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PII: S0048-9697(20)35873-3

DOI: <https://doi.org/10.1016/j.scitotenv.2020.142344>

Reference: STOTEN 142344

To appear in: *Science of the Total Environment*

Received date: 30 June 2020

Revised date: 8 September 2020

Accepted date: 9 September 2020

Please cite this article as: M. Köck-Schulmeyer, A. Ginebreda, M. Petrovic, et al., Priority and emerging organic microcontaminants in three Mediterranean river basins: Occurrence, spatial distribution, and identification of river basin specific pollutants, *Science of the Total Environment* (2020), <https://doi.org/10.1016/j.scitotenv.2020.142344>

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PRIORITY AND EMERGING ORGANIC MICROCONTAMINANTS IN THREE MEDITERRANEAN RIVER BASINS: OCCURRENCE, SPATIAL DISTRIBUTION, AND IDENTIFICATION OF RIVER BASIN SPECIFIC POLLUTANTS

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Abstract

There is a worldwide growing use of chemicals by our developed, industrialized, and technological society. More than 100,000 chemical substances are thus commonly used both by industry and households. Depending on the amount produced, physical-chemical properties, and mode of use, many of them may reach the environment and, notably, the aquatic receiving systems. This may result in undesirable and harmful side-effects on both the human and the ecosystem's health. Mediterranean rivers are largely different from Northern and Central European rivers in terms of hydrological regime, climate conditions (e.g. air temperature, solar irradiation, precipitation), and socio-economics (e.g. land use, tourism, crop types, etc.), with all these factors leading to differences in the relative importance of the environmental stressors, in the classes and levels of the pollutants found and their environmental fate. Furthermore, water scarcity might be critical in affecting water pollution because of the lowered dilution capacity of chemicals.

This work provides raw chemical data from different families of microcontaminants identified in three selected Mediterranean rivers (the Sava, Evrotas, and Adige) collected during two sampling campaigns conducted in 2014 and 2015 in three different matrices, namely, water, sediments, and biota (fish). More than 200 organic micropollutants were analyzed, including relevant groups like pharmaceuticals, personal care products, perfluorinated compounds, pesticides, pyrethroid insecticides, flame retardants, and persistent organic pollutants. Data obtained were summarized with some basic statistics for all compound families and matrices analyzed. Observed occurrence and spatial patterns were interpreted both in terms of compound physical-chemical properties and local environmental pressures. Finally, their spatial distribution was examined and their ecotoxicological risk in the water phase was assessed. This allowed locating, at each basin, the most polluted sites ("hot spots") and identifying the respective river basin specific pollutants (RBSPs), prioritizing them in terms of the potential ecotoxicological risk posed to the aquatic ecosystems.

Keywords: Mediterranean basins; emerging contaminants; water pollution; Adige; Evrotas; Sava; risk assessment; prioritization; river basin specific pollutants

1. Introduction

There is extensive and intensive use of chemicals in our developed, highly technological society. For instance, under the provision of the REACH regulation (EC 2006) more than 100,000 chemical substances have been currently registered by the European Chemicals Agency (ECHA 2019). Significant scientific evidence has led to the recognition that their improper use and release may result in undesirable and harmful side-effects on both the human and ecosystem health (López-Pacheco et al. 2019; Malaj et al. 2014; Ginebreda et al. 2016). Mediterranean rivers are largely different from Northern and Central European rivers in terms of the hydrological regime (e.g. extreme hydrological events like droughts and floods), climate conditions (e.g. ambient temperature, solar irradiation), socio-economics (e.g. land-use changes, tourism, kinds of crops, water abstractions, urban and industrial releases, etc.), all of which leads to differences in the relative importance of the environmental pressures, in the classes and levels of the pollutants found and their environmental fate, etc. Water scarcity might increase the effects of water pollution on the receiving ecosystems (Petrovic et al. 2011) because of lowered dilution capacity of chemicals (Sabater et al. 2019) and combining its effects on the biological communities with other co-occurring environmental stressors (nutrients, altered discharge, dissolved organic matter, etc.) (Sabater et al. 2016). In this context, our study aims at providing both raw chemical occurrence data found in the monitoring of the three Mediterranean rivers (i.e., Adige, Sava, and Evrotas) together with the assessment of their ecotoxicological risk potentially affecting the receiving freshwater ecosystems. The three rivers surveyed, located in the northern Mediterranean basin, cover a broad range of characteristics in terms of basin area, discharge, land uses, or anthropogenic pressures (see Section 2.1).

Environmental chemical water monitoring seeks to provide the necessary data required for preserving the aquatic environment against the adverse effects caused by anthropogenic chemical pollution. In the regulatory context, chemical monitoring plays a key role in the Water Framework Directive (WFD) (EC, Directive 2000/60/EC) for the characterization and control of the so-called ‘chemical and ecological status’ of the water bodies. Despite the huge number of compounds present in environmental samples, only a few of them are covered by the WFD and daughter directives (EC, Directive 2013/39/EU), the so-called ‘priority substances’ for which environmental quality standards (EQS) are set up. Since this is insufficient, in this survey more than 200 organic compounds were monitored encompassing both emerging and regulated pollutants in three

different environmental compartments, namely, water, sediments, and biota (fish). The micropollutants analyzed included relevant groups like pharmaceuticals, personal care products, perfluorinated compounds, polar pesticides, pyrethroids, persistent organic pollutants, and brominated flame retardants. On the other hand, according to the same regulations (WFD Article 4 and Annex V), water authorities must identify river basin specific pollutants (RBSP) of concern to ensure the good chemical and ecological status of the water bodies. This can be done using existing prioritization procedures based on the ecotoxicological risk of every single compound identified (Von der Ohe et al. 2011; Dulio and Von der Ohe 2013; Dulio and Slobodnik 2015; Kuzmanovic et al. 2015; Kuzmanovic et al. 2016).

Though our study is aligned with the WFD principles, it goes beyond and fits the concept of advanced monitoring (Altenburger et al. 2019) that recommends the combined use of information on the occurrence of chemicals and their potentially adverse biological effects to properly assess the potential impact of contamination in the receiving aquatic ecosystems. Under such approach, overarching aims addressed in the study were: (a) the identification of the main pollutants occurring in the three rivers surveyed, considering their partition behavior among the environmental compartments (water, sediments, and biota); (b) the characterization of the spatial distribution of the pollutants monitored in each of the three river basins, with emphasis on the location of the main “hot-spots”; (c) the assessment of the ecotoxicological risk associated to the joint occurrence of many chemicals at every site monitored, and (d) the identification and prioritization of the river basin specific pollutants (RBSP) in terms of the potential ecotoxicological risk posed to the aquatic ecosystems.

The study presented in this paper was carried out under the EU FP7 project GLOBAQUA (Navarro-Ortega et al. 2015).

2. Materials and methods

2.1. Description of the basins

A brief overview of the three river basins under study, namely the Adige, the Sava, and the Evrotas (Fig. 1), is provided below.

Adige basin

The Adige is the second longest river in Italy, with a length of 410 km and a drainage area of 12,000 km². Its source is near the border with Austria and Switzerland and enters the Adriatic Sea south of the Venice lagoon. As in most of the central and southern Alps, the climate in the Adige river basin is characterized by dry winters, snowmelt in the spring, and humid summer and falls (Laiti et al. 2018; Mallucci et al. 2019). Because of its morphology and humid climate, the river basin is well suited for hydroelectric production, and to date, 30 major reservoirs exist in the catchment, for a total storage capacity of 571 106 m³ (8.5% of the long-term mean annual runoff (Chiogna et al. 2016). These reservoirs supply 34 major hydropower plants, for a total installed power of 983 MW, and potential energy production of 4123 GWh/year. Because of hydropower production streamflow is altered, particularly at intermediate and low flow regimes (Bellin et al, 2016; Majone et al., 2016). Earlier snow melting is already affecting the Adige river basin reducing water resources available during the irrigation period (roughly June-August), while the higher temperature recorded in the summer months is expected to cause an increase of water demand in this period (Lutz et al. 2016; Diamantini et al., 2018). This is expected to increase the deficit of water resources in summer, when agricultural and recreational uses reach the highest demand, thereby exacerbating the conflicts between different uses of water resources (La Jeunesse et al. 2016). Diffused pollution by agriculture in the central and lower course of the Adige River represents a relevant environmental pressure factor. In particular, pesticides have been widely used in the widespread apple trees cultivations. Furthermore, hydropower activities can also have severe consequences on contaminant loads transported in the stream either by direct release or induced hydropeaking effects. Another important stressor of the aquatic ecosystem relevant for the upper part of the basin can be the release of persistent organic pollutants (such as DDT) accumulated in the glaciers through long-range atmospheric transport and deposition, and the discharge of emerging pollutants (e.g., pharmaceuticals, and personal care products, in particular, UV filters) from the WWTPs serving the ski resorts.

Evrotas basin

The Evrotas River drains a mid-altitude, medium-sized (2418 km²) Mediterranean basin, located at the South-eastern Peloponnese (Southern Greece). The climate, discharge, and precipitation of the Evrotas basin follow a predictable seasonal pattern, similar to other Mediterranean rivers with hot and dry summers and cool, wet winters. The average annual temperature is 16°C and the mean annual precipitation 803 mm. The Evrotas

basin is representative of a large fraction of Greek territory drained by intermittent rivers exceeding 40% (Skoulikidis et al. 2017). The vast majority of the river basin is covered by natural and semi-natural areas accounting for 61% of the total river basin, followed by agricultural areas that cover 38%, while urban areas account for 1%. The dominant pressures in Evrotas derive mainly from agricultural activities and include overexploitation of water resources for irrigation, disposal of agro-industrial wastes (mainly olive oil mill wastes), agrochemical pollution, and hydromorphological modifications (Karaouzas et al. 2018). Overexploitation of groundwater aquifers and water abstraction from surface waters led to a dramatic long-term discharge reduction, as well as to artificial desiccation of parts of the Evrotas main stem and tributaries, particularly during dry years. Only one Waste Water Treatment Plant (WVTP) exists in the basin (town of Sparta), while villages are served by permeable and impermeable cisterns. From an ecological point of view, the Evrotas basin can be characterized as a unique biodiversity hotspot in Greece, with many local endemic plants and vertebrates. As a result of desiccation, massive fish deaths occur in summer and the three native range-restricted endemic fish species of the Evrotas River, and especially the Evrotas chub *Squalius keadicus*, are threatened (Vardakas et al. 2017).

Sava basin

The Sava River (length 945 km) is the major drainage basin of South-Eastern Europe and the largest tributary to the Danube River. The 97,713.20 km² large catchment is extended over Slovenia, Croatia, Bosnia, and Herzegovina, and Serbia. In the Alpine headwater regions mean annual precipitation range between 2000 and 3000 mm per year with a mean annual temperature of 6 °C. At the confluence of the Sava with the Danube, annual precipitation decreases sharply to around 660 mm per year, and temperature increases to 13 °C. The important characteristic of the Sava is its quick response to precipitation that is also reflected in the short mean residence time of approximately 2 years estimated in the river. The population in the Sava basin is about 8.2 million (46% of the total population of all the riparian countries). The basin is covered by forest and semi-natural areas (55%) and agricultural surfaces (42%). The Sava River provides drinking water (surface and groundwater) for the population in large cities. The upper reaches are largely influenced by carbonate mineral weathering, the middle by agricultural activity, and biological processes related to eutrophication, while the lower reaches are influenced mostly by stressors related to high pollution from industrial processing along with untreated municipal wastewater discharges.

2.2. Sampling campaigns and protocols

Two sampling campaigns, from the three target river basins, were carried out between June 2014 and September 2015. In summary, 152 samples were collected including 61 water samples, 54 sediment samples, and 37 fish samples. The sampling protocol for each environmental matrix (water, sediment, and fish) is presented in Table S1 (Supplementary Information).

2.3. Selection of target compounds

In this study, 268 target compounds were selected for monitoring, however because of the variety of physicochemical properties covered not all were analyzed in the three matrices. Table 1 shows the list, classification, and acronyms of the 7 main families and 17 subfamilies analyzed, namely, flame retardants (FR), personal care products (PCP), polar pesticides (PES), pyrethroid insecticides (PYR), perfluorinated compounds (PFC), pharmaceutically active compounds (PhAC) and persistent organic pollutants (POP). For the sake of statistical consistency, only those compounds that were traced in at least 35% of the water samples were used, except for the flame retardants and pyrethroid insecticides that were analyzed mainly in sediment and fish samples, due to their hydrophobic physicochemical properties. In consequence, 224 out of the initial 268 compounds were considered in this study. The full list of compounds analyzed under each family/subfamily is given in Table S2 (Supplementary Information). More details about the sampling per river basin are given in Table 2.

2.4. Statistical data analysis

For both water and sediments the two results per compound and monitoring site obtained in the two campaigns were averaged taking into account the limit of detection (LOD) and limit of quantification (LOQ) of the analytical method used. In particular, results shown in Table 2 were calculated according to the Directive 2009/90/EC, i.e. values below the LOQ were considered as half the corresponding limit ($bLOQ = LOQ/2$); whereas for total concentration, values below the LOD were considered as zero ($bLOD = 0$). The detection frequency (DF) corresponds to the maximum percentage of detection of individual compounds in a sampling point. The median, average, and maximum concentration that are given in Table 2 were calculated based on the sum of all individual compounds from the same family detected in a sample. Principal

component analysis (PCA) was performed using PASW Statistics 18 (Predictive Analytics Software — SPSS Inc., USA). To avoid dealing with missing values (values below the LOD), the PCA was performed at the family level (i.e., the sum of PCP, PES, etc.). In all cases, a 95% confidence interval was considered.

2.5. Risk-based prioritization of pollutants: identification of river basin specific pollutants (RBSP)

River basin specific pollutants (RBSP) in the water phase were identified and prioritized following the NORMAN network prioritization methodology (Dulio and Von der Ohe, 2013; Dulio and Slobodnik, 2015). Briefly, target analytes detected in the water phase at each river basin were ranked using two risk indicators, namely the *Frequency of Exceedance (FoE)* and the *Extent of Exceedance (EoE)*, that was subsequently added to yield a final ranking score (RS between 0 and 2). The *Frequency of Exceedance (FoE)* takes into consideration the “extension” of the temporal or spatial occurrence of the contaminant, whereas the *Extent of Exceedance (EoE)* captures the “intensity” of the occurrence, i.e., the maximum or peak concentrations exceeding a reference ecotoxicological safe level. These indicators (and associated scores) were calculated for every single compound and basin. Method and calculation details are described in Tables S3 to S5 (Supplementary Information). For calculation purposes, “non detected” values were treated as 0.

2.6. Site Ecotoxic Risk

The single compound risk associated with a sample was quantified in terms of its Risk Quotient (RQ), defined as the ratio of its measured concentration (MEC) respect to the predicted non-effect concentration (PNEC). For the majority of compounds, the list of lowest PNECs compiled in the NORMAN network database (<https://www.norman-network.com/nds/ecotox/>) was used and, if not available, retrieved from published literature (Table S2, Supplementary Information). The site-specific risk RQ_{site} was quantified (eq. 1) by summing up the RQ 's for all the compounds present:

$$RQ_{site} = \sum_{i=1}^n RQ_i \quad (1)$$

where RQ_i is the risk quotient of each compound i at the site. Such aggregation is based on the ‘Concentration Addition’ (CA) approach, which assumes a common action mode. This is not strictly true, but since the modes of action of many studied compounds are still unknown, the CA is generally accepted as a first-tier approach (Backhaus and Faust, 2012). In the present article, RQ s were calculated only for the water phase.

3. Results and discussion

3.1. Chemical compounds identified

3.1.1. Distribution of compounds amongst the environmental compartments

To have some insight on the distribution of the compounds analyzed amongst the three environmental compartments (water, sediments, and fish), we plotted in Fig. 2 the respective mean concentrations of the different compounds vs. their octanol-water partition constant (K_{ow}), which captures the compound's distribution behavior between the water phase and a hydrophobic phase like sediment or biota. Logarithms of the K_{ow} ($\log K_{ow}$) spanned from *ca.* -3 to 14, with the minimum and maximum values corresponding to the herbicide glyphosate (-3.4), and the brominated flame retardant decabromodiphenyl ethane (13.6) respectively. About 90% of the compounds' $\log K_{ow}$ values were comprised in the range 1–8 (Table S2, Supplementary Information). As one may expect, the compounds found in the water phase (Fig. 3A) were only those of medium to high polarity (i.e., $\log K_{ow} \leq 6$). DPs of the different compound families in the three matrices analyzed are presented in Table 2. Water DPs were dominated by PhACs and PCPs in the three rivers. Some POP families like Polycyclic Aromatic Hydrocarbons (PAH) were present in the Sava River, while halogenated persistent insecticides and PCBs were detected in the Adige, possibly because of long-range atmospheric transport and deposition (Carrera et al. 2001; Villa et al. 2003; Bizzotto et al. 2009; Kirchgeorg et al. 2016), though direct release from hydropower stations should be also taken into consideration. These low polar compounds were found in the three compartments, while organophosphate flame retardants (OPFR) and pyrethroid insecticides were exclusively identified in sediments (Fig. 3B) and fish (Fig. 4). Fish bioaccumulation levels do not only depend on the physical-chemical properties of the compounds and environmental exposure. Other biological and ecological aspects such as the size or weight (proxies of the age) of the specimen analyzed, the feeding habits (bottom vs. surface feeders), and the position in the trophic chain (super-predators, predators, herbivores-detritivores) should be taken into consideration as well. However, as shown in Fig. 4A and B, in general, it seems that site pollution exposure was the dominating factor. A more detailed description of the different compound families' occurrence is given in the next sections (see Table 2).

3.1.2. *Pharmaceutically Active Compounds (PhACs)*

The predominant pharmaceutical class detected in water samples throughout the studied river basins was the analgesics/anti-inflammatory with maximum concentration per site of 230 ng/L (Sava), 850 ng/L (Adige), and 167 ng/L (Evrotas). Others, in order of contribution, were antihypertensives, diuretics, antibiotics, lipid regulators, and cholesterol-lowering statin drugs. The highest concentrations amongst all studied PhACs in water samples in the three river basins were the anti-inflammatories acetaminophen, ibuprofen, diclofenac, and naproxen. This fact may be explained by their availability as non-prescription (“over the counter”) drugs, and the widespread practice of self-medication. The highest total concentration of PhACs per site was detected in the Adige (2041 ng/L), followed by Sava (461 ng/L) and Evrotas (368 ng/L), the latter two at similar levels. The higher total concentrations of PhACs in the Adige River compared to the other basins may be explained by the joint effect of low streamflow and increased tourist fluxes during winter months. Even though many big cities (i.e. Zagreb, Beograd) are located on the Sava River, the overall concentration levels of PhACs were lower in comparison to the other two basins. This finding could be attributed to the fact that the Sava River is much larger (97,713.20 km² catchment size) than the other studied rivers and, therefore, effluents were subjected to a higher dilution. Considering the three basins studied, our measurements indicated that the accumulation of PhACs in sediments was moderate in comparison to water samples, with total maximum values per site of 60.5 ng/g dw (Adige), 217 ng/g dw (Evrotas) and 19 ng/g dw (Sava). Only PhACs of low polarity may sorb onto sediments and suspended particles (Ruegner et al. 2019). On the other hand, compounds with basic properties (pKa > 7) such as the macrolide antibiotic clarithromycin (pKa 8.9), the diuretic hydrochlorothiazide (pKa 7.9), the beta-blocker metoprolol (pKa 14.1), and the analgesic acetaminophen (pKa 9.38) showed tendency to bind to sediments. Hence, the predominant PhAC classes detected in sediment samples throughout the studied river basins were antibiotics, lipid regulators and, diuretics, with the highest total concentration detected in the Evrotas River, maybe because of its prolonged seasonal desiccation. Maximum levels on fish per specimen analyzed were 56.5 ng/g dw (Adige), 82.5 ng/g dw (Evrotas), and 290 ng/g dw (Sava).

3.1.3. *Personal Care Products (PCPs)*

Regarding the water phase (Mandarić et al. 2017; Molins-Delgado et al. 2018), all the investigated categories of PCPs, i.e. organic UV filters, fragrances, insect repellents, and paraben preservatives were detected in the

three basins studied, with DFs of 59% (Adige), 82% (Evrotas) and 78% (Sava) respectively. The highest frequency of detection was that of UV filters with maximum values between 74% for 3-(4-methylbenzylidene)camphor (4MBC) (Sava) and 67% for the UV stabilizers of the benzotriazole family (benzotriazoles) (Adige). Total PCP were relatively high, with maximum values as high as 3054 ng/L (Adige), 3568 ng/L (Evrotas), and 4603 ng/L (Sava), respectively, being the most relevant compounds benzophenone-type UV filters (2860 ng/L, BP1 in the Adige) and insect repellents.

DFs in sediments, were 13% (Adige), 17% (Evrotas) and 33% (Sava) respectively. Low to medium-polar compounds, such as benzophenone-type UV filters, were also found, but at low concentrations, i.e., 0.6 – 904 ng/g dw. The most contaminated sediments were those collected in the second campaign in Evrotas at very low flows, with a maximum of 700 ng/g dw of 4MBC (Diaz-Cruz et al. 2018; Ruegner et al. 2019).

The concentration levels and the occurrence frequency of the target PCPs in the fish samples collected were quite different in the three rivers. In the Evrotas, *Squalius laietanus* bioaccumulated the benzophenone-type UV filters, and methylbenzotriazole (Diaz-Cruz et al. 2018). In the Sava River total concentrations of PCPs in fish samples were relatively high in two species (i.e. *Onchorhynchus mykiss* and *Squalius cephalus* (55.3 and 73 ng/g dw, respectively), and very low in *Barbus Barbus*. These concentrations corresponded to the benzophenone-type UV filters, ethyl 4-aminobenzoate (ethyl PABA), 3-(4-methylbenzylidene)camphor (4MBC), and the benzotriazoles. Adige's fish samples presented the highest PCPs' bioaccumulation values by far. Total concentrations ranged from 137.5 to 42608 ng/g dw, which were measured in *Leuciscus cephalus*, *Salmo trutta fario*, *Salmo trutta malinoratus*, *Thymallus thymallus*, and *Cottus gobio*.

3.1.4 Perfluorinated compounds (PFC)

Overall, the concentration of PFCs in water samples was generally in the range 0.9 – 3.5 ng/L and coherent with the level of industrial pressure of the site and the dilution capacity of the receiving waterbody, with a low impact in the case of Adige and high dilution factors in the Sava. PFCs were not analyzed in the Evrotas River. In agreement with these findings, sediments presented trace concentration levels in both the Sava and Adige River (>1 ng/g dw). The compounds found at major concentrations and also more frequently detected were perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) that are the more stable congeners. It is worth noting that PFOS has been included in the last update of the list of the WFD priority

compounds (Directive 2013/39/EU), with an environmental quality standard (EQS) of 0.65 ng/L which was exceeded in some of the sites monitored. On the other hand, the bioaccumulation in fish presented trace PFC concentrations in the same individuals that gave positive responses also for other groups of contaminants (Abalos et al. 2019).

3.1.5. *Flame retardants (FRs)*

Classic (polybromodiphenyl ethers, PBDEs) and emerging HFRs such as decabromodiphenyl ethane and halogenated norbornenes, as well as OPFRs were analyzed in 52 sediments and 27 fish samples from the three river basins studied (Giulivo et al. 2017). The highest contamination was found in the Adige and Sava rivers, whereas lower values were recorded in the Evrotas. The maximum concentration levels per site in sediment samples ranged between 6.8 (Evrotas) and 24.9 ng/g dw (Sava), and between 20.1 (Evrotas) and 297.3 ng/g dw (Sava), for HFRs and OPFRs respectively. The most relevant compounds were decabromodiphenylethane and the PBDE BDE-209 in the HFR group, as well as 2-ethyldiphenylphosphate and isodecyldiphenylphosphate in the OPFR. Regarding the levels in fish, the maximum concentrations per site ranged between 10.8 and 37.2 ng/g dw and between 8.4 and 37.4 ng/g dw, for HFRs and OPFRs respectively. In contrast with sediment samples, similar or lower concentrations of OPFRs compared to HFRs were found in the fish samples, highlighting the higher bioaccumulation potential of the latter. In agreement with this finding, biota to sediment accumulation factors were calculated and higher values were obtained for HFRs compared to those of OPFRs.

PBDEs were the main contributors to HFR contamination while emerging brominated FRs were barely detected. Different OPFR patterns were observed in each river studied, but tributylphosphate, tris(chloroethyl)phosphate, and isopropylphenylphosphate were the most abundant OPFRs found in fish in the three basins.

3.1.6. *Polar pesticides (PES)*

All the polar pesticides analyzed in the water samples were detected, though levels were usually in the pg or low ng/L range, and the frequency of detection was generally low. Concentrations above 50 ng/L were found only for simazine (up to 54.8 ng/L), irgarol (up to 275 ng/L), diuron (up to 287.7 ng/L), and glyphosate (up to

1870 ng/L) in the Sava River, and for glyphosate in the Adige River (up to 135 ng/L). In sediments, the levels and frequency of detection were even lower, with concentrations above 5 ng/g only in the case of fenitrothion in one sample of the Evrotas River (7.9 ng/g dw), diuron in one sample of the Sava River (12.4 ng/g dw), and dichlorvos in a sample of the Adige River (168 ng/g dw, respectively). In fish, only 8 out of the 48 polar pesticides analyzed were detected, and the levels and frequency of detection were generally low as well. Metolachlor was the most ubiquitous compound (found in 23 samples), and levels above 1 ng/g dw were only measured for the relatively non-polar pesticide quinoxifen in the Adige (11.4 ng/g) and the Sava (41.8 ng/g dw) rivers, and to a less extent the herbicides terbutylazine and irgarol (2.7 and 1.7 ng/g dw respectively) in fishes from the Evrotas River.

3.1.7. Pyrethroid Insecticides (PYR)

PYRs were analyzed only in the water samples collected in the Evrotas river basin, with maximum values per site up to 4.7 ng/L. In this river basin, sediment and fish samples reached levels up to 14 ng/g dw, and 0.7 ng/g dw respectively. These contamination levels were similar to those obtained found in the Sava river basin, with maximum concentrations up to 5.4 ng/g dw in sediments, and 6.9 ng/g dw in fish samples. In the case of the Adige river basin, PYR contamination was slightly lower with maximum concentrations up to 1.6 ng/g dw in sediments and 0.65 ng/g dw in fish samples.

As regards the most abundant PYRs compounds, permethrin presented the highest concentrations in sediment samples from the Adige and Sava river basins. In fish samples collected in these two rivers, besides permethrin also cypermethrin, cyhalothrin, fenvalerate, and fluvalinate were identified. In the case of the Evrotas, permethrin was found basically in water samples, whereas cypermethrin and deltamethrin were the most abundant in sediment and fish samples.

3.1.8. Persistent Organic Pollutants (POPs)

Polycyclic Aromatic Hydrocarbons (PAHs) were the class of POPs most frequently detected in the water samples of the Sava and Adige rivers, present in nearly all samples at ng/L levels. In the Adige River, both PAHs and PCBs were ubiquitously found at trace levels (similar to those previously reported in other remote areas), due to their broad environmental distribution. In contrast, in the Sava River, the concentrations of POPs measured were generally low considering the influence of urban and industrial emissions.

In sediments, the frequency of detection of POPs was comparatively higher than in water, and the concentrations in the Sava River were higher than in the other basins, as expected. The maximum concentration levels in the Sava River were close to 9300 ng/g dw, while the lowest concentrations, with a maximum per site of 640 ng/g dw, were observed in the Evrotas River. In fish samples, contaminated individuals coincided with those also found to contain other organic microcontaminants such as PFC. Other halogenated POP groups like halogenated insecticides and PCBs were roughly found at similar levels. The maximum total concentrations were 740 ng/g dw in the Sava River (Abalos et al. 2019), while in the Evrotas only one sample gave positive, with a maximum concentration of 70 ng/g dw. In the Adige, the maximum concentration in fish was 254 ng/g dw.

3.2. Contamination profile per basin

3.2.1. Overview

The concentration profiles of the different pollutant groups for the three rivers are depicted in Figs. 3A and 3B for the water and sediment phases, and Fig. 4 for the biota. In general, the six families of emerging pollutants analyzed were found in the three basins surveyed in one or more of the matrices studied. To have a better insight into the possible sources of pollution we carried out a PCA for the three environmental matrices studied (Fig. 5). For water and sediments, PCA was performed using the averaged values per compound and site resulting from the two monitoring campaigns. For water and fish, two components explained more than 80% of the total variance (water: 49.4% and 33.4%; fish: 55.1% and 33.3%), while sediments required a third component (39%, 30.1%, and 27.7%). In the case of water, the loadings of PC1 were positively dominated by PES and negatively by PhACs, whereas for PC2 the largest positive contributors were PCP and PhACs. This can be roughly explained as PC1 and PC2 reflecting the agriculture and the urban sources of pollution respectively. The first two components of sediment were dominated by PhACs, PCPs, FR, and POPs thus reflecting unequivocally urban pollution, while the third component has pesticides as the main contributor and likely pointing to agriculture sources. The fish PCA was explained by mixed profiles not easily interpretable, i.e., PFCs, PES, POP, PhACs and FR for PC1, and PCPs and POPs for PC2. Regarding the scores, water PCA was not successful for achieving a good separation of the three river sites, while the sediments' PCA was only able to partially discern the Evrotas sites. Fish PCA scores enabled to distinguish the sites of the three rivers,

being those of the Adige the ones that were more clearly separated. The three river basins are briefly commented in the following paragraphs.

3.2.2. *Adige basin*

Adige results show evidence of the strong tourism impact on the river quality. Concentrations detected during winter at A2 (located downstream a WWTP serving an important ski resort) are higher than those detected in other sites. Concentrations were in general higher during winter than during summer, a variation that could be jointly attributed to low flow conditions, cold-water temperatures, and an increased number of tourist arrivals. During summer the pattern was reversed, and higher concentrations occurred in downstream sites where the presence of urbanized areas is larger (e.g., A7). The lower part of the catchment (A7) had the most contaminated sediments during both campaigns, likely because of the accumulation of the transported materials along the basin. Analyses conducted in fish tissues evidenced higher concentrations in sites located downstream, particularly those located up and downstream to the municipality of Trento (A6 and A7, respectively).

3.2.3. *Evrotas basin*

Organic micropollutants loads were mainly found at the section below the effluent of the Sparta WWTP (sites E7 and E8) and, to a lesser degree, the section below the confluence of the Kolliniatiko stream to the main Evrotas stream (sites E3 and E4, up and downstream to the confluence respectively) carrying loads from the upstream Kollines village.

3.2.4. *Sava basin*

In the upper Sava, two multi-stress hot spots at S2 and S4 were identified in water samples. Pollution at Radovljica (S2) was related to upstream local workshops and metalworks, while at Vrhovo (S4) a big hydropower reservoir, receiving pollutants from Sava tributary Boben, impacted by the chemical and glass industry and former chloro-alkali industry constituted the main pollution sources. At middle Sava stretch, a big city Zagreb (S6) is impacted by indicators of urban pollution, such as PhACs and PCPs. At lower Sava stretches Jasenovac (S7), Slavonski Brod (S8), Županja (S9), Sremska Mitrovica (S10), and Beograd (S12) pollutants arising from agricultural activities local oil refineries, heavy metallic industry, site mining industry, and river transport contributed to contamination of the Sava River water. In Beograd (S12) representatives from urban pollution were detected. Due to the same reasons, sediments were contaminated at almost all the

same sampling sites as water samples. Contamination in Litija (S3) sediment during high water discharges was related to flooded riverbanks with slag deposits from former mining activities, while contamination with metals and metalloids and organic microcontaminants at Čatež (S5) were related to agricultural activities. The contamination of sediments by organic microcontaminants at Šabac (S11) was related to urban pollution. At the lower Sava stretches, big fish and predator fish were contaminated with halogenated POPs (dioxin-like substances), which were biomagnified through the food chain.

3.3. Risk-based prioritization of pollutants: identification of river basin specific pollutants (RBSP)

3.3.1. Adige basin

Eight compounds yielding a final risk score (total score) exceeding 0 were considered relevant. The results are presented in Figure 6 and Table S3. The frequency of exceedance (number of sites where the measured concentration exceeds the PNEC), seems to be the dominating factor in the total score. Eight compounds exceeding PNEC are coincident with those of the highest total score. The most relevant pollutants were contaminants of urban origin such as PhACs belonging to the families of psychiatric drugs (carbamazepine and venlafaxine), anti-inflammatories (diclofenac and ibuprofen), and antibiotics (clarithromycin). Another relevant group was insecticides, *i.e.* the neonicotinoid imidacloprid and the persistent halogenated compound 4,4'-DDE (environmental transformation product of 4,4'-DDT). Finally, the PABA derivative 2-ethylhexyl-4-(dimethylamino)benzoate yielded a final score exceeding 0. PABA derivatives are used in sunscreens as a UV filter.

The occurrence of pharmaceuticals and personal care products in Alpine rivers was associated with the seasonal variation of river streamflow (low flow in winter and high flow in summer), as well as the fluctuation of tourist arrivals during the year. In the case of the Adige River, results highlighted the strong tourism impact on the Alpine river quality (Mandaric et al, 2017) and this is also reflected in the highest scores for several pharmaceutical compounds. Diffuse pollution by agriculture in the central and lower course of the Adige River represents a relevant environmental pressure factor. In particular, pesticides have been widely used in the widespread apple trees cultivations. On the other hand, hydroelectric related activities could also have a relevant influence on contaminant loads, both by direct release and flow alterations (hydropeaking effects). Another important pollution source relevant for the upper part of the basin might be the release of persistent

pollutants (e.g., POPs such as DDT) accumulated in the glaciers through long-range atmospheric transport and deposition (Carrera et al. 2001; Villa et al. 2003; Bizzotto et al. 2009; Kirchgeorg et al. 2016), as well as, the discharge of emerging pollutants (e.g., PhACs and PCPs, in particular, UV filters) from the WWTPs serving the ski resorts.

3.3.2. *Evrotas basin*

Nine compounds yielding a total score exceeding 0 were considered relevant. The results are presented in Figure 6 and Table S4. The frequency of exceedance (number of sites where the measured concentration exceeds the PNEC), is the dominating factor in the total score. Eight compounds exceeding PNEC (out of nine) are coincident with those of the highest total score. Concerning the sources, the highest occurrence corresponded to contaminants of mostly agricultural origin such as the herbicides irgarol, diflufenican, and 2,4-dichlorophenoxyacetic acid (2,4-D), and the insecticides azinphos ethyl, methiocarb, thiacloprid, and diazinon. The only compounds of urban origin were some PhACs like the anti-inflammatory ibuprofen and the antibiotic azithromycin.

Such a result is in agreement with the dominant pressures in the Evrotas River being mainly from agricultural activities (predominantly olive and orange tree cultivations), resulting to agrochemical pollution and pollution from the disposal of agro-industrial waste (mainly olive mills), aggravated by the overexploitation of water resources for irrigation that leads to a decrease in flow during the summer months often to the point of the partial desiccation of the river.

3.3.3. *Sava basin*

Fourteen compounds yielding a final score (total score) exceeding 0 were considered relevant. The results are presented in Figure 6 and Table S5. As previously observed in Adige and Evrotas, the frequency of exceedance (number of sites where the measured concentration exceeds the PNEC) is the dominating factor in the Score total. However, in Sava river 8 compounds exceeding PNEC (out of 9) are coincident with those of the highest total score, while the remaining 6 were dominated with the second indicator considering the extent of local effects. Regarding the compounds, the highest relevance was found for contaminants of industrial origin mixed with agricultural and urban. Compounds considered relevant were the following (ranked

according to the Risk Score): the POP halogenated insecticide DDT (mostly 2,4-DDT), together with its transformation products 4,4-DDE and 4,4-DDD, the organophosphate insecticides azinphos ethyl, and diazinon; the herbicides irgarol and diuron; some PCBs (PCB18, 28, 31 and 153), the PAH dibenzo(a,h)anthracene; the anti-inflammatory ibuprofen; the PFC perfluorooctane sulfonate (PFOS); and the PCPs 2-(2-benzotriazolyl)-p-cresol and 4MBC.

The upper reaches are largely influenced by carbonate mineral weathering, the middle by agricultural activity, and biological processes related to eutrophication, while the lower reaches are influenced mostly by stressors related to high pollution from industrial processing along with untreated municipal wastewater discharges. Stressors, which are indicators of urban pollution, such as pharmaceuticals and personal care products are more pronounced downstream of big cities, such as Zagreb. In the middle part and especially after the confluence with the heavily polluted Bosna River, Sava is highly impacted by the oil refinery, heavy metallic industry, site mining industry, and agricultural activities. In the lower part, the river is highly impacted by the oil refinery, heavy industry, and agricultural activities. Moreover, the site in Belgrade is highly influenced by untreated sewage discharge. The area is highly vulnerable to flood events. The excess of nutrients is more pronounced during the low flow regime and is mostly related to untreated sewage discharge. Other stressors related to urban pollution, such as pharmaceuticals, are more pronounced during low water regime.

3.4. Total risk per site

The total risk per site for the three basins studied is shown in Fig. 7, specifying as well the contribution of the different families. RQs greater than 1 indicate a potential ecotoxicological risk for the receiving aquatic ecosystems. The three basins exhibited different profiles and intensities. The highest peak values were found in the Evrotas river (RQ up to 80), notably at sites E01, E02 and E04. The risk was mostly driven by PES, with main compounds being the triazine herbicide irgarol (E04), and the insecticides azinphos-ethyl (site E01), permethrin (sites E02 and E01), and to less extent diazinon. The risk in the Adige basin, with RQ values in the range 2 to 20, was dominated by pharmaceuticals. The highest risk values were located in the upper basin (sites A02 and A03) and the compounds accounting for the greatest amount of risk were the anti-inflammatories diclofenac and ibuprofen, followed by the psychiatric drugs carbamazepine and venlafaxine (see the previous section). The Sava River showed risk quotients up to 20 (sites S06 and S12) but, in contrast

with the other two basins, it exhibited a variety of risk profiles with contributions of the different families, which point to different pollution pressure sources. Thus, for instance, risk at sites S02 and S08 were dominated by the perfluorinated compound PFOS (industrial origin), at site S06 by the insecticide azinphos ethyl and at sites S05, S10, and S12 by the POPs 4,4-DDE and PCB38 (legacy pollutants from agriculture and industrial origin respectively).

4. Conclusions

In the present article, we have described and interpreted the results of an extensive chemical monitoring survey carried out in three Mediterranean rivers, namely the Adige, Evrotas, and Sava Rivers.

The three basins are very different, both in terms of geophysical conditions (drainage area, climate, and hydrology) and anthropogenic pressures and impacts (agricultural, urban, and industrial). This was evident in the respective pollution profiles found, as described in the previous sections. Despite these obvious differences, it is worth mentioning the common effect of treated (or untreated) discharges of urban effluents in the three rivers often giving rise downstream to pollution “hot spots”. This is the case of ski-resorts and Trento in the Adige, Sparta in the Evrotas, and Zagreb, Beograd, and other towns in the Sava. The footprint of this widespread urban pollution was well reflected in the fact that personal care products (PCP’s) and pharmaceutical compounds (PhAc’s) were the families with the highest contribution to organic pollution in the water phase. In turn, persistent organic pollutants (POPs), personal care products (PCP’s), and flame retardants (FR’s) were the highest contributors in sediments. The occurrence of some banned organochlorine POPs (i.e., DDT and PCBs) is remarkable and attributable to long-range atmospheric transport followed by deposition in cold mountain areas (Adige), and legacy or present pollution (Sava). Because of the broad range of polarities covered by the different pollutants analyzed (well reflected in their corresponding physical-chemical properties like the octanol-water partition coefficient K_{ow}), our study highlights the convenience of combining the monitoring of different environmental compartments (water, sediments, and biota) to obtain a comprehensive insight into the pollution patterns of the aquatic systems. Biota (fish) was found to be a good indicator of pollution, with the differences observed in bioaccumulation in the specimens analyzed were more likely attributable to the site-specific pollution rather than the species themselves (Figures 4A and 4B), irrespectively of their feeding habits along the water column (bottom vs. benthic feeders) or their position in

the trophic web (predators *vs.* herbivores-detritivores), clearly exemplified in the case of Salmonids and Cyprinids.

Our chemical monitoring data have been assessed in terms of ecotoxicological risk. This allowed firstly to estimate the ecotoxicological risk per monitoring site summing up of the single risk quotients of the occurring compounds (under the assumption of concentration addition), and secondly, to prioritize the most relevant compounds per basin, that are identified as River Basin Specific Pollutants (RBSP), as required by the WFD.

As regards the first aspect, as one may expect, highly polluted sites in terms of concentration roughly coincide with those exhibiting the higher ecotoxicological risk expressed as RQ. However, it is worth noting that when the main compounds contributing to either concentration or risk are examined and compared the pictures are not always the same. As mentioned, the pollution load in the water phase was dominated by PhACs and PCPs in the three rivers. Contrastingly, the risk was driven only in the Adige by these two pollutant families, while pesticides were the main contributor in the Evrotas, and POPs, PFCs, and PCPs in the Sava. Such a difference emphasizes the convenience of including the ecotoxicological risk assessment as a complementary tool in the interpretation of chemical monitoring data. Finally, compounds identified as RBSPs provide a good picture of the main pressures affecting the basins. Thus, for instance, while the urban pressure is common to the three rivers (reflected in some PhACs like ibuprofen), the contribution of agriculture is remarkable in the Evrotas (pesticides like irgarol, permethryn, azinphos-methyl, etc.), and present or past industrial pollution in the Sava (DDE, PCBs, PFOS).

Both aspects have an obvious interest from the management point of view regarding the implementation of the River Basin Management Plans and the corresponding Programmes of Measures foreseen in the WFD.

Acknowledgments

This work has been supported by the European Communities 7th Framework Programme Funding under Grant agreement no. 603629-ENV-2013-6.2.1-Globaqua and partly by the Generalitat de Catalunya (Consolidate Research Group 2017-SGR-01404) and by the Spanish Ministry of Science, Innovation and Universities (Projects CEX2018-000794-S and IBERAQUA-NET RED2018-102737-T). Special thanks are due to all partners of the GLOBAQUA consortium and the peer review panel for ensuring quality results and a fruitful collaboration within the frame of the project.

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Credit author statement

Marianne Köck-Schulmeyer: data curation, project administration, writing – original draft preparation; **Antoni Ginebreda:** conceptualization, writing – original draft preparation; **Mira Petrovic:** investigation, writing – review & editing; **Monica Giulivo:** investigation; **Òscar Aznar-Alemany:** investigation; **Ethel Eljarrat:** investigation, writing – review & editing; **Jennifer Valle-Sistac:** investigation; **Daniel Molins-Delgado:** investigation; **M.Silvia Diaz-Cruz:** investigation, writing – review & editing; **Luis Simón Monllor-Alcaraz:** investigation; **Nuria Guillem-Argiles:** investigation; **Elena Martínez:** investigation; **Miren López de Alda:** investigation, writing – review & editing; **Marta Llorca:** investigation; **Marinella Farré:** investigation; **Juan Manuel Peña:** investigation; **Ladislav Mandaric:** investigation; **Sanlra Pérez:** investigation; **Bruno Majone:** investigation, writing – review & editing; **Alberto Bellin:** investigation, writing – review & editing; **Eleni Kalogianni:** investigation, writing – review & editing; **Nikolaos Th. Skoulikidis:** investigation, writing – review & editing; **Radmila Milačić:** investigation, writing – review & editing; **Damià Barceló:** conceptualization, supervision.

Declaration of interests

x ☐ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

**PRIORITY AND EMERGING CONTAMINANTS IN THREE MEDITERRANEAN RIVER
BASINS: OCCURRENCE, SPATIAL DISTRIBUTION, AND IDENTIFICATION OF RIVER
BASIN SPECIFIC POLLUTANTS**

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Alcaraz¹, Nuria Guillem-Argiles¹, Elena Martínez¹, Miren López de Arca¹, Marta Llorca¹, Marinella Farré¹,
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Nikolaos Th. Skoulikidis⁴, Radmila Mlačić⁵, Damià Barceló^{1,2}.

Figures

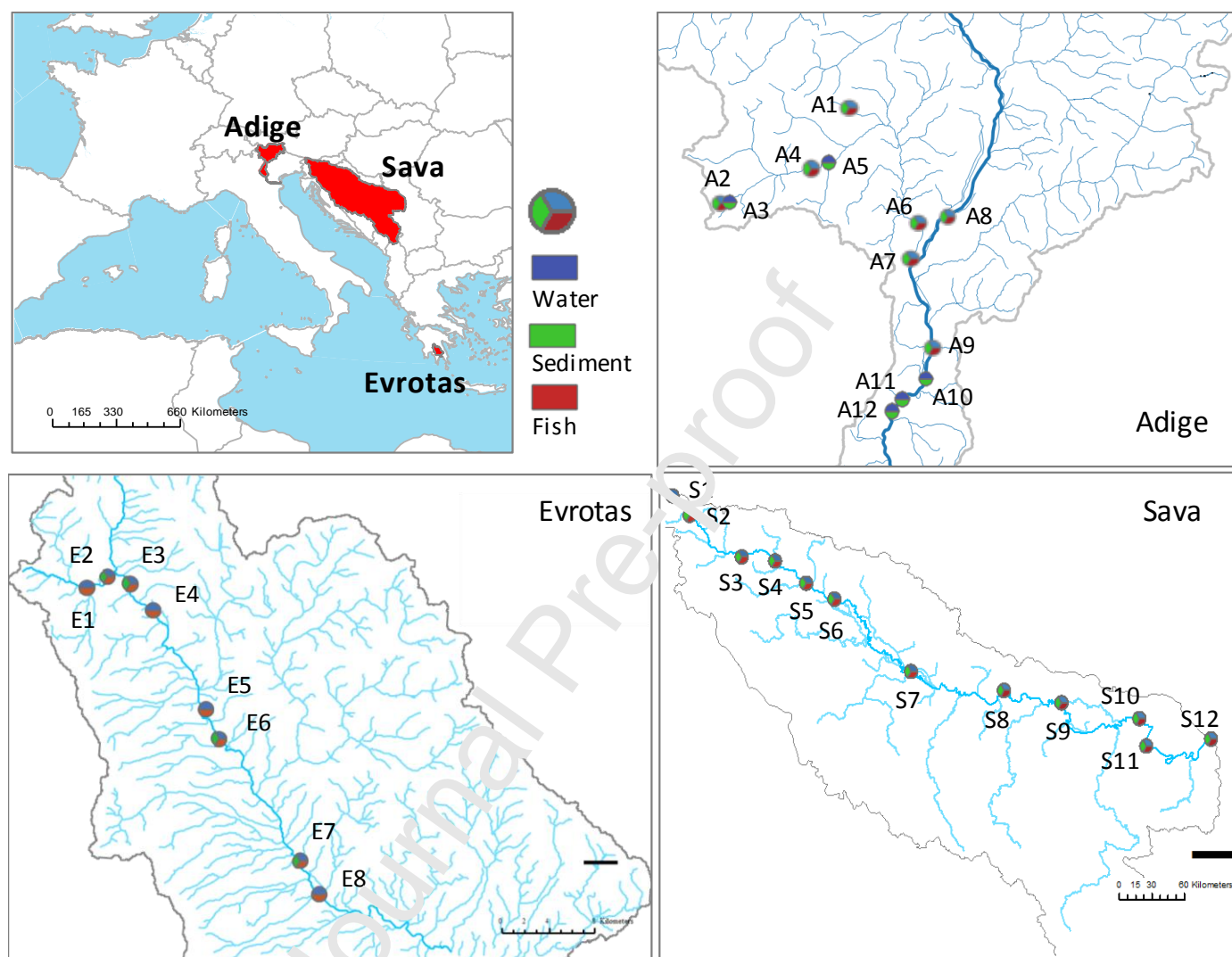


Figure 1. Maps of the river basins showing the location of the sampling points.

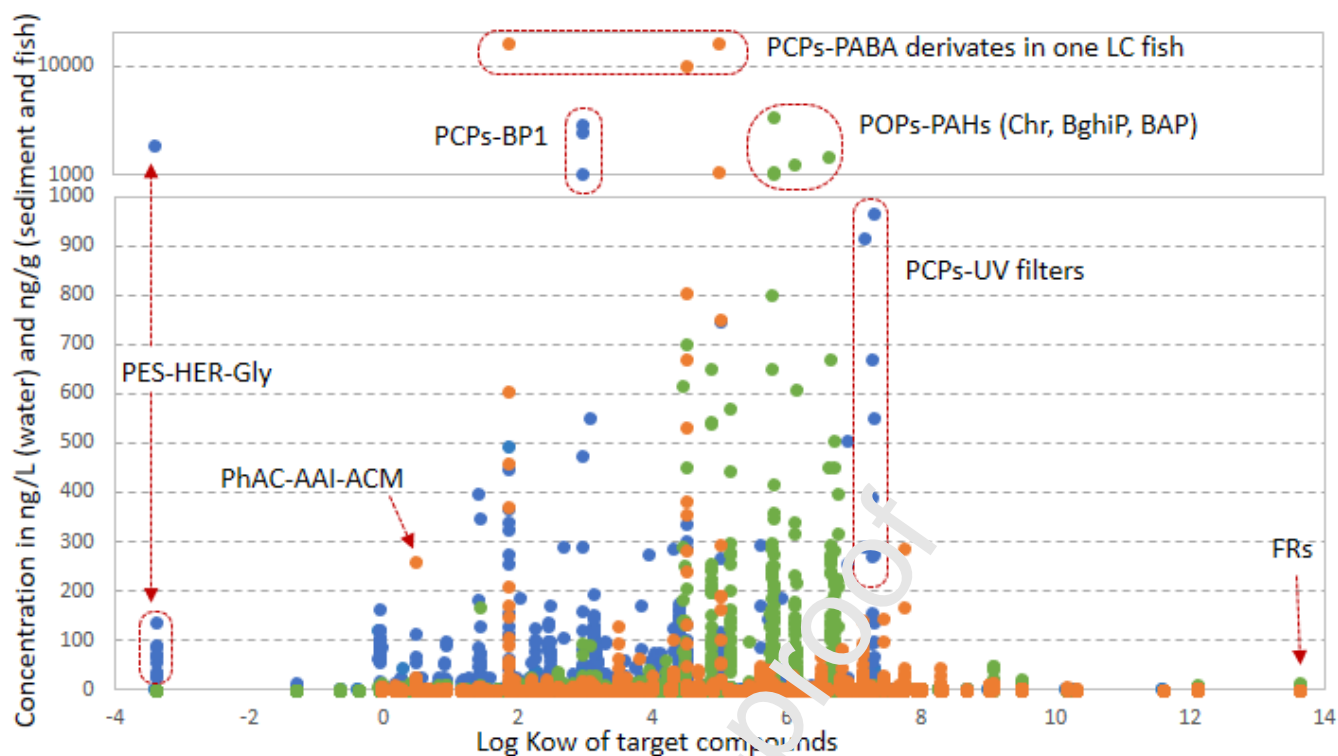


Figure 2. Plot showing the relationships between mean water concentrations vs. Log K_{ow} of each target compound in all individual samples (blue: water; green: sediment; orange: fish). Families and compounds' acronyms are listed in Table S2 (Supplementary Information); LC fish: *Leuciscus cephalus*

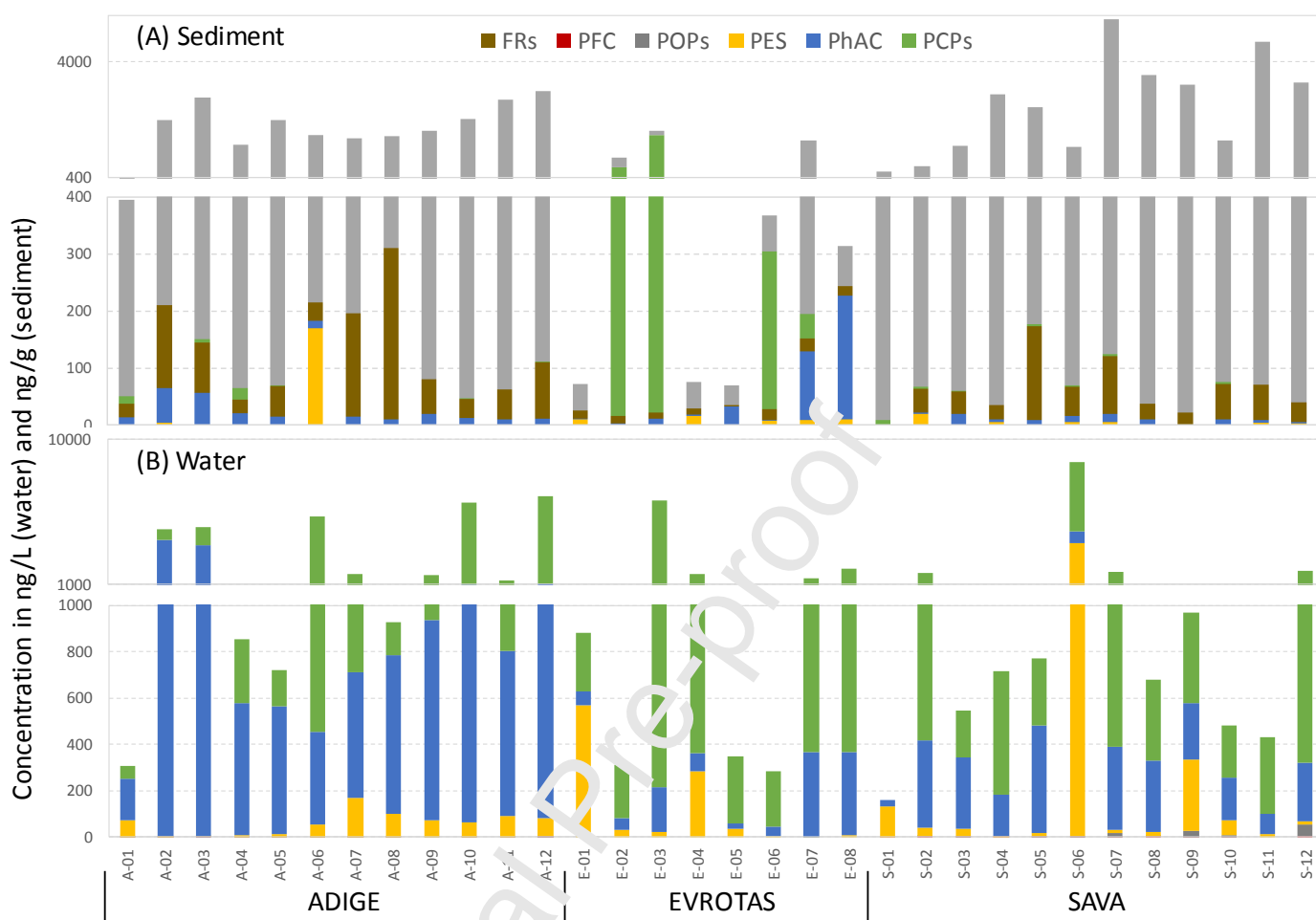


Figure 3. Mean concentration of target pollutants in sediment (A) and water (B) samples from the three river basins. FR, flame retardants; PFC, perfluorinated compounds; POP, persistent organic pollutants; PES, pesticides; PhAC, pharmaceutical active compounds; PCP, personal care products.

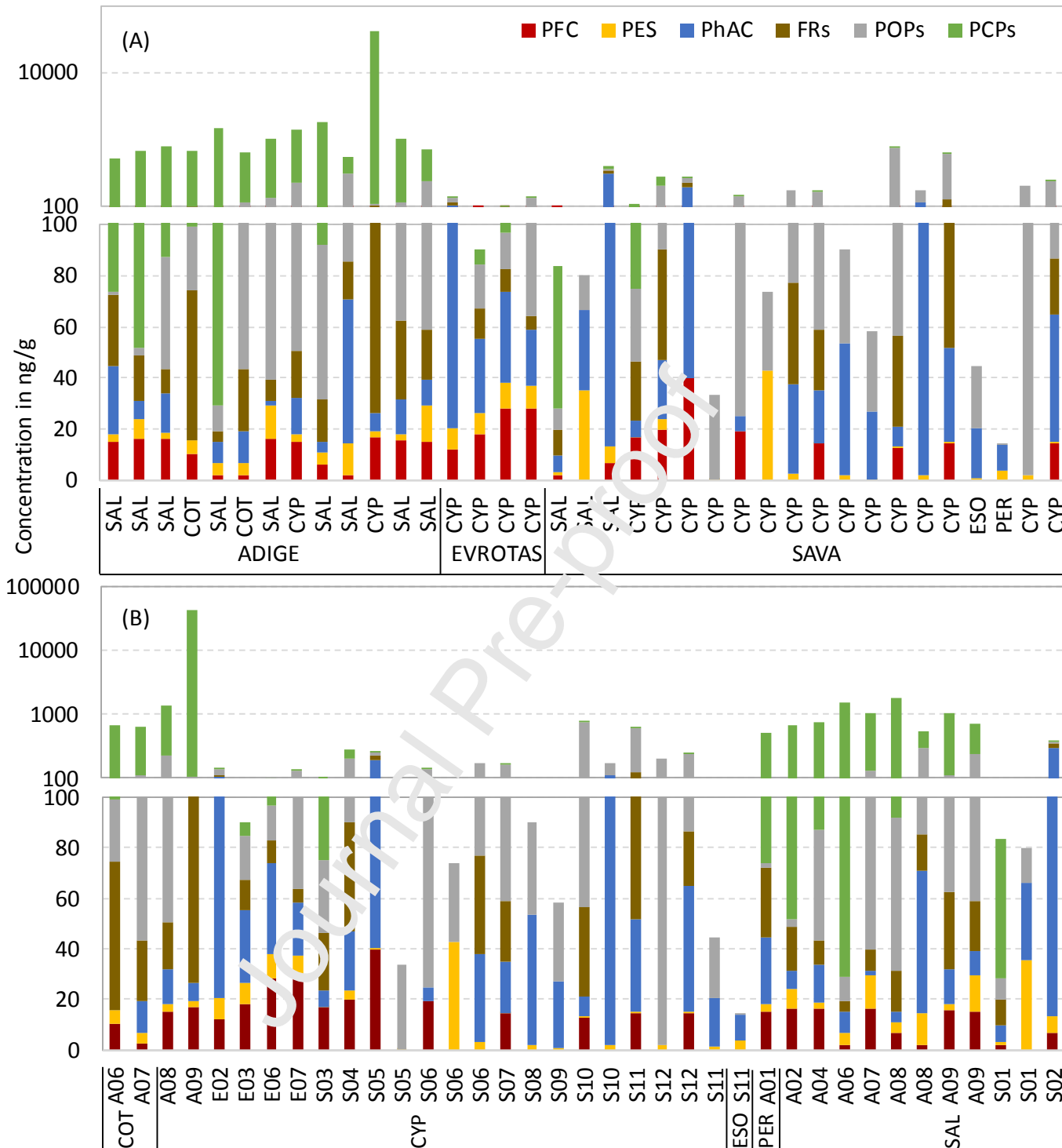


Figure 4. Mean concentration of target pollutants in fish: (A) Per river and (B) per taxa. SAL: Salmonidae; COT: Cottidae; CYP: Cyprinidae; ESO: Esocidae; PER: Percidae. PFC, perfluorinated compounds; PES, pesticides; PhAC, pharmaceutical active compounds; FR, flame retardants; POP, persistent organic pollutants; PCP, personal care products.

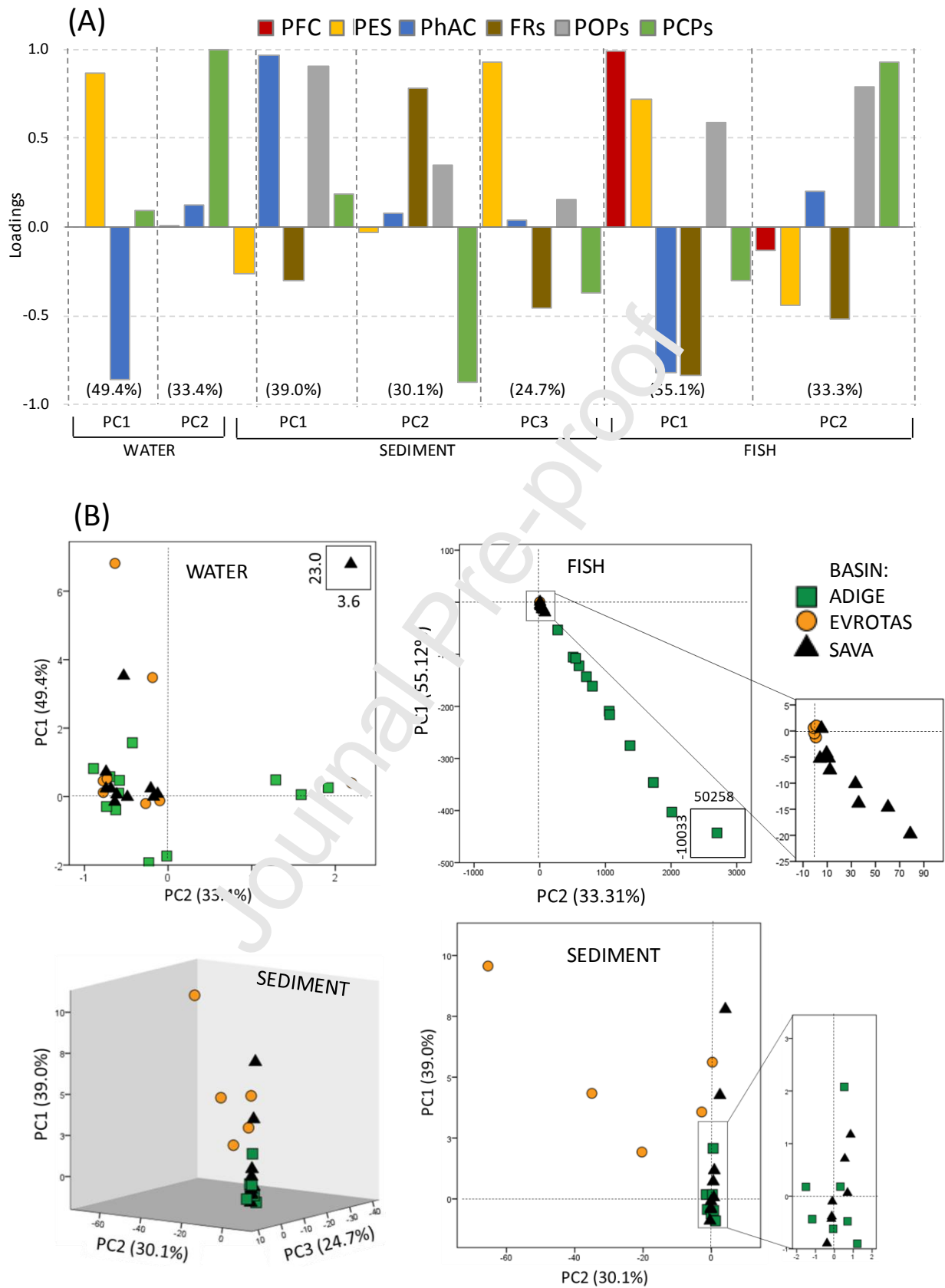


Figure 5. Principal component analysis (PCA) performed using the compound families for the three matrices water, sediments, and fishes: (A) loadings and (B) scores of the first components (two for water and fish and three for sediment). PFC, perfluorinated compounds; PES, pesticides; PhAC, pharmaceutical active compounds; FR, flame retardants; POP, persistent organic pollutants; PCP, personal care products.

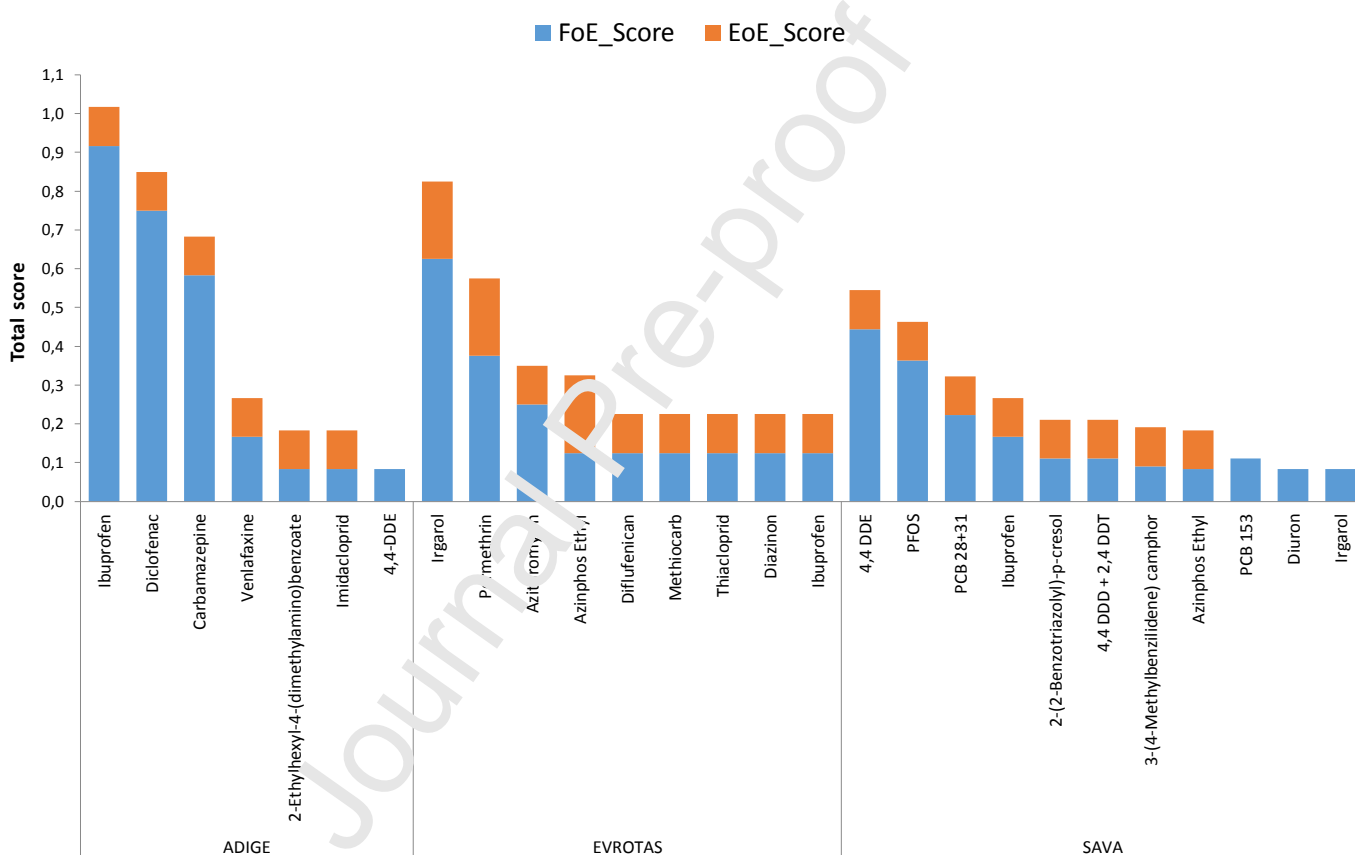


Figure 6. River basin specific pollutants prioritized according to the NORMAN methodology (Section 2.5) for the three investigated rivers.

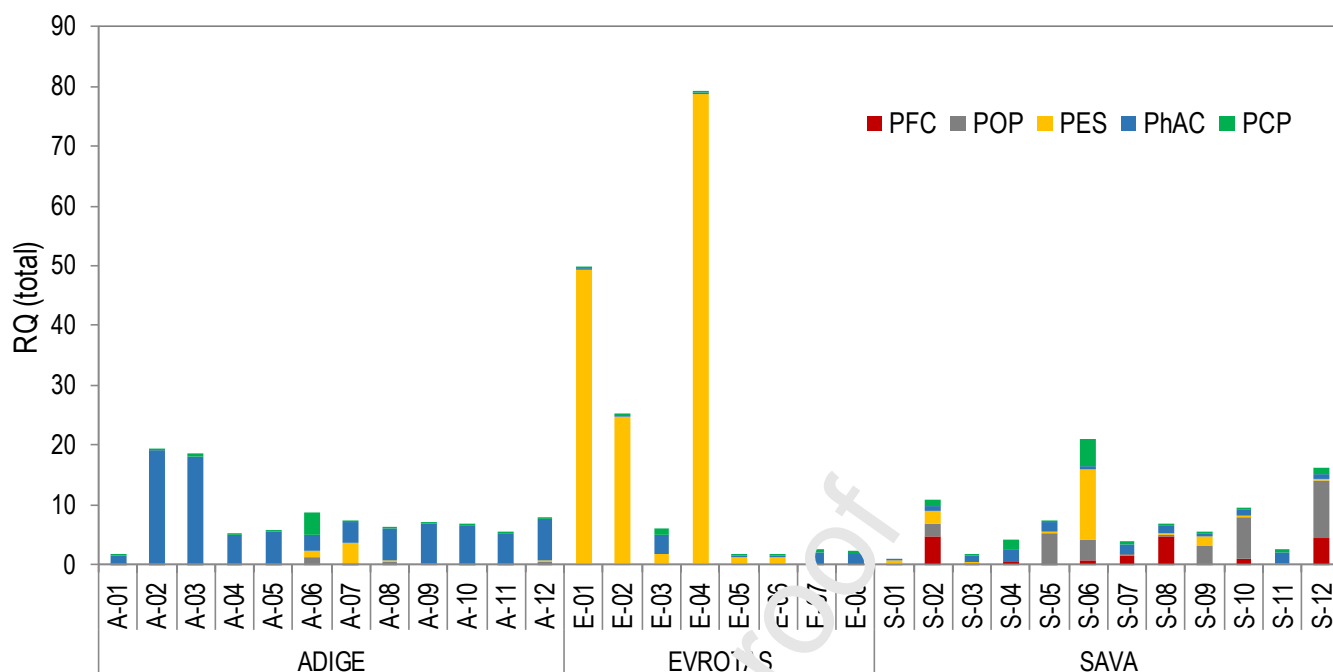
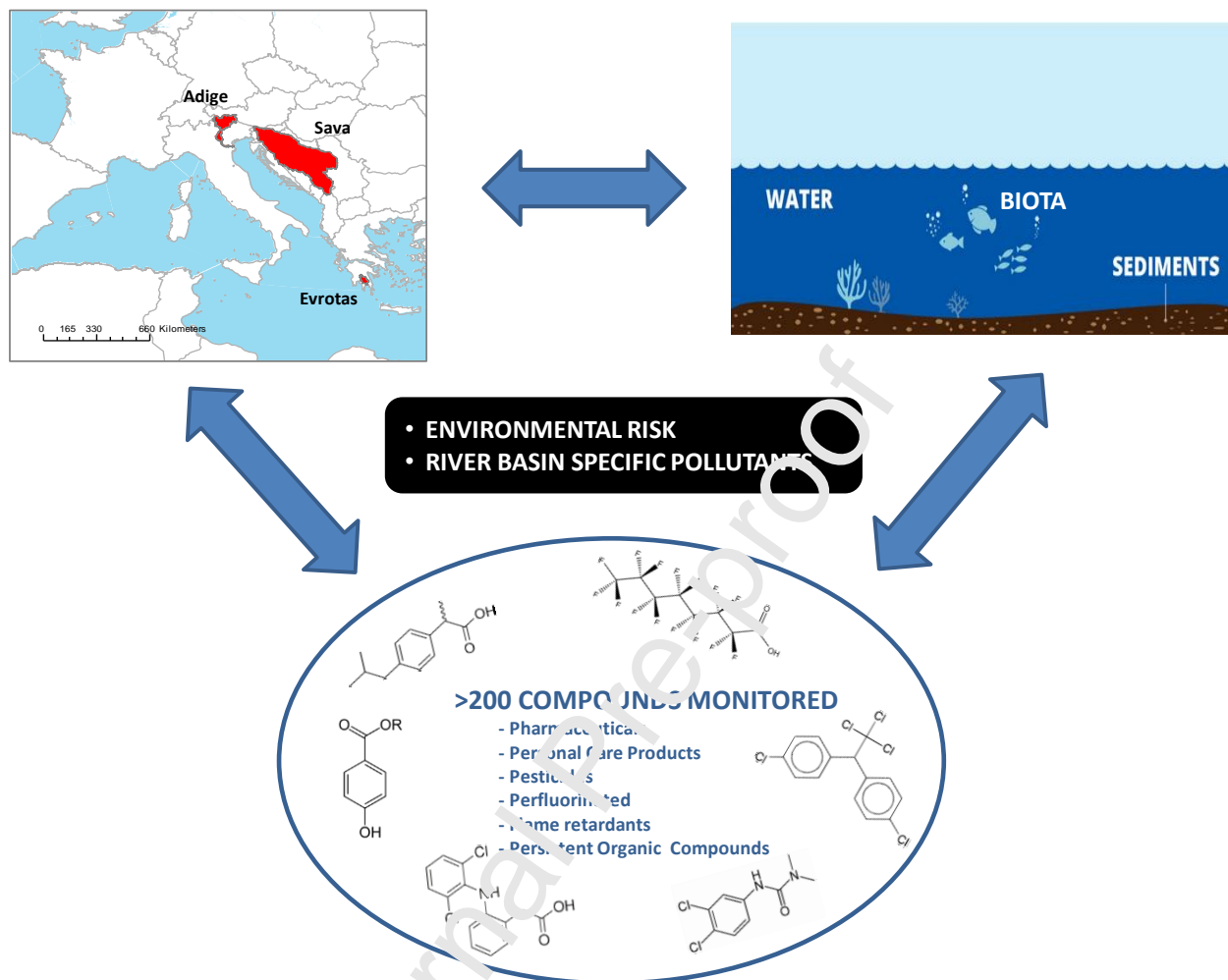


Figure 7: Cumulated risk per site for the three river basins, showing the contribution of the different compound families: PFC, perfluorinated compounds; POP, persistent organic pollutants; PES, pesticides; PhAC, pharmaceutical active compounds; PCP, personal care products.

Graphical abstract:



Highlights:

- **More than 200 emerging contaminants monitored in three Mediterranean River Basins**
- **Three environmental compartments (water, sediments, biota) investigated**
- **River basin “hot spots” characterized through ecotoxicological risk assessment**
- **River basin specific pollutants identified**

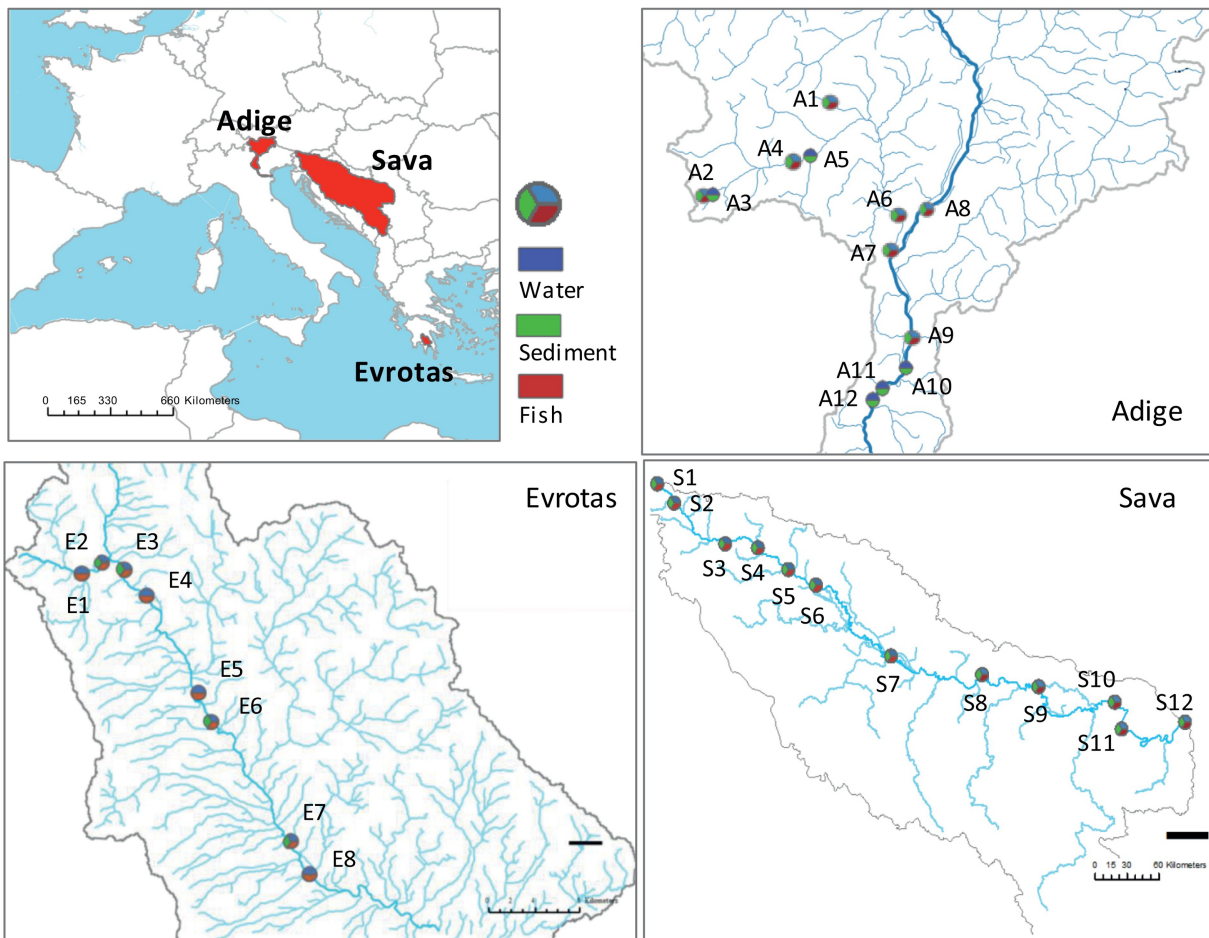


Figure 1

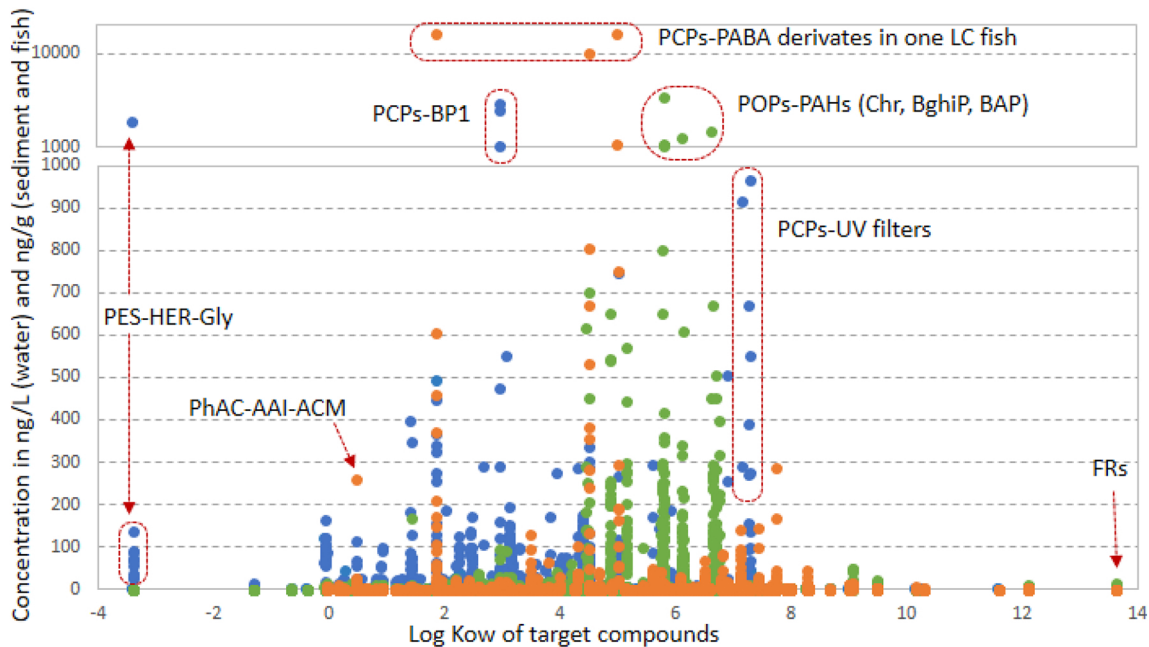


Figure 2

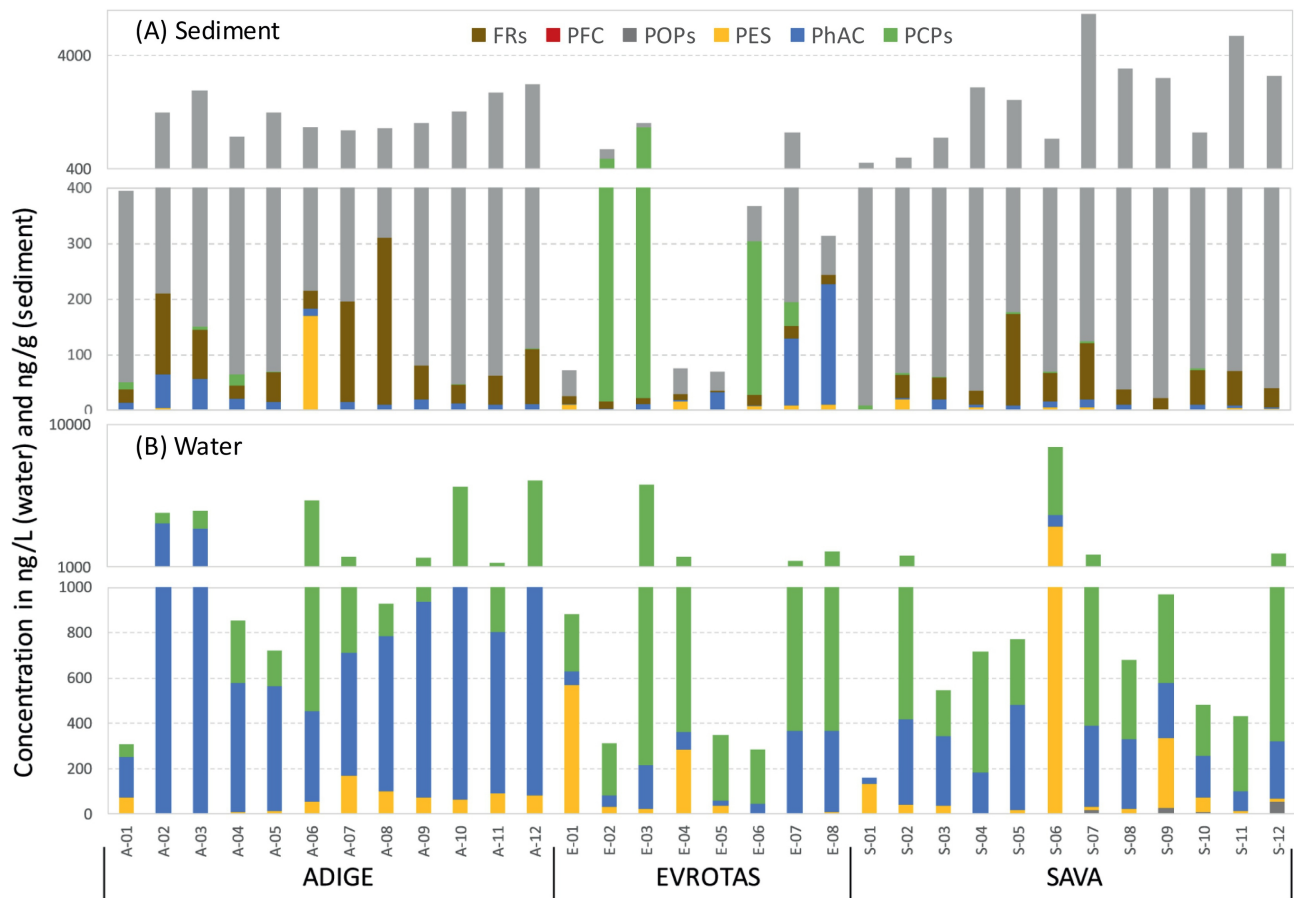


Figure 3

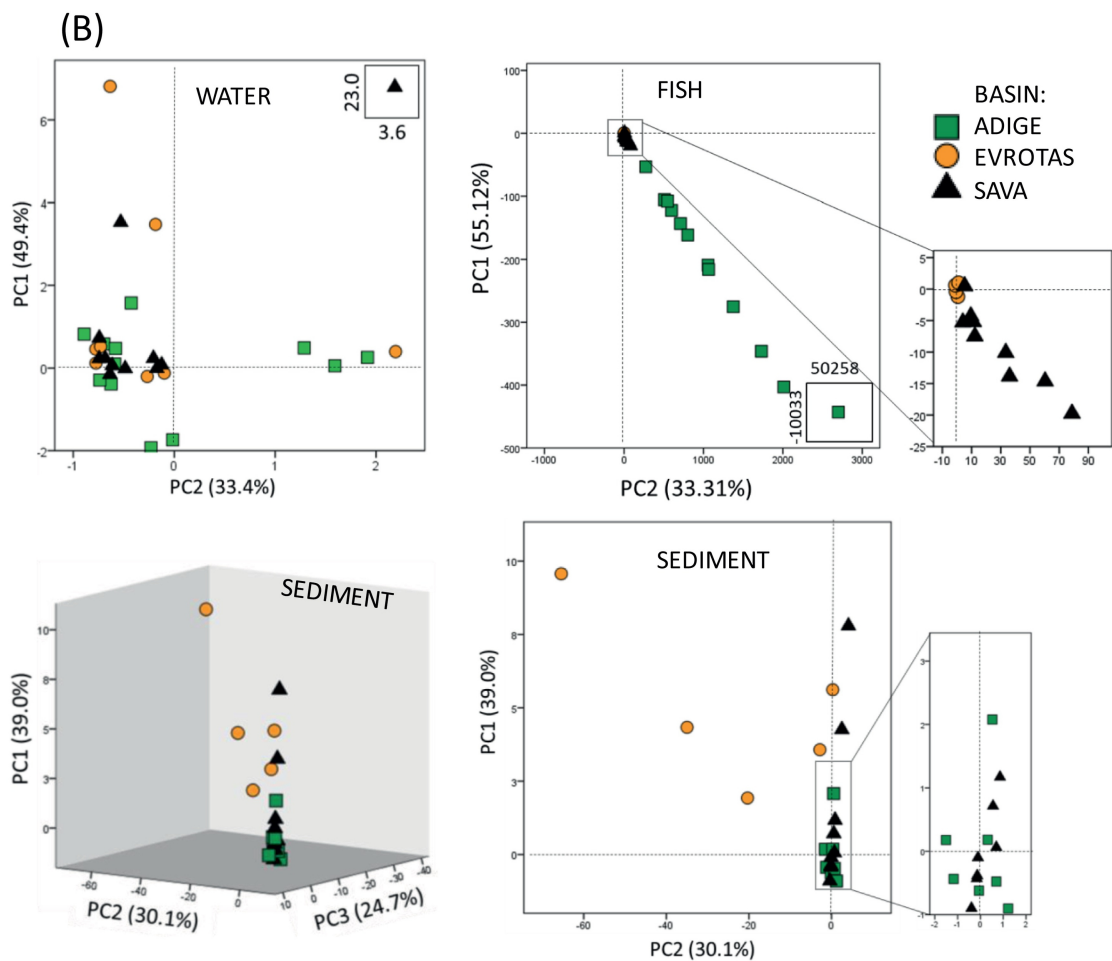
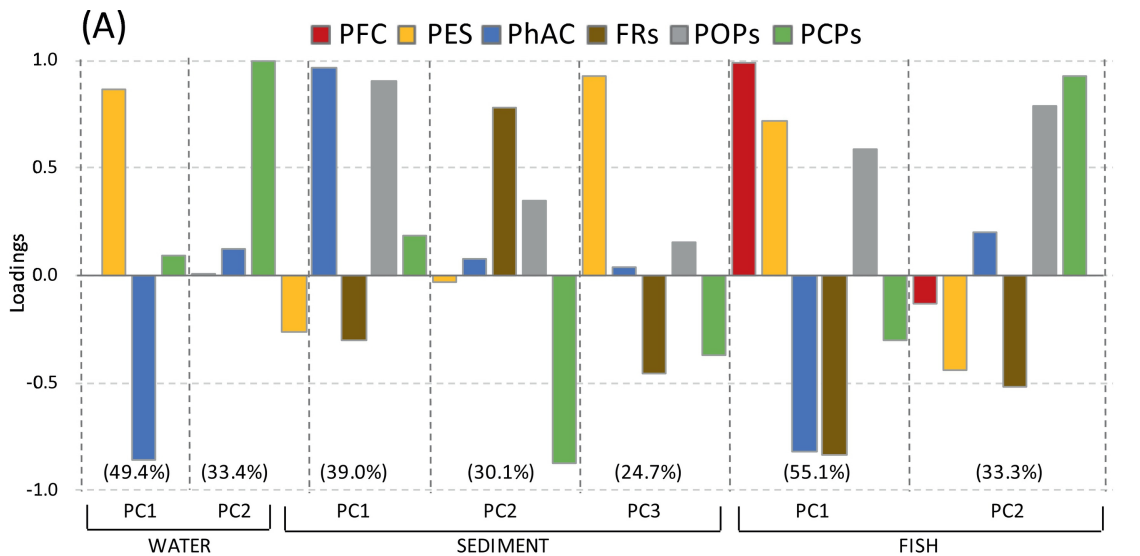


Figure 5

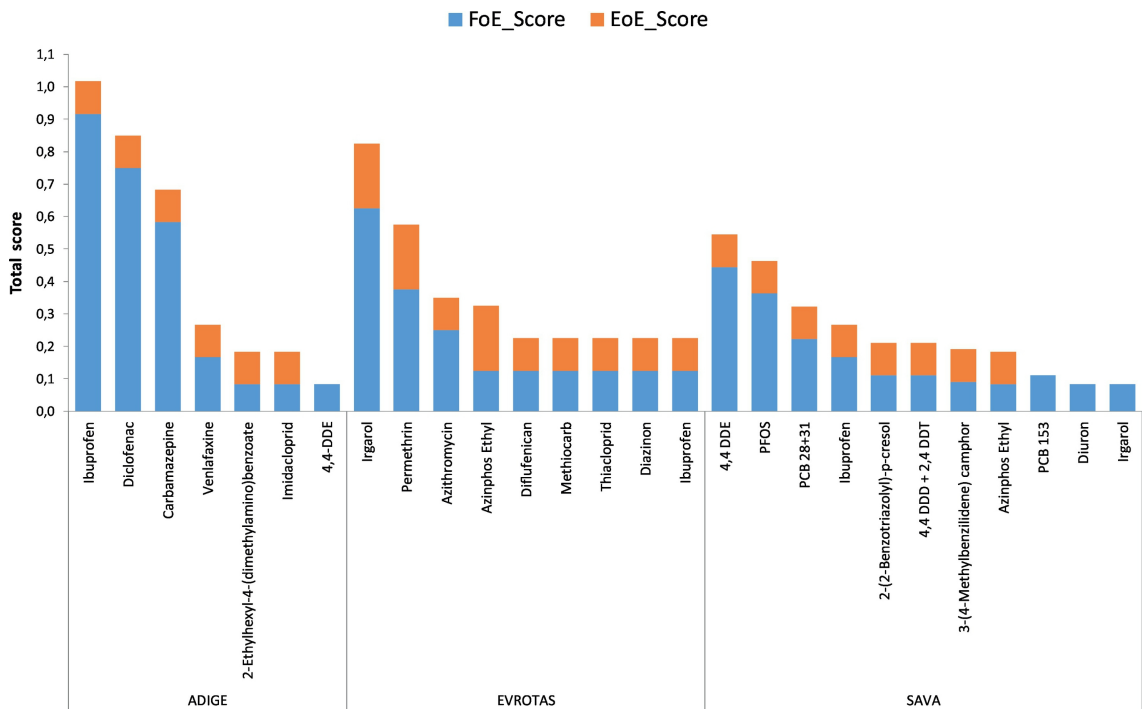


Figure 6

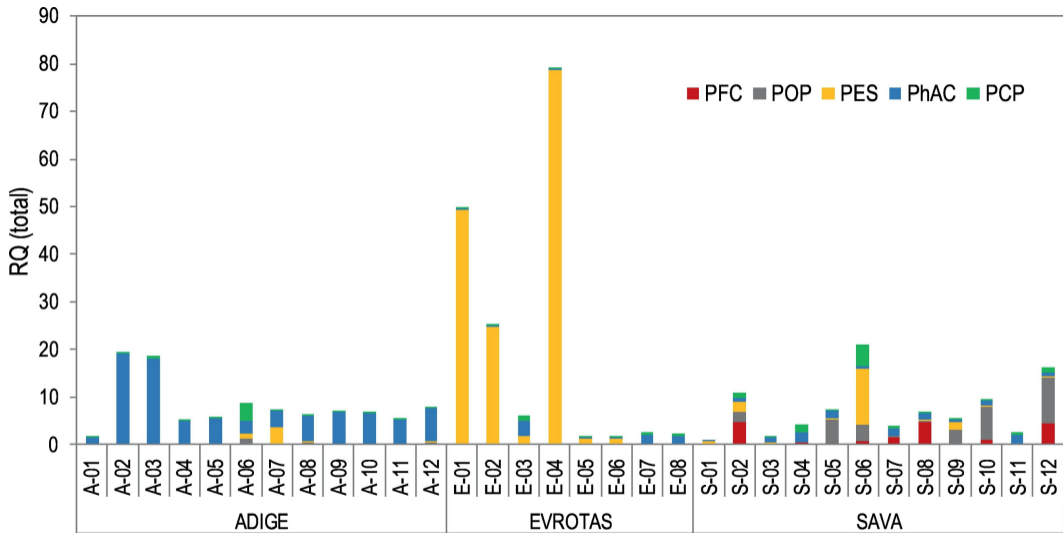


Figure 7