{sed}$ were derived from $PNEC_{water}$ (Table S12). Due to the limited toxicity studies on several of the PFAAs selected, read-across from closely related substances (PFOS and PFOA) was used for obtaining $PNEC_{oral}$ and then the estimation of the corresponding RCR values, assuming some uncertainty. The use of PFOS and PFOA as analogues for toxicity is conservative and it could be considered a worst-case and protective approach in relation to ecological effects for aquatic organisms and fish-eating predators. The quotients calculated ($RCR_{water}$, $RCR_{sed}$ and $RCR_{oral, \; fish}$) were below 1: $RCR_{water}$ ranged from $1.39 \times 10^{-8}$ to $1.51 \times 10^{-5}$, $RCR_{sed}$ ranged from $5.36 \times 10^{-9}$ to $1.58 \times 10^{-5}$ and $RCR_{oral, \; fish}$ from $6.16 \times 10^{-7}$ to $5.07 \times 10^{-5}$ (Table S13). These values below 1

suggested that the PFAA concentration in surface water did not involve a significant risk for the aquatic system.

## 4. Conclusions

The presence of 20 PFAAs in surface water has been periodically evaluated through the course of a major European watershed (Tagus River basin in its Spanish section). PFOS was the predominant compound with the highest measured levels. The ΣPFAA concentrations detected at sampling points located in urban and industrial areas were statistically higher than those at background or remote areas, pointing out the metropolitan areas of big cites as main pollution sources. Besides, the estimated mass flow rates of ΣPFAAs also indicated that the PFAA discharge was more closely related to industrial or urban sources, showing a great influence of the human impact in the pollution of the river course. The annual average PFOS concentrations detected in Tagus River were above the annual average environmental quality standards (AA-EQS) established in the Directive, 2013/39/EU for inland surface waters. The reported concentrations are of interest since these surface waters may be treated by water treatment plants to generate tap water, where the presence of PFAAs could have human health implications. However, the quotients calculated ($RCR_{water}$, $RCR_{sed}$ and $RCR_{oral, fish}$; < 1) in the environmental exposure assessment, suggested that the PFAA concentration in surface water did not involve a significant risk to the aquatic ecosystem.

## Declaration of competing interest

The authors declare that there is no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.113511.

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