

1 **Drugs of abuse and their metabolites in river sediments: analysis,**
2 **occurrence in four Spanish river basins and environmental risk**
3 **assessment**

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34 **Abstract**

35 The environmental impact produced by the presence of drugs of abuse in sediments has
36 been scarcely studied to date, even though many of them may adsorb onto particulate
37 matter due to their physical-chemical properties. This study presents an analytical
38 method for the determination of 20 drugs of abuse and metabolites in sediments. The
39 validated method was satisfactory in terms of linearity ($r^2 > 0.99$), recovery (90-135%),
40 repeatability (relative standard deviations $< 15\%$), sensitivity (limits of quantification
41 < 2.1 ng/g d.w, except for cannabinoids), and matrix effects (ionization suppression
42 $< 40\%$). The method was applied to the analysis of 144 sediments collected in four
43 Spanish river basins. Cocaine, methadone, and its metabolite 2-ethylidene-1,5-dimethyl-
44 3,3-diphenylpyrrolidine (EDDP) were the most ubiquitous compounds (detection
45 frequencies $> 36\%$), whereas cannabinal, Δ^9 -tetrahydrocannabinol (THC), and
46 methadone were the most abundant compounds (up to 44, 37, and 33 ng/g d.w,
47 respectively). The presence of EDDP, THC, and methadone in the sediments of 28
48 locations may pose a risk to sediment-dwelling organisms. To the author's knowledge,
49 this is the most extensive study conducted so far on the occurrence of drugs of abuse in
50 sediments, and the first time that sediment-water distribution coefficients for EDDP,
51 methadone, MDMA, and diazepam are reported from field observations.

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55 **KEYWORDS:** Illicit drugs; Benzodiazepines; Liquid chromatography-mass
56 spectrometry; Pressurized liquid extraction; Hazard quotients.

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58 **1. Introduction**

59 In recent years, the number of studies conducted to assess the consumption of drugs of
60 abuse has increased worldwide due to the effects that these compounds produce to
61 health (e.g., 585,000 people died as a consequence of illicit drug use in 2017 (UNODC,
62 2018)). Likewise, their occurrence and fate in the environment have increasingly
63 become a matter of scientific concern. The release of these substances into the aquatic
64 environment is directly related to their consumption, production, and direct disposal into
65 the sewage system or other water compartments. In the best-case scenario, these
66 substances and/or their metabolites are collected in the sewage network and conducted
67 to a wastewater treatment plant (WWTP) where they are only partially eliminated
68 (Baker and Kasprzyk-Hordern, 2013). In the receiving water bodies, the concentrations
69 present in the WWTP effluents are diluted to different extents, and with that the
70 negative effects that they can cause on the aquatic organisms (Postigo et al., 2012).
71 However, in areas that suffer water scarcity, the dilution factor is very low, and during
72 drought or low-flow periods WWTP discharges represent the largest fraction of the total
73 river flow, and thus, the effects of these substances on the aquatic ecosystem functions
74 may be of relevance (Navarro-Ortega et al., 2012).

75 The occurrence of illicit drugs and their metabolites in surface water, including rivers,
76 streams, lakes, and creeks has been extensively studied worldwide (Pal et al., 2013;
77 Yadav et al., 2017). From these studies, it can be concluded that the illicit drugs and
78 metabolites most commonly detected in surface waters are benzoylecgonine, cocaine,
79 norcocaine, norbenzoylecgonine, cocaethylene, amphetamine, methamphetamine, 3,4-
80 methylenedioxyamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA),
81 morphine, cannabis, codeine, methadone, and 2-ethylidene-1,5-dimethyl-3,3-
82 diphenylpyrrolidine (EDDP). All of them are usually measured at concentrations below
83 100 ng/L, except for benzoylecgonine, amphetamine, and codeine that have been found
84 at concentrations up to 316 ng/L (González-Mariño et al., 2010), 309 ng/L (Martínez
85 Bueno et al., 2011), and 341 ng/L (Baker and Kasprzyk-Hordern, 2013), respectively.
86 The occurrence of legal drugs of abuse, like benzodiazepines and the antidepressant
87 citalopram, in water was reviewed by Cunha et al. (Cunha et al., 2017).
88 Benzodiazepines, the most commonly prescribed psychoactive pharmaceuticals in 2018
89 (INCB, 2019), are overall more abundant in surface water than illicit drugs, reaching
90 occasionally the $\mu\text{g/L}$ level, as it was the case for alprazolam in the Cascavel River,

91 Brazil (5,900 ng/L) (Nunes et al., 2015), and oxazepam (1,400 ng/L) in the Vilaine
92 River basin, France (Piel et al., 2013). Given their overall medium to high polarity, it is
93 expected that these compounds remain in the aqueous phase and for this reason, most of
94 the studies conducted so far have focused on environmental water matrices. However,
95 some of these substances, such as cannabinoids and the opioid methadone and its main
96 metabolite EDDP, present hydrophobic properties ($\log K_{ow} > 3$) that make them
97 susceptible to adsorb onto organic-rich solid matrices (Postigo et al., 2010).

98 Sediments can accumulate a large variety of organic contaminants and consequently,
99 they become contaminant sources during re-suspension processes (Matić Bujagić et al.,
100 2019). To date, very few studies have investigated the occurrence of drugs of abuse in
101 sediments, and overall, they were multi-residue studies that included a limited number
102 of drugs of abuse. The illicit drugs and metabolites most commonly investigated in
103 sediments so far are cocaine, benzoylecgonine, amphetamine, methamphetamine,
104 methadone, and Δ^9 -tetrahydrocannabinol (THC). They were found at concentrations
105 ranging from not detectable to 200 ng/g (Álvarez-Ruiz et al., 2015; Carmona et al.,
106 2017; Klosterhaus et al., 2013; Langford et al., 2011; Wilkinson et al., 2018). Similar
107 concentrations were also measured in sediments for the benzodiazepines alprazolam,
108 diazepam, and lorazepam (Beretta et al., 2014; Matic Bujagic et al., 2019; Picó et al.,
109 2020; Vazquez-Roig et al., 2012).

110 Given the low concentrations of this type of compounds in sediments, it is necessary to
111 apply highly sensitive and selective analytical methodologies for their determination.
112 Extraction of these substances from solid matrices has been achieved with pressurized
113 liquid extraction (PLE) (Arbeláez et al., 2014; Baker and Kasprzyk-Hordern, 2011;
114 Langford et al., 2011; Mastroianni et al., 2013; Senta et al., 2013), ultrasonic-assisted
115 extraction (UAE) (Álvarez-Ruiz et al., 2015; Carmona et al., 2017; Gago-Ferrero et al.,
116 2015; Wilkinson et al., 2018) or solid-liquid extraction (SLE) (Klosterhaus et al., 2013).
117 PLE was the preferred technique because of its high extraction efficiency, due to the
118 application of high temperature and pressure, and automation, which leads to highly
119 reproducible results and allows saving time and solvent consumption (Álvarez-Ruiz et
120 al., 2015; Biel-Maeso et al., 2017; Montesano et al., 2017). The extracts obtained need
121 to be cleaned-up before their analysis. Extract clean-up has been accomplished using
122 solid-phase extraction (SPE), while analyte determination has been commonly done
123 with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

124 The objective of this work was to validate an analytical methodology based on PLE
125 extraction, SPE clean-up and LC-MS/MS determination for the simultaneous analysis of
126 20 drugs of abuse and metabolites in sediment samples and to apply this method to the
127 analysis of 144 river sediment samples collected along four Spanish rivers in two
128 sampling campaigns to (i) study the occurrence and distinct geographical and temporal
129 distribution of the target drugs of abuse among river basins and between sampling
130 campaigns, as well as between the water and sediment compartments, and (ii) assess the
131 environmental risk posed by them to aquatic organisms, as well as the compounds of
132 highest concern, by applying the hazard quotient (HQ) approach. The sampled areas
133 were selected because of their Mediterranean character, which makes them subject to
134 water scarcity periods and prone to a greater accumulation of emerging pollutants.
135 Moreover, a previous study had revealed the presence of up to 80% of the targeted
136 drugs of abuse in the water of these river basins at maximum concentrations of 144 ng/L
137 (Mastroianni et al., 2016).

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139 **2. Material and methods**

140 **2.1. Reagents and materials**

141 The compounds investigated included cocaine-related compounds (cocaine (COC) and
142 its metabolites benzoylecgonine (BE) and cocaethylene (CE)), amphetamine-type
143 stimulants (ATS) (amphetamine (AM), methamphetamine (MA), 3,4-
144 methylenedioxymethamphetamine (MDMA), ephedrine (EPH)), opiates/opioids
145 (morphine (MOR), heroin (HER) and its exclusive metabolite 6-acetylmorphine
146 (6ACM), methadone (METH) and its metabolite EDDP), hallucinogens (lysergic acid
147 diethylamide (LSD) and its metabolite 2-oxo-3-hydroxy-LSD (OH-LSD)), cannabinoids
148 (THC, its metabolite 11-hydroxy- Δ^9 -THC (OH-THC), cannabidiol (CBD), and
149 cannabiol (CBN)), and benzodiazepines (alprazolam (ALP) and diazepam (DIA)).
150 Unfortunately, 11-nor-9-carboxy- Δ^9 -THC (THC-COOH), the THC metabolite most
151 investigated in environmental samples, was not included in the method because the
152 analytical standard was not available in the lab at the time of the study. The main
153 physical-chemical properties of these compounds are provided in Table 1.

154 **Table 1.** CAS number, main physical-chemical properties, and predicted no-effect concentration (PNEC) of the target analytes in water and
 155 sediments.

Compound	CAS number	Molecular formula	Molecular weight	Log K _{ow} ^a	Log K _{oc} ^a	PNEC _{water} (µg/L) ^b	PNEC _{sed} (ng/g) ^b
Cocaine (COC)	50-36-2	C ₁₇ H ₂₁ NO ₄	303.35	2.30	3.28	2.28 ^c	3.65 ^c
Benzoylcegonine (BE)	519-09-5	C ₁₆ H ₁₉ NO ₄	289.33	-1.32*	2.55	2.33	3.73
Cocaethylene (CE)	529-38-4	C ₁₈ H ₂₃ NO ₄	317.38	2.66*	3.54	1.55	2.48
Amphetamine (AM)	300-62-9	C ₉ H ₁₃ N	135.21	1.76	3.05	24.80	39.66
Methamphetamine (MA)	537-46-2	C ₁₀ H ₁₅ N	149.23	2.07	3.21	9.74	15.57
3,4-methylenedioxymethamphetamine (MDMA)	537-46-2	C ₁₁ H ₁₅ NO ₂	193.24	2.28	2.70	47.60	76.11
Ephedrine (EPH)	299-42-3	C ₁₀ H ₁₅ NO	165.23	1.13	1.92	69.90	111.77
Morphine (MOR)	57-27-2	C ₁₇ H ₁₉ NO ₃	285.34	0.89	3.47	5.38	8.60
6-acetylmorphine (6ACM)	2784-73-8	C ₁₉ H ₂₁ NO ₄	327.37	1.55	4.42	3.33	5.32
Heroin (HER)	561-27-3	C ₂₁ H ₂₃ NO ₅	369.41	1.58	3.86	0.53	0.85
Methadone (METH)	76-99-3	C ₂₁ H ₂₇ NO	309.45	3.93	4.86	0.84	1.34
2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	30223-73-5	C ₂₀ H ₂₃ N	277.40	4.94*	5.67	0.14	0.22
Lysergic acid diethylamide (LSD)	50-37-3	C ₂₀ H ₂₅ N ₃ O	323.43	2.95	5.38	0.39	0.62
2-oxo-3-hydroxy-LSD (OH-LSD)	111295-09-1	C ₂₀ H ₂₅ N ₃ O ₃	355.43	0.39*	2.68	-	-
Δ ⁹ -tetrahydrocannabinol (THC)	1972-08-3	C ₂₁ H ₃₀ O ₂	314.46	6.97	5.79	0.07	0.12
11-hydroxy-Δ ⁹ -tetrahydrocannabinol (OH-THC)	36557-05-8	C ₂₁ H ₃₀ O ₃	330.46	5.33	4.55	0.28	0.45
Cannabidiol (CBD)	74219-29-7	C ₂₁ H ₃₀ O ₂	314.46	8.01*	6.44	0.17	0.27
Cannabinol (CBN)	521-35-7	C ₂₁ H ₂₆ O ₂	310.43	7.23*	5.79	0.08	0.13
Alprazolam (ALP)	28981-97-7	C ₁₇ H ₁₃ ClN ₄	308.77	2.12	6.33	0.08	0.12
Diazepam (DIA)	439-14-5	C ₁₆ H ₁₃ ClN ₂ O	284.74	2.82	4.05	0.29	0.46

156 ^a Data were obtained from the ChemSpider database. Predicted data were generated using the US Environmental Protection Agency's EPISuiteTM

157 (* Estimated)

158 ^b Data were obtained from NORMAN Ecotoxicology DataBase

159 ^c PNEC_{water} obtained from Mendoza et al. (2014) and PNEC_{sed} by applying the following equation: $PNEC_{sed} = PNEC_{water} * 2.6 * (0.615 + 0.019 * K_{oc})$

High-purity (> 97%) standard solutions of the above-mentioned target compounds and isotopically labeled analogs were purchased from Cerilliant (Round Rock, TX, USA) as solutions in methanol (MeOH) or acetonitrile (ACN) at a concentration of 1 mg/mL or 0.1 mg/mL.

Working standard mixture solutions were prepared in MeOH at different concentrations in the range of 0.1 and 1,000 ng/mL by appropriate dilution of individual stock solutions. All of them contained the isotopically labeled compounds at a fixed concentration so that they could be used as surrogate standards (SS) in the quantification process. 6ACM-d₆, AM-d₅, CE-d₃, EDDP-d₃, EPH-d₃, HER-d₉, LSD-d₃, MA-d₁₄ and MDMA-d₅ were added at a final concentration of 20 ng/mL, whereas ALP-d₅, BE-d₃, CBD-d₃, COC-d₃, DIA-d₅, METH-d₃, MOR-d₃, OH-THC-d₃ and THC-d₃ were added at 50 ng/mL. All standard solutions were stored in the dark at – 20 °C until use.

All solvents used were HPLC-grade and were supplied by Merck (Darmstadt, Germany), as well as formic acid (> 98%) and activated neutral aluminum oxide (Al₂O₃) (99% purity). Ammonium formate (> 99%) used as a mobile phase modifier was purchased from Fluka Analytical (Sigma Aldrich).

Cellulose filters (0.45 µm pore size) placed in PLE cells to prevent the transfer of fine particles into the extract and plugging of the system were purchased from Dionex Corporation (Sunnyvale, CA, USA). Evolute ABN cartridges (50 µm, 200 mg, 6 mL) used for SPE clean-up were provided by Biotage (Uppsala, Sweden).

Nitrogen gas (99.995%) used for extract evaporation was produced by a nitrogen generator system (Centralair, San Sebastian, Spain).

2.2. Sample collection

A total of 144 river sediment samples were collected as grab samples from 75 different locations (Figure 1) along four Spanish river basins, namely, Llobregat, Ebro, Jucar, and Guadalquivir, during two sampling campaigns conducted in September, October and November 2010 and 2011. Sediments were collected with a van Veen drag and placed in an aluminum tray that was wrapped with aluminum foil. They were kept at 4°C during transport to the laboratory. Once in the laboratory, sediments were freeze-dried

using a LyoAlfa 6-50 freeze-drier (Telstar S.A., Barcelona, Spain), finely ground with a mortar, and sieved through 125 μm mesh to obtain a homogeneous sediment sample. Finally, samples were stored at $-20\text{ }^{\circ}\text{C}$ until analysis. Table 2 shows the total organic carbon (%) content of the sediment samples collected during 2011.

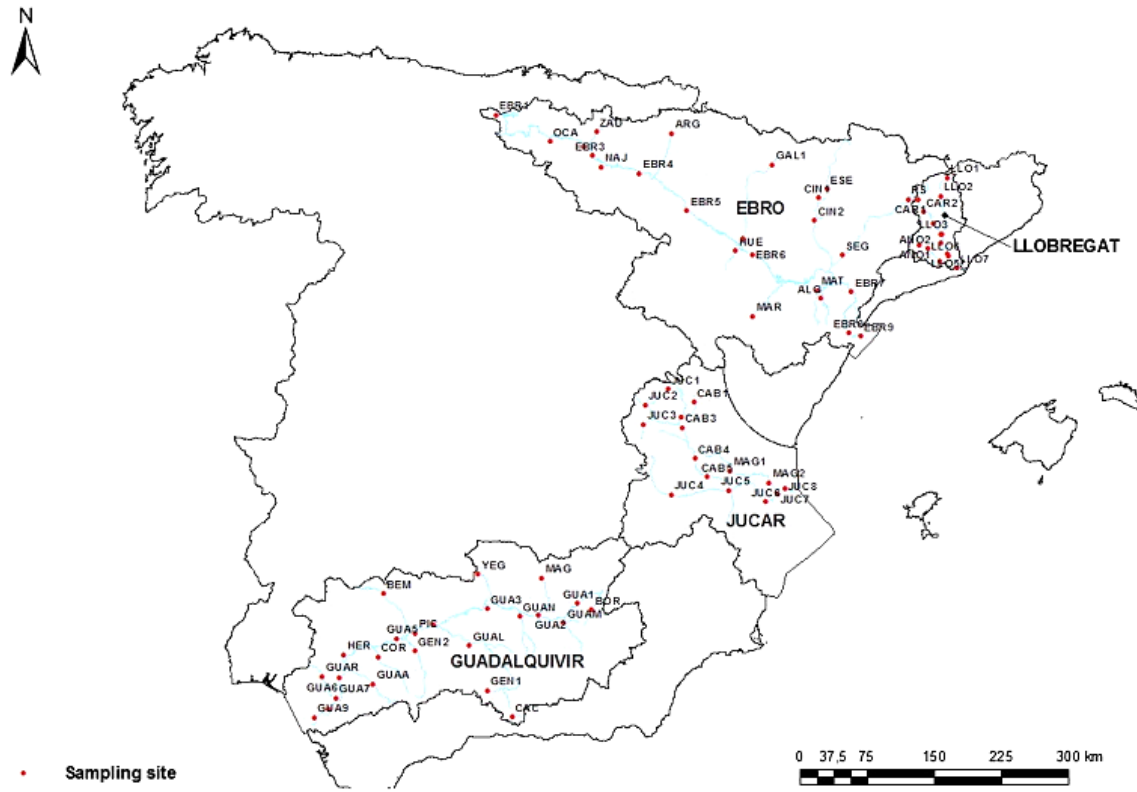


Figure 1. Map showing the location of the sediment sampling stations in each river basin.

Table 2. Total organic carbon (TOC, % C) content of the sediment samples collected during the 2011 sampling campaign.

	Main River	TOC (% C)	Tributary River	TOC (% C)	Tributary River	TOC (% C)
Llobregat basin	LLO1	1.33	CAR1	2.1		
	LLO2	2.24	CAR2	2.81		
	LLO3	1.25	CAR3	1.31		
	LLO4	2.03	CAR4	1.82		
	LLO5	0.56	ANO1	1.06		
	LLO6	0.65	ANO2	4.79		
	LLO7	2.14	ANO3	1.23		
Ebro basin	EBRO1	2.78	OCA	1.98	ESE	0.34
	EBRO2	2.58	ZAD	5.22	CIN1	0.84
	EBRO3	3.95	NAJ	2.85	CIN2	1.83
	EBRO4	1.35	ARG	1.14	RS	2.98
	EBRO5	2.27	GAL1	0.42	SEG	4.86
	EBRO6	3.77	GAL2	2.53	MAT	2.42
	EBRO7	3.05	HUE	1.23	ALG	0.56
	EBRO9	0.71	MAR	2.82		
Jucar basin	JUC1	1.20	CAB1	3.83		
	JUC2	0.65	CAB2	1.89		
	JUC3	1.99	CAB3	1.41		
	JUC4	0.96	CAB4	1.95		
	JUC5	3.43	CAB5	0.85		
	JUC6	0.51	MAG1	2.94		
	JUC7	2.43	MAG2	1.92		
	JUC8	2.55				
Guadalquivir basin	GUA1	0.79	BOR	2.19	GUA-A	1.07
	GUA2	0.68	GUA-M	0.63	GUA-R	1.87
	GUA3	0.69	MAG	0.77		
	GUA4	1.20	GUA-N	0.63		
	GUA5	0.98	YEG	3.43		
	GUA6	0.67	GUA-L	0.84		
	GUA7	0.39	PIC	0.50		
	GUA8	1.14	BEM	0.41		
	GUA9	0.88	CAC	1.00		
			GEN1	0.68		
			GEN2	1.52		
			COR	0.59		
			HER	1.07		

2.3. Sample preparation

Preparation of sediment samples was performed following an analytical methodology previously described for the determination of drugs of abuse in sewage sludge samples (Mastroianni et al., 2013).

Briefly, 1 g of freeze-dried river sediment was transferred into 11 mL stainless extraction cells containing a cellulose filter at the bottom of the cell and partially filled with activated Al_2O_3 (approximately 5 g, activated at 350 °C during 15 min). Then, the SS mixture was added (10 ng and 25 ng of the SS present in the calibration curve at a concentration of 20 ng/mL and 50 ng/mL, respectively (see section 2.1), to ensure the same SS concentration in the final extract and the calibration curve). Cells were left overnight under a fume hood at room temperature to allow interaction of the SS with the matrix and methanol evaporation. The next day, cells were filled up with activated Al_2O_3 and covered with another cellulose filter. PLE was done using an ASE 200 (Dionex Corporation, Sunnyvale, CA, USA). The PLE conditions applied to the extraction process were: pressure, 1250 psi; temperature, 50°C; preheating time, heating time and static time, 5 min each, number of static cycles, one; flush volume, 60%; and purge time, 1 min. The extraction solvent used was a mixture of MeOH/H₂O (9:1, v/v).

The PLE extract obtained (about 14 mL) was evaporated to an approximate volume of 1.5 mL under a gentle stream of N₂ with a TurboVap LV evaporator (Zymark, Hopkinton, MA, USA), diluted with HPLC-grade water to a final volume of 25 mL, and purified through SPE with an SPE vacuum manifold (J.T. Baker, The Netherlands) using a polymeric Evolute ABN cartridge (200 mg, 6 mL), and a gravity-assisted flow. Before extract loading, the cartridge was sequentially conditioned and equilibrated with 6 mL of MeOH and 6 mL of H₂O. After loading the extract, the sorbent was washed with 3 mL of H₂O followed by 3 mL of a mixture of H₂O/MeOH (95:5, v/v), to remove undesired matrix components in the final extract. Then, the sorbent was vacuum dried for 15 min and after dryness, analytes were eluted with 3 mL of MeOH followed by 3 mL of a mixture of MeOH/formic acid (99:1, v/v). Finally, the combined eluted fractions were evaporated under nitrogen to dryness with a PIERCE ReactiTherm III evaporator (Rockford, IL, USA) and reconstituted with 0.5 mL of MeOH for LC-MS/MS analysis.

2.4. LC-MS/MS analysis

Analysis of the extracts was performed with an HPLC SymbiosisTM Pico System (Spark Holland, Emmen, The Netherlands) connected in series with a 4000 QTRAP hybrid triple quadrupole-linear ion trap (QqLIT) mass spectrometer (Applied Biosystem-Sciex, Foster City, CA, USA). LC separation was achieved with a Purospher Star RP-18 end-capped column (125 mm × 2.0 mm, 5 μm) (Merk, Darmstadt, Germany) and a mobile phase of formic acid/ammonium formate buffer (20 mM) and ACN. The ionization of the compounds was achieved with a Turbo Ion Spray source operating in the positive ionization mode (ESI+). Mass acquisition was performed in the selected reaction monitoring mode (SRM) recording two SRM transitions per compound and one per SS. The conditions used for the LC-MS/MS determination of the target compounds are described in detail elsewhere (Mastroianni et al., 2013).

2.5. Method performance

The performance of the methodology in sediments was evaluated in terms of linearity, sensitivity, recovery, repeatability, and matrix effects.

The linearity of the method was evaluated between 0.1 and 1,000 ng/mL (equivalent to 0.05 and 500 ng/g d.w.) through the analysis of eleven methanolic standard solutions at different concentrations covering the aforementioned range. A calibration curve was constructed for each analyte using the internal standard approach by plotting the area ratio between the analyte and its corresponding surrogate standard and applying weighted least-squares linear regression. A weighting factor of $1/x^2$ was used to reduce the influence of the high concentrations in the linear model.

Analyte recoveries were calculated from replicate analysis (n=6) of river sediment samples fortified at two levels, 10 ng/g d.w. and 25 ng/g d.w. Absolute recoveries were calculated by comparing the analyte peak areas obtained in fortified samples (after subtracting the peak area corresponding to the amount of analyte in the blank if present) and in standard solutions at equivalent concentrations. Relative recoveries were calculated by comparing the absolute recoveries obtained for each analyte and its corresponding surrogate standard.

63 The method repeatability was calculated as the relative standard deviation (RSD) of the
64 response (analyte/surrogate standard) after the replicate analysis (n=6) of river sediment
65 samples fortified at 10 ng/g d.w. and 25 ng/g d.w.

66 The sensitivity of the method was evaluated through the limit of detection (LOD) and
67 limit of quantification (LOQ) observed for each analyte. Average LODs and LOQs were
68 experimentally estimated from the analysis of river sediment samples as the
69 concentration of the analyte giving a signal to noise ratio of 3 and 10, respectively. In
70 the case that the target compounds were not detected in any sample, LODs and LOQs
71 were estimated from the signal observed in river sediment samples fortified at the lower
72 level (10 ng/g d.w., n=6).

73 Matrix effects (ME) were evaluated by comparing the peak area obtained for each
74 analyte in the river sediment extract fortified (25 ng/g d.w.) at the end of the sample
75 treatment procedure, i.e., after the PLE and SPE steps (A_{sediment}) (after subtracting the
76 peak area corresponding to the amount of the analyte in the blank if present (A_{blank})),
77 and a standard solution at an equivalent concentration (A_{standard}) (50 ng/mL), according
78 to the following equation:

$$\text{ME (\%)} = \left[\frac{(A_{\text{sediment}} - A_{\text{blank}}) - (A_{\text{standard}})}{A_{\text{standard}}} \right] * 100$$

79 Negative values indicate MS signal suppression by matrix components, whereas
80 positive values indicate signal enhancement. Values close to 0 indicate the absence of
81 matrix effects.

82

83 **2.6. Statistical analysis**

84 Data were statistically analyzed to compare the occurrence of drugs of abuse among
85 river basins and between sampling campaigns. Since data were not normally distributed,
86 non-parametric tests were applied. The Wilcoxon Rank-Sum test was used to compare
87 compound distribution between sampling campaigns (two independent samples). Then,
88 a multivariate analysis consisting of the adjustment of the Quantile Regression Models
89 (Median Regression Models) was used to predict the median concentration of each drug
90 of abuse in each basin in the two sampling campaigns. The differences between the

91 sampling campaigns median predictions and their 95% confidence interval were
92 estimated.

93 The Kruskal-Wallis test was used to compare the distribution of each compound
94 (present in at least three basins) among the four basins. If significant differences among
95 groups were obtained, they were subsequently investigated by applying the Wilcoxon
96 Rank-Sum test to each pair of basins. False Discovery Rate (FDR) correction for
97 multiple testing was applied to reduce the number of “false positives”.

98 The relationship between the concentrations of a specific drug found in the sediment
99 and water compartments of the different investigated sampling stations was evaluated
100 using the Spearman’s correlation test.

101 All the statistical analyses were done using the software R and considering a confidence
102 level of 95% ($\alpha=0.05$).

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104 **3. Results and discussion**

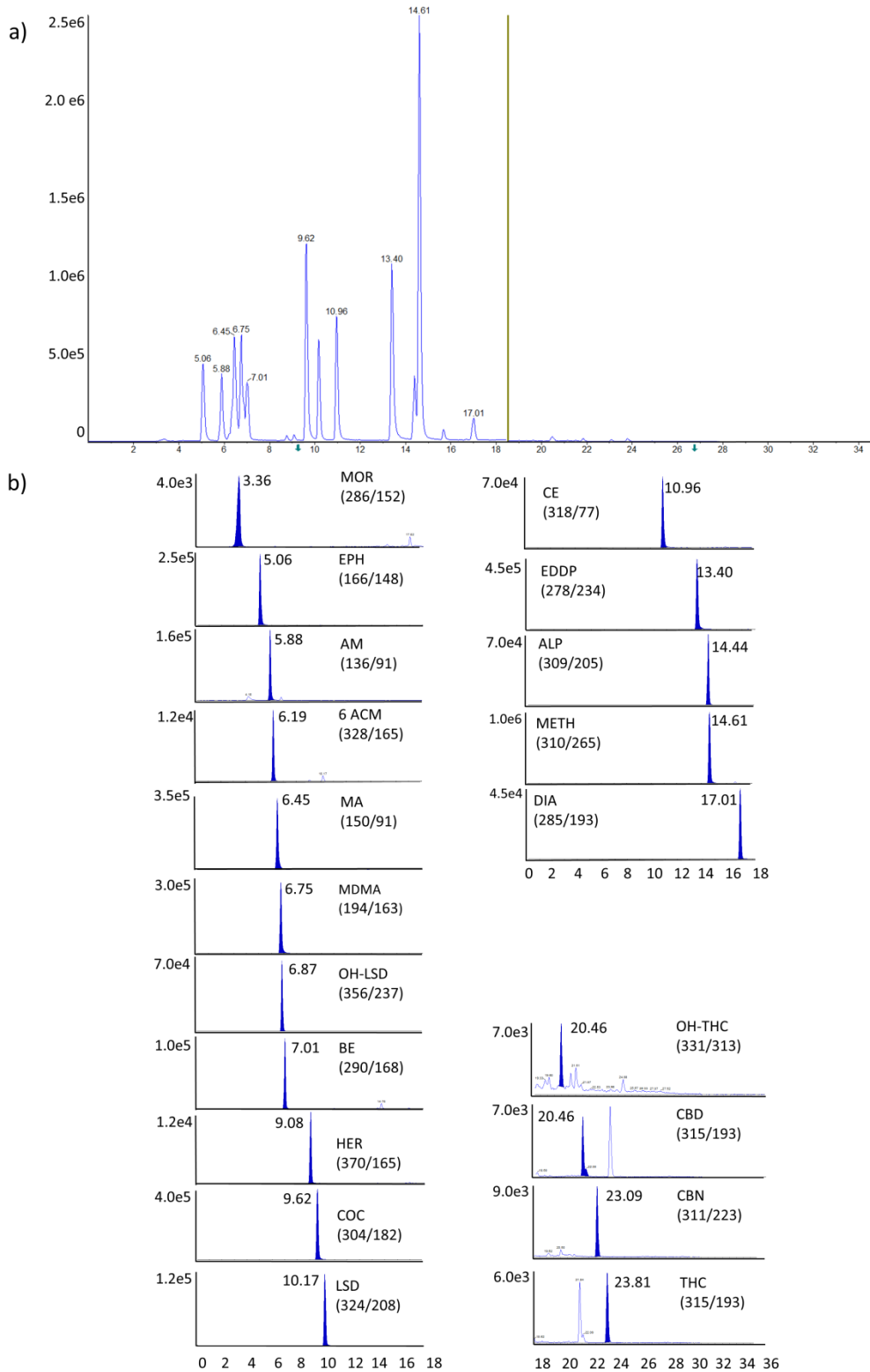
105 **3.1. Method performance**

106 Table 3 shows the method performance in terms of linearity, recovery, repeatability, and
107 sensitivity. Figure 2 shows the total ion chromatogram (TIC) and the extracted ion
108 chromatograms (XIC) of the target analytes after PLE-SPE-LC-MS/MS analysis of a
109 sediment sample fortified at a concentration of 25 ng/g d.w.

110 The linearity of the method was satisfactory for all analytes. Coefficients of
111 determination obtained for at least six-point calibration curves were higher than 0.99.

112 As for method precision and accuracy, absolute recoveries were in good agreement at
113 the two concentration levels tested for all compounds. Most of the compounds presented
114 absolute recoveries between 40 and 78% except amphetamine, morphine, EDDP, THC,
115 cannabidiol, cannabinal, and alprazolam that presented absolute recoveries below 32%.
116 Despite this, the relative recoveries obtained for all compounds (between 90 and 113%),
117 except OH-THC at the low concentration level (135%) and cannabinal at both levels
118 (141%), indicate that the use of isotopically labeled analogs as surrogate standards
119 allows correcting analyte losses during sample preparation as well as matrix effects. In
120 the case of OH-THC and cannabinal, the high relative recoveries obtained can be

121 attributed to their presence in the matrix used for validation at concentrations close to
 122 the spiking levels.



123
 124 **Figure 2.** Total ion chromatogram (TIC) (a) and extracted ion chromatograms (XIC) (b)
 125 of the target analytes after PLE-SPE-LC-MS/MS analysis of a sediment sample fortified
 126 at a concentration of 25 ng/g d.w.

127 The repeatability of the method was satisfactory with RSD values (n=6) below 15% for
128 all the compounds at the two levels tested. Such good repeatability of the method can be
129 attributed to the partial automation of the sample treatment process by using PLE.

130 Regarding sensitivity, LODs and LOQs were below 1.1 and 2.1 ng/g d.w., respectively,
131 except in the case of the cannabinoids that presented LODs between 0.84 and 2.3 ng/g
132 d.w. and LOQs between 3.2 and 13 ng/g d.w. The comparatively lower sensitivity
133 observed for cannabinoids can be explained by several factors: low absolute recovery,
134 high matrix suppression ionization effects, and/or low signal response provided by the
135 instrumentation under positive ionization. The analysis of cannabinoids under favorable
136 negative electrospray ionization conditions would have required an additional
137 chromatographic run with a basic mobile phase. In this context, the simultaneous
138 analysis of all target analytes, in detriment of cannabinoids sensitivity, was prioritized
139 to save chemicals, reagents, and time.

140 Table 3 also summarizes the matrix effects observed during sediment analysis. For most
141 of the compounds, matrix effects were negligible ($ME \leq 20\%$). Only nine compounds,
142 namely, benzoylecgonine, ephedrine, 6-acetylmorphine, LSD, its metabolite OH-LSD,
143 OH-THC, cannabidiol, cannabinol, and alprazolam, were affected by matrix
144 components to a higher extent ($>20\%$), but ionization suppression of their MS signal did
145 not surpass 40%.

146

147

148

149 **Table 3.** Method performance in terms of linearity, analyte recoveries (absolute and relative), repeatability (RSD), matrix effects, and sensitivity
 150 (limits of detection and quantification) in river sediments.

	Linearity	Absolute recovery [%], (n=6)		Relative recovery (repeatability), [% , (RSD)] (n=6)		Matrix effect (%, n=3)	Sensitivity	
	r^2	10 ng/g d.w.	25 ng/g d.w.	10 ng/g d.w.	25 ng/g d.w.	25 ng/g d.w.	LOD (ng/g d.w.)	LOQ (ng/g d.w.)
COC	0.9968	62	70	101 (3.0)	105 (5.3)	-11	0.11	0.16
BE	0.9986	71	68	109 (5.0)	97 (4.8)	-20	0.02	0.04
CE	0.9952	72	78	102 (4.1)	99 (4.6)	-8.3	0.01	0.08
AM	0.9958	26	31	105 (4.8)	103 (3.6)	-16	1.1	2.1
MA	0.9944	61	64	95 (4.1)	99 (4.9)	-12	0.01	0.03
MDMA	0.9916	67	67	95 (4.1)	101 (4.9)	-6.9	0.03	0.06
EPH	0.9984	51	48	102 (3.7)	95 (5.3)	-28	0.07	0.21
MOR	0.9976	15	16	113 (8.8)	104 (12)	-14	0.13	0.70
6ACM	0.9978	62	67	106 (7.3)	106 (9.4)	-23	0.04	0.11
HER	0.9994	59	62	97 (5.1)	104 (11)	-17	0.13	0.35
METH	0.9924	71	77	97 (2.8)	102 (6.2)	-12	0.12	0.16
EDDP	0.9982	31	28	102 (3.2)	98 (9.0)	-4.7	0.16	0.41
LSD	0.9996	58	66	90 (8.7)	98 (5.4)	-29	0.02	0.08
OH-LSD	0.9972	66	63	106 (14)	94 (5.7)	-34	0.05	0.24
THC	0.9978	18	24	92 (4.6)	105 (4.9)	-16	0.84	3.2
OH-THC	0.9900	44	46	135 (6.5)	112 (6.8)	-29	1.9	5.1
CBD	0.9997	24	29	100 (5.0)	99 (8.8)	-24	2.2	5.9
CBN	0.9943	28	32	141 (6.8)	141 (3.8)	-40	2.3	13
ALP	0.9984	25	52	97 (2.3)	101 (4.4)	-29	0.12	0.35
DIA	0.9998	58	66	91 (6.8)	99 (6.9)	-15	0.04	0.10

151 RSD: Relative standard deviation; LOD: Limit of detection; LOQ: Limit of quantification.

152 **Table 4.** Frequency of detection (%), and median (ng/g d.w.) and maximum (ng/g d.w.) concentrations of the target drugs of abuse and
 153 metabolites found in all the samples analyzed during the two sampling campaigns (“All basins”), and in each river basin in 2010 and 2011
 154 (values separated by “/”).

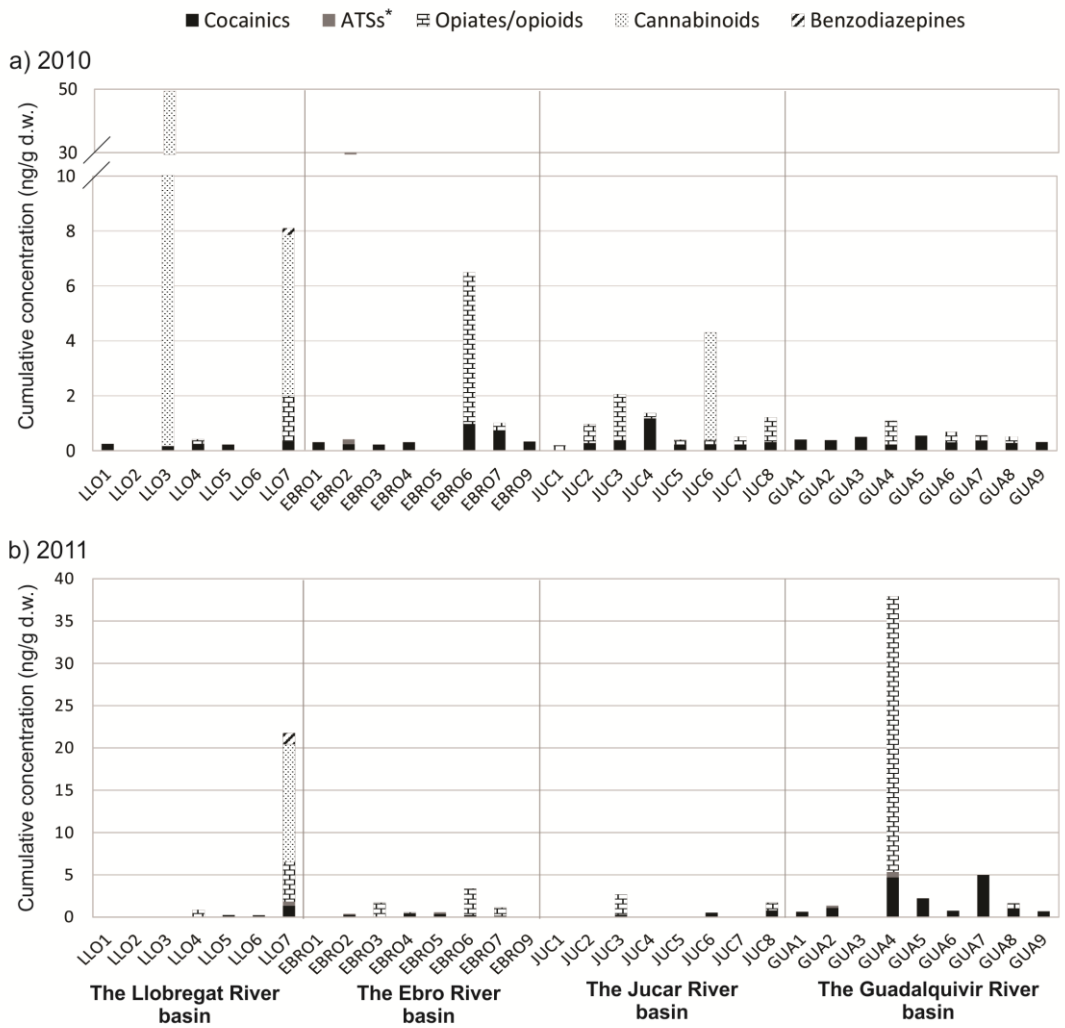
	All basins			Ebro basin			Llobregat basin			Jucar basin			Guadalquivir basin		
	Freq. (%) (n= 144)	Median ^a (ng/g d.w.)	Max. (ng/g d.w.)	Freq. (%) (n=19/23)	Median ^a (ng/g d.w.)	Max. (ng/g d.w.)	Freq. (%) (n=14/14)	Median ^a (ng/g d.w.)	Max. (ng/g d.w.)	Freq. (%) (n=12/15)	Median ^a (ng/g d.w.)	Max. (ng/g d.w.)	Freq. (%) (n=24/23)	Median ^a (ng/g d.w.)	Max. (ng/g d.w.)
COC	74	0.34	5.0	100/35	0.31/0.38	1.0/0.44	93/50	0.25/0.40	0.47/0.72	100/47	0.27/0.53	1.2/4.6	100/74	0.30/0.75	0.75/5.0
BE	2.1	0.46	0.81	-/-	-/-	-/-	-7.1	-/0.81	-/0.81	-/13	-/0.37	-/0.46	-/-	-/-	-/-
CE	-	-	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
AM	-	-	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
MA	2.1	0.25	0.63	-/4.3	-/0.18	-/0.18	-/-	-/-	-/-	-/-	-/-	-/-	-/8.7	-/0.44	-/0.63
MDMA	13	0.17	0.83	26/13	0.17/0.19	0.31/0.43	21/21	0.17/0.17	0.20/0.83	25/-	<LOQ/-	-/-	4.1/-	<LOQ	-/-
EPH	3.5	0.48	0.48	5.3/13	0.40/0.48	0.40/0.48	-7.1	-/0.48	-/0.48	-/-	-/-	-/-	-/-	-/-	-/-
MOR	-	-	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
6ACM	-	-	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
HER	-	-	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
METH	51	0.30	33	42/39	0.70/0.65	3.7/2.7	43/32	0.37/0.89	1.1/5.7	100/33	0.21/0.49	1.1/0.56	75/43	0.25/0.37	1.7/33
EDDP	36	1.6	16	42/52	5.1/2.4	9.5/7.8	43/57	1.2/2.9	3.8/16	33/42	0.64/0.91	0.76/1.9	29/8.3	0.89/5.3	5.8/9.7
LSD	-	-	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
OH-LSD	-	-	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
THC	5.6	6.1	37	16/-	6.1/-	36/-	21/7.1	6.3/14	37/14	8.3/-	3.9/-	3.9/-	-/-	-/-	-/-
OH-THC	-	-	-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-	-/-	-/-
CBD	0.7	15	15	5.3/-	15/-	15/-	-/-	-/-	-/-	8.3/-	<LOQ/-	-/-	-/-	-/-	-/-
CBN	2.1	28	44	5.3/-	44/-	44/-	7.1/-	13/-	13/-	8.3/-	<LOQ/-	-/-	-/-	-/-	-/-
ALP	0.7	<LOQ	-	-/-	-/-	-/-	-/6.7	-/<LOQ	-/-	-/-	-/-	-/-	-/-	-/-	-/-
DIA	6.9	0.24	1.3	11/-	0.24/-	0.24/-	21/21	0.19/0.80	0.25/1.3	8.3/6.7	0.20/0.13	0.20/0.13	-/-	-/-	-/-

155 ^aOnly values above the limit of quantification (LOQ) were considered in the calculation of the median concentration.

156 -: not detected; <LOQ: below limit of quantification.

157 **3.2. Occurrence of drugs of abuse and their metabolites in river sediments**

158 The frequency of detection and median and maximum concentrations of the target drugs
 159 of abuse and metabolites in all sediment samples, as well as in each river basin and
 160 sampling campaign are summarized in Table 4. Cumulative levels of all investigated
 161 compounds in each sample are shown in Figures 3 and 4.

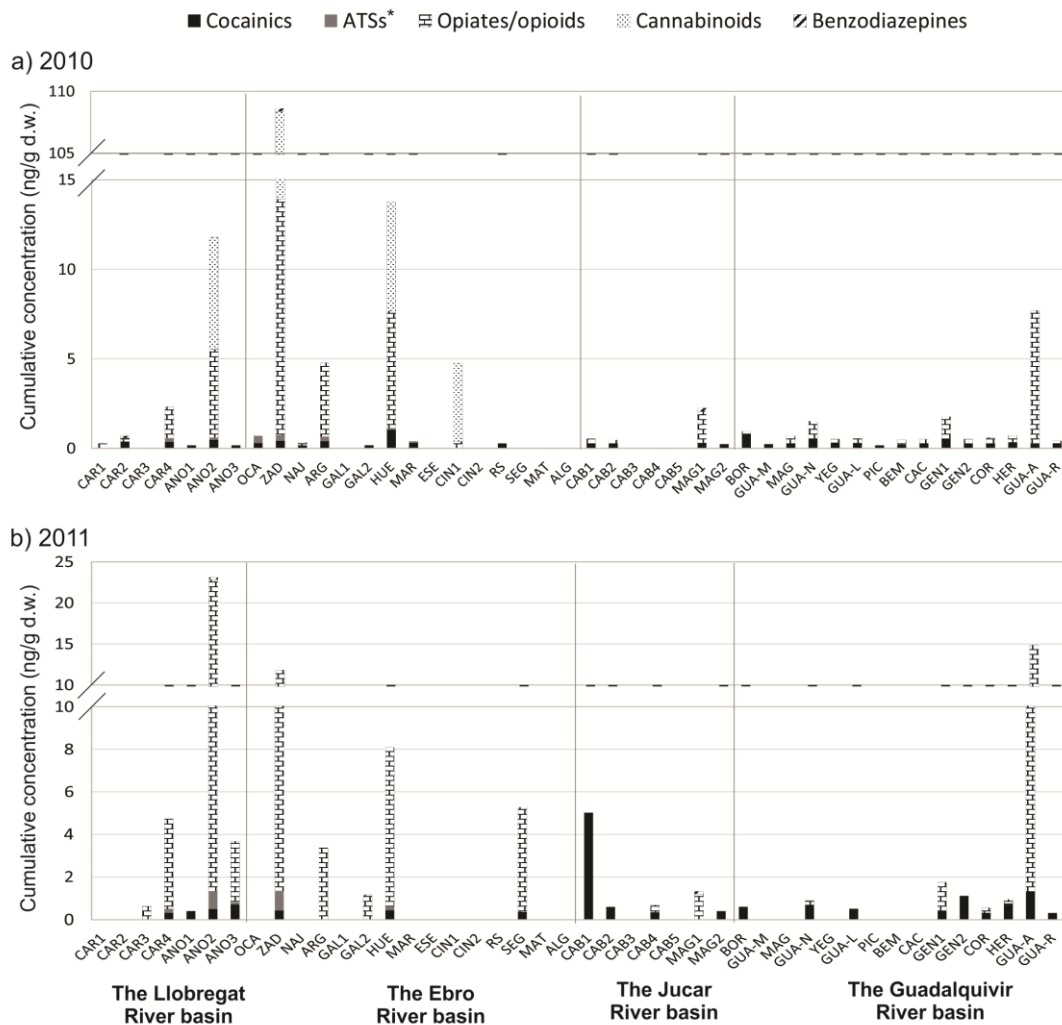


*ATSS: Amphetamine-type stimulants

162

163

164 **Figure 3.** Cumulative concentration (ng/g, d.w.) of drugs of abuse classes in the main
 165 rivers in 2010 (a) and 2011 (b).



*ATSS: Amphetamine-type stimulants

166

167 **Figure 4.** Cumulative concentration (ng/g, d.w.) of drugs of abuse classes in the
 168 tributary rivers in 2010 (a) and 2011 (b).

169

170 Cocaethylene, amphetamine, morphine, 6-acetylmorphine, heroin, LSD and its
 171 metabolite (OH-LSD), and OH-THC were not detected in any sample. The most
 172 ubiquitous compounds were cocaine, found in 74% of the analyzed samples, followed
 173 by methadone and its metabolite EDDP, present in 51 and 36% of the analyzed samples,
 174 respectively. MDMA was found in 13% of the samples and the remaining compounds,
 175 *viz.*, benzoylecgonine, methamphetamine, ephedrine, THC, cannabidiol, cannabinol,
 176 alprazolam, and diazepam were detected in less than 7% of the samples. In general,
 177 concentrations were very low. The maximum concentrations were measured for

178 cannabiniol (44 ng/g d.w.), THC (37 ng/g d.w.), and methadone (33 ng/g d.w.). In terms
179 of median concentrations, calculated only with values above the method LOQ, all
180 quantified compounds were measured at median concentrations in all basins below 1.6
181 ng/g d.w., except the cannabinoids THC, cannabidiol, and cannabiniol that were found at
182 median concentrations of 6.1, 15 and 28 ng/g d.w, respectively. However, it should be
183 pointed out that these high median concentrations are obtained from the detection of
184 THC in only 5.6% of the 144 samples analyzed (three samples in both the Ebro and the
185 Llobregat basins, and one sample in the Jucar basin), only one sample in the Ebro basin
186 in the case of cannabidiol, and two samples in the Ebro and the Llobregat basin in the
187 case of cannabiniol.

188 As for each family of compounds, cocaine was more ubiquitous and abundant than its
189 human metabolite, benzoylecgonine, which is more polar and is usually present at a
190 higher concentration in surface water (Mastroianni et al., 2016). Cocaine and
191 benzoylecgonine were detected at maximum concentrations of 5.0 ng/g d.w. (GUA7)
192 and 0.81 ng/g d.w. (LLO7), respectively, in sediment samples collected in 2011 in the
193 main rivers of the Guadalquivir and the Llobregat basins. These concentrations were
194 higher than those found in sediments of the San Francisco Bay (USA) (benzoylecgonine
195 was not detected and cocaine was detected at 2.2 ng/g) (Klosterhaus et al., 2013), and
196 lower than those found in the Beiyunhe River, China (benzoylecgonine: 3.1 ng/g d.w.;
197 cocaine: 10 ng/g d.w.) (Hu et al., 2019). In a study previously conducted in Spain,
198 similar concentrations of benzoylecgonine (0.95 ng/g) and higher concentrations of
199 cocaine (30 ng/g) were measured in sediments of the Turia River basin, Valencia
200 (Álvarez-Ruiz et al., 2015).

201 Within the amphetamine-type stimulants, MDMA was the most ubiquitous compound
202 with a frequency of detection of 13%, while ephedrine and methamphetamine were only
203 detected in 3.5 and 2.1% of the samples, respectively, and amphetamine was not
204 detected. Maximum levels of MDMA (0.83 ng/g d.w.) were found in a tributary river of
205 the Llobregat basin, Anoia River (ANO2), in 2011. Maximum levels of ephedrine (0.48
206 ng/g d.w.) and methamphetamine (0.63 ng/g d.w.) were found in a tributary river of the
207 Ebro basin, Zadorra River (ZAD), in 2011 and the main Guadalquivir River in 2011
208 (GUA4), respectively. As compared with this study, higher concentrations of
209 amphetamine (6.9 ng/g d.w.) and methamphetamine (9.1 ng/g d.w.) were found in

210 sediments of China (Hu et al., 2019) and also in the San Francisco Bay (USA) where
211 amphetamine was detected at maximum levels of 3.3 ng/g (Klosterhaus et al., 2013).

212 As for opiates/opioids, neither morphine nor heroin or its metabolite 6ACM was
213 positively identified in the investigated samples. Methadone and EDDP were found at
214 maximum concentrations of 33 ng/g d.w. (GUA4) and 16 ng/g d.w. (ANO2),
215 respectively, in samples collected in 2011 in the main river of the Guadalquivir basin
216 and a tributary river of the Llobregat basin. The maximum methadone concentration
217 measured in this study was higher than the maximum concentration found in sediments
218 collected in the Turia River basin, Valencia (Spain) (0.53 ng/g) (Álvarez-Ruiz et al.,
219 2015).

220 As for cannabinoids, cannabinal and cannabidiol were found at maximum
221 concentrations of 44 and 15 ng/g d.w., respectively, in a tributary river of the Ebro basin
222 (ZAD) in 2010, and THC was found at a maximum concentration of 37 ng/g d.w. in the
223 main river of the Llobregat basin (LLO3) in 2010. Despite this, THC levels found were
224 not as high as those found in the Turia River, Valencia (Spain), where the THC
225 concentration in sediments reached 200 ng/g (Carmona et al., 2017).

226 Alprazolam was detected in only one sample but at levels below the method LOQ.
227 Higher levels of alprazolam (maximum concentrations of 87 ng/g d.w.) were found in
228 sediment samples collected in the lakes Al-Hufuf and Al-Oyun in Saudi Arabia (Picó et
229 al., 2020). The other investigated benzodiazepine, diazepam, was detected in 6.9% of
230 the analyzed samples at maximum concentrations of 1.3 ng/g d.w. in the main river of
231 the Llobregat basin (LLO7) in 2011. The maximum diazepam concentration found in
232 the present study was lower than that found in the Danube River (Serbia) (48 ng/g)
233 (Radović et al., 2015), and higher than the maximum concentrations found in sediment
234 samples collected in the Salvador Bay (Brazil) (0.71 ng/g d.w.) (Beretta et al., 2014). In
235 the Turia River (Spain) (Carmona et al., 2017), and the Douro and the Lima Rivers
236 (Portugal) (Santos et al., 2016), diazepam was either not present or at levels below the
237 LOD of the corresponding method.

238

240 3.3. Spatial and temporal variability of drugs of abuse in river sediments

241 Figure 3 shows the cumulative levels of the different classes of drugs of abuse and
242 metabolites investigated along the four main rivers in the two sampling campaigns. The
243 concentrations measured in tributary rivers are depicted in Figure 4.

244 The most polluted river basin in terms of drugs of abuse and taking into account both,
245 concentrations found in the main river and its tributaries, and the two sampling
246 campaigns conducted was the Ebro River basin, with a total cumulative level of drugs
247 of abuse on average of 91 ng/g d.w. The Ebro River basin was followed by the
248 Llobregat River basin (65 ng/g d.w.), the Guadalquivir River basin (47 ng/g d.w.), and
249 the Jucar River basin (14 ng/g d.w.). The Llobregat and the Ebro River basins presented
250 higher cumulative levels in 2010 than in 2011 (74 vs 56 ng/g d.w. in the case of the
251 Llobregat basin, and 143 vs 38 ng/g d.w. in the case of the Ebro basin), while the
252 Guadalquivir River basin presented higher cumulative levels during 2011 than during
253 2010 (72 vs 22 ng/g d.w, respectively). In the case of the Jucar River similar cumulative
254 levels were found in both years (15 and 13 ng/g d.w. respectively).

255 The most polluted sampling locations were ZAD in 2010 (the Ebro River basin) and
256 LLO3 in 2010 (the Llobregat River basin), with cumulative levels of 109 and 50 ng/g
257 d.w., respectively, followed by GUA4 (the Guadalquivir River basin) in 2011 with a
258 cumulative level of 38 ng/g d.w. Cannabinoids were the chemical class that contributed
259 the most to ZAD and LLO3 total concentrations, whereas opioids were the most
260 abundant class in GUA4. The samples ANO2 (the Llobregat River basin) in 2011,
261 LLO7 (the Llobregat River basin) in 2011, GUA-A (the Guadalquivir River basin) in
262 2011, HUE (the Ebro River basin) in 2010, ANO2 (the Llobregat River basin) in 2010
263 and ZAD (the Ebro River basin) in 2011, presented cumulative levels of drugs of abuse
264 between 23 and 12 ng/g d.w., while the rest of sediment samples contained cumulative
265 levels below 10 ng/g d.w. Similar to the results obtained for surface water collected in
266 the same sampling locations (Mastroianni et al., 2016), the highest accumulation of
267 drugs of abuse was found in small tributary rivers located downstream of medium-
268 (40.000 inhabitants) to large-size (2M inhabitants) urban areas, like ZAD (Gasteiz),
269 ANO2 (Igualada), GUA-A (Sevilla) or HUE (Zaragoza) or close to WWTP discharge
270 points like GUA4 (WWTP from Córdoba). The lower dilution capacity of tributaries as

271 compared to the main rivers of the wastewater effluent discharges may favor the
 272 accumulation of drugs of abuse and/or their metabolites in sediments. High cumulative
 273 levels were also found in main river locations like LLO3 and LLO7, which correspond
 274 to the middle and the final section of the Llobregat River. Unlike other river basins,
 275 where the pollution gradient decreases downstream the main river due to its increasing
 276 flow and hence dilution capacity, in the Llobregat River pollution could increase from
 277 its head to its mouth due to a growing population density and number of WWTPs
 278 downstream. WWTP discharges may even represent almost 100% of the Llobregat
 279 River flow in drought periods (Boleda et al., 2009; Osorio et al., 2012). Our findings are
 280 in agreement with other studies conducted in this basin, that reported the highest levels
 281 of organic micropollutants like pharmaceuticals (Osorio et al., 2016) and endocrine
 282 disruptors (Gorga et al., 2015) in sediments samples collected near the mouth of the
 283 river.

284 To study differences in the occurrence of drugs of abuse between river basins and
 285 sampling campaigns, the most detected compounds (cocaine, methadone, EDDP, and
 286 MDMA), as well as the sum of all detected compounds (“Sum”) and the sum of all
 287 detected compounds excluding cannabinoids (“Sum No Cannabinoids”), were
 288 statistically evaluated. Table 5 shows the p-values obtained after applying the Wilcoxon
 289 Rank-Sum test to assess differences in the distribution of drugs of abuse between
 290 sampling campaigns stratifying by river basin.

291

292 **Table 5.** Comparison of compound`s distribution between 2010 and 2011 stratifying by
 293 river basin (Wilcoxon Rank-Sum test p-value).

Compound	Llobregat	Ebro	Jucar	Guadalquivir
Cocaine	0.52	<0.01*	0.92	0.04*
MDMA	0.83	0.34	0.08†	0.35
Methadone	0.79	0.64	0.02*	0.05*
EDDP	0.40	0.69	0.72	0.04*
Sum	0.80	0.35	0.21	0.54
Sum No Cannabinoids	0.87	0.50	0.87	0.54

294 *p< 0.05

295 †p< 0.10

296

297 **Table 6a.** Predicted median concentration (ng/g d.w.) in 2010 and 2011 obtained with the Quantile Regression Models (Median Regression
 298 Models) in the Ebro and the Llobregat River basins. Difference between 2011 and 2010 predicted medians and its 95% confidence interval.
 299

Compounds	Year	Ebro basin				Llobregat basin			
		Predicted median conc. (ng/g d.w.)	95%Ci ^a	Δ median (ng/g d.w.)	95%Ci ^a	Predicted median conc. (ng/g d.w.)	95%Ci ^a	Δ median (ng/g d.w.)	95%Ci ^a
Cocaine	2010	0.3	(0.19;0.42)	(-0.25*)	(-0.41;0.09)	0.22	(0.09;0.36)	(-0.01)	(-0.20;0.19)
	2011	0.05	(-0.54;0.16)			0.22	(0.08;0.35)		
MDMA	2010	0.08	(0.03;0.13)	(-0.06)		0.03	(-0.03;0.09)	(-0.02)	
	2011	0.02	(-0.03;0.06)			0.01	(-0.04;0.07)		
METH	2010	0.06	(0.00;0.12)	0		0.11	(0.04;0.17)	(-0.05)	(-0.14;0.49)
	2011	0.06	(0.01;0.11)			0.06	(-0.01;0.13)		
EDDP	2010	0.03	(-0.13;0.18)	0.06	(-0.15;0.28)	0.15	(-0.04;0.33)	0.06	(-0.20;0.32)
	2011	0.09	(-0.05;0.23)			0.21	(0.26;0.39)		
Sum	2010	0.41	(-0.03;0.85)	(-0.10)	(-0.70;0.50)	0.32	(-0.19;0.83)	0.27	(-0.46;1.00)
	2011	0.31	(-0.09;0.71)			0.59	(0.08;1.10)		
Sum No Cannabinoids	2010	0.39	(0.03;0.76)	(-0.08)	(-0.58;0.42)	0.32	(-0.11;0.74)	0.27	(-0.34;0.88)
	2011	0.31	(-0.02;0.64)			0.59	(0.16;1.01)		

300
 301 *Statistically significant difference between predicted median in 2011 and 2010 concentrations (p-value ≤ 0.05)

302 ^a 95% Confidence Interval

303
 304

305

306 **Table 6b.** Predicted median concentration (ng/g d.w.) in 2010 and 2011 obtained with the Quantile Regression Models (Median Regression
307 Models) in the Jucar and the Guadalquivir River basins. Difference between 2011 and 2010 predicted medians and its 95% confidence interval.

Compounds	Year	Jucar basin				Guadalquivir basin			
		Predicted median conc. (ng/g d.w.)	95%Ci ^a	Δ median (ng/g d.w.)	95%Ci ^a	Predicted median conc. (ng/g d.w.)	95%Ci ^a	Δ median (ng/g d.w.)	95%Ci ^a
Cocaine	2010	0.24	(0.11;0.37)	(-0.13)	(-0.32;0.05)	0.31	(0.20;0.41)	(0.32*)	(0.17;0.47)
	2011	0.11	(-0.03;0.24)			0.63	(0.52;0.74)		
MDMA	2010	0.01	(-0.04;0.07)	0		0.01	(-0.03;0.06)	0	
	2011	0.01	(-0.04;0.07)			0.01	(-0.03;0.06)		
METH	2010	0.20	(0.13;0.26)	(-0.14*)	(-0.23;-0.04)	0.20	(0.15;0.25)	(-0.14*)	(-0.21;0.06)
	2011	0.06	(-0.01;0.13)			0.06	(0.01;0.11)		
EDDP	2010	0.03	(-0.15;0.20)	0		0.03	(-0.12;0.17)	0	
	2011	0.03	(-0.15;0.20)			0.03	(-0.12;0.17)		
Sum	2010	1.01	(0.46;1.56)	(-0.49)	(-1.23;0.26)	0.56	(0.16;0.95)	0.13	(-0.44;0.69)
	2011	0.53	(0.03;1.02)			0.68	(0.28;1.08)		
Sum No Cannabinoids	2010	0.47	(0.06;0.89)	0.05	(-0.54;0.64)	0.56	(0.23;0.88)	0.13	(-0.34;0.59)
	2011	0.53	(0.11;0.94)			0.68	(0.35;1.01)		

308

309 *Statistically significant difference between predicted median in 2011 and 2010 concentrations (p-value ≤ 0.05)

310 ^a95% Confidence Interval

311

312 **Table 7.** Comparison of compound`s distribution between the four basins stratifying by year (Kruskal-Wallis test p-values). If statistical
 313 differences were shown, pairwise basin comparisons were performed (Wilcoxon Rank-Sum test p-values). False Discovery Rate (FDR)
 314 correction for multiple testing was applied.

Compound	All basins ^a		Ebro-Llo ^b		Ebro-Juc ^b		Ebro-Gua ^b		Llo-Juc ^b		Llo-Gua ^a		Juc-Gua ^b		FDR ^b		
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	
Cocaine	0.06 [†]	<0.01*		0.41		0.31		<0.01*		0.78		0.04*		0.06 [†]			Ebro- Gua, Llo-Gua
MDMA	0.13	0.06 [†]															
Methadone	0.36	0.79															
EDDP	0.58	0.01*		0.70		0.22		0.01*		0.20		0.01*		0.19			Ebro- Gua, Llo-Gua
Sum	0.51	0.65															
Sum No Cannabinoids	0.26	0.66															

315 Ebro= the Ebro River basin; Llo= the Llobregat River basin; Juc= the Jucar River basin; Gua= the Guadalquivir River basin

316 * p< 0.05

317 [†] p< 0.10

318 ^a Null hypothesis in Kruskal-Wallis test (H₀: the compound`s distribution in the four independent basins are equal) was rejected if corresponding p-value < 0.05

319 ^b Null hypothesis in Wilcoxon Sum-Rank test (H₀: the compound`s distribution in the independent pair of basins tested are equal) was rejected if corresponding p-value was
 320 lower than the corresponding corrected significance level $\alpha_{corrected}$ obtained after applying the False Discovery Rate correction due to Multiple Testing (six multiple pairs
 321 compared). Overall significance level was = 0.05

322

323

324 Concentrations of drugs of abuse detected in sediment samples collected in 2011 were
325 statistically significantly different than those collected in 2010 for cocaine in the Ebro
326 and the Guadalquivir basins, methadone in the Jucar and the Guadalquivir basins, and
327 EDDP in the Guadalquivir basin. The whole set of data obtained in this study was then
328 employed for a multivariate analysis based on the quantile regression model (median
329 regression model) to predict in both years the median concentration of drugs of abuse in
330 the investigated basins (Tables 6a and 6b). Compared to 2010, in 2011 statistically
331 significant ($\alpha=0.05$) lower median concentrations of cocaine in the Ebro basin (Δ
332 median -0.25, 95% CI -0.41; 0.09) and methadone in the Jucar (Δ median -0.14, 95% CI
333 -0.23; -0.04) and the Guadalquivir (Δ median -0.14, 95% CI -0.21; -0.06) basins were
334 predicted, while a higher median concentration of cocaine in the Guadalquivir (Δ
335 median 0.32, 95% CI 0.17; 0.47) basin was predicted in 2011 compared to 2010. There
336 was a reduced flow, and hence lower dilution factor and higher diffusion rates of the
337 water concentrations into the sediments, in most of the sampling locations investigated
338 in 2011 compared to 2010 (data not shown). This could explain the statistically
339 significant higher cocaine concentrations found in the Guadalquivir basin found in
340 2011. However, hydrological conditions cannot explain the larger concentrations of
341 cocaine in the Ebro river basin and methadone in the Jucar and the Guadalquivir river
342 basins found in 2010. Storm events may also play a relevant role in the desorption of
343 organic pollutants from sediments. Additionally, other factors such as the patterns of
344 consumption of drugs of abuse by the surrounding population, the efficiency of the
345 WWTPs to remove the drugs of abuse, or natural attenuation processes (like
346 photodegradation and biodegradation) may be responsible for the overall high
347 concentrations of drugs of abuse in the water and consequently in the sediments
348 observed in 2010.

349 Differences in the distribution of drugs of abuse between basins stratifying by year were
350 also studied. The results are shown in Table 7. Statistically significant different
351 distributions were only found for cocaine and EDDP in 2011. To find the basins where
352 there was a difference in the distribution of these compounds, a posthoc analysis of each
353 pair of basins was done (Wilcoxon Sum Rank test) and the p-value obtained was
354 corrected by a multiple comparison method (False Discovery Rate, FDR). For both
355 compounds, EDDP and cocaine, statistically different distributions were obtained
356 between the Ebro and the Guadalquivir, and between the Llobregat and the

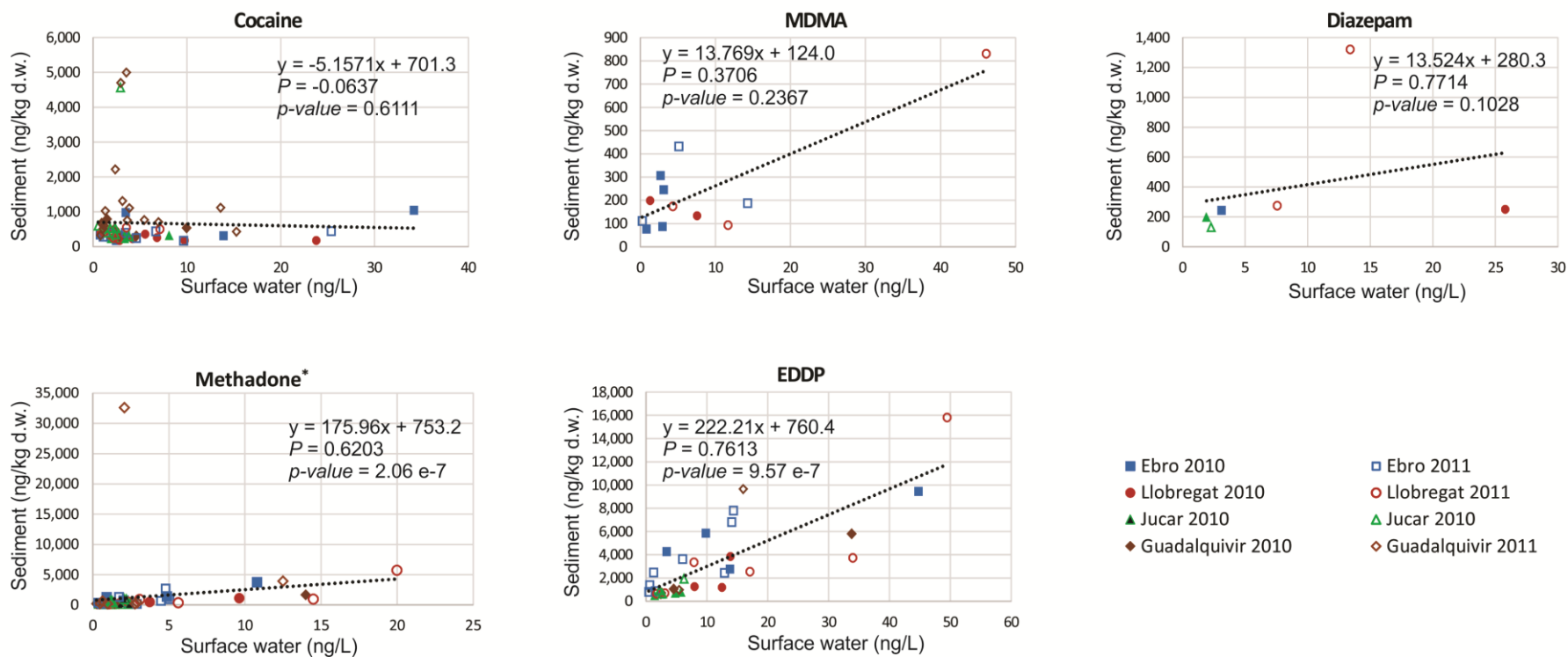
357 Guadalquivir in 2011. These differences are due to the remarkably high concentrations
358 of cocaine found in the Guadalquivir and EDDP in the Ebro and the Llobregat rivers in
359 2011 (Tables 6a and 6b), and could be associated with a different consumption pattern
360 of cocaine and methadone by the population living in these areas, provided that EDDP
361 comes mainly from methadone consumption and to a minor extent from methadone
362 photolysis (Postigo et al., 2011). However, since official data on the annual
363 consumption of drugs is only available for the whole Spanish territory, without
364 distinguishing among regions (except in the single case of cannabis) (OEDA, 2019), a
365 solid association of these results with distinct human consumption habits is not possible.
366 Besides, other factors such as drug trafficking, apart from those aforementioned, could
367 also play a role (the Guadalquivir River is a trafficking route used to introduce drugs,
368 particularly cannabis, in Spain, and hence, in the European markets).

369

370 **3.4. Distribution of drugs of abuse in the sediment and water compartments**

371 The distribution of drugs of abuse and metabolites between the sediment and the
372 aqueous phase in the various sampling sites investigated was evaluated through the
373 experimental determination of the sediment-water distribution coefficient (K_D). This
374 coefficient corresponds with the average value of the ratios between the sediment and
375 water concentrations obtained in each sampling location. K_D was calculated only for
376 compounds that were found to be present in more than 6% of the sediment samples
377 analyzed, *viz.*, cocaine, MDMA, diazepam, methadone, and EDDP. The source data
378 used, reported in this study for sediments and elsewhere for water (Mastroianni et al.,
379 2016), and the experimental K_D estimated for these compounds are summarized in
380 Table 8. The compounds presenting the greatest tendency to become absorbed into the
381 sediments rather than to remain in the aqueous phase were methadone (K_D : 619 L/kg,
382 $\log K_D$: 2.79) and EDDP (K_D : 474 L/kg, $\log K_D$: 2.68), followed by cocaine (K_D : 281
383 L/kg, $\log K_D$: 2.45), MDMA (K_D : 88 L/kg, $\log K_D$: 1.95) and diazepam (K_D : 64 L/kg,
384 $\log K_D$: 1.79). Additionally, the Spearman's correlation test carried out with the data set
385 (Figure 5) showed a significant correlation (Spearman p-value < 0.05) for methadone
386 and EEDP, suggesting a good equilibrium of these compounds between both
387 compartments. The K_D value reported for cocaine differs from those obtained in
388 previous studies (K_D : 840 L/kg (Plósz et al., 2013); 469.5 L/kg (Hu et al., 2019)),
389 possibly due to the different physical-chemical characteristics of the samples

390 investigated in each work, or exceptional events altering surface water concentrations
391 and normal diffusion rates of this compound (e.g. delivered disposal of cocaine into the
392 water). K_D values for amphetamine, methamphetamine, ketamine, ephedrine,
393 benzoylecgonine, and morphine have been previously reported (Hu et al., 2019), but, to
394 the author's knowledge, this is the first time that K_D values for EDDP, methadone,
395 MDMA, and diazepam are reported from field observations.



396

397 **Figure 5.** Correlation between drugs of abuse distribution in surface water and sediment. K_D is the slope of the regression line (P : Spearman
 398 correlation coefficient; * $p\text{-value} < 0.05$ were considered statistically significant).

399

400
401

Table 8. Concentrations of cocaine, MDMA, diazepam, methadone, and EDDP in the sampling stations where they were positively identified in both the water and sediment compartments and experimental K_D obtained.

		Cocaine (n=65)			MDMA (n=11)			Diazepam (n=6)			Methadone (n=58)			EDDP (n=34)			
		Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	
Llobregat basin	2010	LLO3	24	171	7.2												
		LLO4	6.8	252	37						1.7	164	96				
		LLO5	4.2	223	53												
		LLO7	5.6	353	63			26	249	9.7	3.8	472	126	13	1180	94	
		CAR1									0.57	276	483				
		CAR2	0.89	380	426						0.46	194	422				
		CAR4	0.81	374	461						2.3	510	221	8.0	1235	154	
		ANO1	2.8	170	60												
		ANO2	1.6	474	298	7.6	133	18			9.6	1100	114	14	3835	276	
		ANO3	9.8	170	17												
		2011	LLO4									1.1	191	176	3.1	680	219
			LLO5	2.2	244	110											
			LLO6	2.7	216	80											
			LLO7	3.6	540	151			13	1320	99	15	945	65	34	3715	109
		CAR3												1.8	635	359	
		CAR4	1.9	330	178	4.3	174	40			3.1	885	286	7.9	3345	422	
		ANO1	1.2	403	342												
		ANO2	7.1	498	70	46	830	18	7.6	274	36	20	5700	285	50	15800	319
		ANO3				12	93	8			5.6	333	59	17	2535	148	
Ebro basin	2010	EBRO4	14	310	22												
		EBRO6	3.5	975	280						0.92	1260	1370	3.4	4260	1249	
		EBRO9	0.73	333	455												

402 **Table 8.** (continued)

		Cocaine (n=65)			MDMA (n=11)			Diazepam (n=6)			Methadone (n=58)			EDDP (n=34)			
		Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)	
Ebro basin	2010	ZAD			2.7	306	113	3.1	243		11	3740	346	45	9450	211	
		NAJ	2.5	173	70						0.67	162	241				
		ARG				3.1	245	78			4.9	1370	281	14	2760	200	
		HUE	34	1040	30	3.0	87	29			2.0	700	345	9.9	5850	593	
		MAR	2.8	321	114	0.84	76	90									
		CIN1									2.0	391	193				
		RS	3.4	276	80												
		2011	EBRO2	1.1	281	260	0.27	111	419								
			EBRO3								0.37	338	915	0.62	1375	2236	
			EBRO4	1.3	405	307					0.59	171	291				
			EBRO5	3.5	391	113											
			EBRO6	4.6	243	52					0.89	685	771	1.3	2460	1937	
			EBRO7	9.7	166	17					2.9	1666	57	0.47	795	1688	
		ZAD	6.7	437	65	5.1	432	84		4.8	2685	561	14	7800	542		
		ARG								5.0	945	188	13	2430	188		
		GAL2								1.0	302	299	1.6	865	554		
		HUE	25	444	17	14	188	13		4.5	650	145	14	6800	482		
		SEG	1.9	363	196					1.7	1305	759	6.0	3615	599		
Jucar basin	2010	JUC1									2.4	197	81				
		JUC2	2.2	276	125						1.8	198	110	1.4	491	343	
		JUC3	2.8	381	137						2.2	925	420	5.8	755	131	
		JUC5	1.9	227	117						0.77	174	224				
		JUC6	2.5	234	92						1.2	167	136				

403 **Table 8.** (continued)

			Cocaine (n=65)			MDMA (n=11)			Diazepam (n=6)			Methadone (n=58)			EDDP (n=34)		
			Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)
Jucar basin	2010	JUC7	3.4	225								1.1	289	255			
		JUC8	8.1	316								1.2	298	244	2.6	605	237
		CAB1										1.0	277	279			
		CAB2	1.8	268								0.41	207	499			
		MAG1	3.4	307					1.9	197		2.1	1075	524	4.8	675	140
	2011	JUC3	3.8	287								1.3	486	379	6.3	1915	306
		JUC6	2.4	525													
		JUC8	1.8	525											2.2	905	404
		CAB1	2.9	4560													
		CAB2	0.54	595													
		CAB4	2.4	346													
		MAG1							2.3	131		1.2	555	474	2.7	645	237
		MAG2	1.5	399													
Guadalquivi r basin	2010	GUA4										0.79	236	300	2.4	625	256
		GUA6	4.6	307								1.0	381	369			
		GUA7										0.45	186	415			
		GUA8										0.42	235	554			
		BOR	1.5	790								0.31	165	532			
		GUA-N	2.3	500								1.1	305	274	2.5	720	286
		YEG										0.24	198	818			
		GUA-L										0.38	251	668			

404 **Table 8.** (continued)

		Cocaine (n=65)			MDMA (n=11)			Diazepam (n=6)			Methadone (n=58)			EDDP (n=34)		
		Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K _D (L/kg)
Guadalquivir basin	2010	GEN1	9.9	535							1.4	185	128	4.5	1050	230
		GEN2	2.9	269							0.69	249	359			
		HER									0.43	165	379			
		GUA-A									14	1660	119	34	5800	172
		GUA-R									0.43	193	446			
	2011	GUA1	1.0	630												
		GUA2	3.9	1100												
		GUA4	3.0	4695							2.1	32600	15673			
		GUA5	2.4	2215												
		GUA6	3.7	750												
		GUA7	3.6	4990												
		GUA8	1.3	1020							0.65	620	957			
		GUA9	0.94	680												
		BOR	1.2	600												
		GUA-N	7.0	700							2.8	200	72			
		GUA-L	1.1	510												
		GEN1	15	424							2.9	368	126	5.5	960	175
		COR	0.81	322							0.50	236	468			
		HER	5.5	760							0.98	194	198			
		GUA-A	3.1	1305							13	3910	313	16	9650	603
Average all basins				281			88			64			619			474

405 3.5. Environmental risk assessment

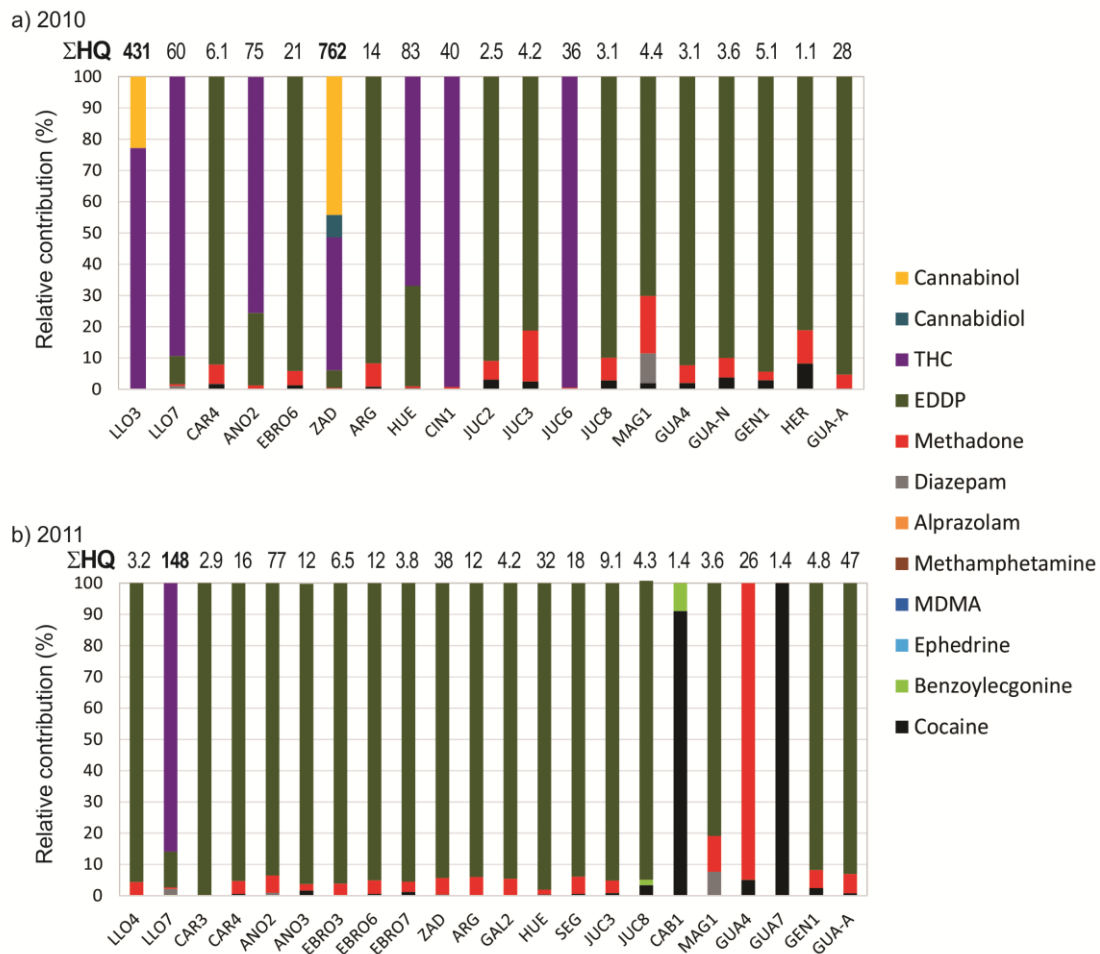
406 The accumulation of drugs of abuse and their metabolites in sediments may pose a
407 toxicological risk for aquatic organisms living or feeding on/in river sediments since
408 these substances are biologically active and their chronic effects are relatively unknown
409 (Ginebreda et al., 2014). To assess the environmental risk, the Hazard Quotient (HQ)
410 approach, where the measured environmental concentration (MEC) of a given
411 compound is compared with its predicted non-effect concentration (PNEC), at which no
412 toxic effects are expected, was applied. The PNEC values in sediments ($PNEC_{sed}$)
413 (Table 1) were extracted from the NORMAN Ecotoxicology Database for all
414 compounds except for cocaine, as it was not covered by the database. In this case, the
415 $PNEC_{sed}$ was calculated from the $PNEC_{water}$ value reported in Mendoza et al. (2014) by
416 applying the equilibrium partitioning approach that uses the NORMAN database to
417 convert $PNEC_{water}$ values (predicted by QSAR models or obtained experimentally) into
418 $PNEC_{sed}$ values (Table 1) (NORMAN, 2020).

419 To jointly consider the effects produced by the mixture of the drugs of abuse
420 investigated, the toxicological risk caused by their presence in each sample was
421 evaluated by applying a concentration addition model (Ginebreda et al., 2010), i.e., in
422 each sample, the total HQ was calculated as the sum of the individual HQ of each drug
423 or metabolite positively identified in the sample. When $\sum HQ < 1$, sampling sites were
424 not considered hazardous, whereas $\sum HQ$ values between 1 and 10 indicated potentially
425 hazardous sites, and $\sum HQ > 10$ pointed out the most hazardous sites for aquatic
426 organisms living or feeding on/in sediments. Tables 9 and 10 show HQ values obtained
427 for each sediment sample collected in 2010 and 2011 sampling campaigns, respectively.
428 The relative contribution weight of each compound to overall HQ values in those cases
429 where $\sum HQ > 1$ are depicted in Figure 6.

430 $\sum HQ$ values < 1 were obtained for 72.5% (2010) and 70.6% (2011) of the sampling
431 locations, indicating low or no potential risk for sediment-dwelling organisms. On the
432 contrary, $\sum HQ$ values between 1 and 10 were obtained for 13.0% (2010) and 14.7%
433 (2011) of the samples, and $\sum HQ > 10$ were obtained for 14.5% (2010) and 14.7%
434 (2011) of the investigated sediments, indicating risk for the aquatic organisms living or
435 feeding on/in sediments in those sampling locations. However, it should be noted that,
436 in most cases, these high values of HQ are due to the low $PNEC_{sed}$ values of specific
437 target analytes (Table 1) rather than to a high accumulation of drugs of abuse.

438 The maximum \sum HQ values were obtained for the sample collected in ZAD in 2010
439 (\sum HQ: 762) due to the contribution of cannabinal (HQ: 338), THC (HQ: 324),
440 cannabidiol (HQ: 55), and EDDP (HQ: 43), the sample collected in LLO3 in 2010
441 (\sum HQ: 431) mainly due to the contribution of THC (HQ: 333) and cannabinal (HQ: 98)
442 and the sample collected in LLO7 in 2011 (\sum HQ: 148) due to the main contribution of
443 THC (127). The remaining sampling locations presented \sum HQ < 83 (Tables 9 and 10).
444 Overall, the compounds that contributed the most to the toxicity of the samples were
445 EDDP and THC in 2010 and EDDP in 2011 (Figure 6). Methadone contributed also to
446 the toxicity of many samples but its relative contribution was low (below 20% in all
447 samples except in GUA4 in 2011).

448 In both sampling campaigns, the sampling locations LLO7, CAR4, and ANO2 in the
449 Llobregat River basin; EBRO6, ZAD, ARG, and HUE in the Ebro River basin; JUC3,
450 JUC8, and MAG1 in the Jucar River basin, and GUA4, GEN1, and GUA-A in the
451 Guadalquivir River basin presented \sum HQ values > 1 so they could be considered sites
452 with certain toxicological risk. Nevertheless, it is important to stress that these results
453 correspond to the analysis of grab samples and hence they are not necessarily
454 representative of a long-term exposure scenario.



455

456 **Figure 6.** Relative contribution (%) of different drugs of abuse to the hazard quotient
 457 obtained in each sample showing a toxicological risk ($\Sigma HQ > 1$).

458

459

460 **Table 9.** Hazard Quotient (HQ) values calculated for each compound at each sampling point during the 2010 sampling campaign.

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	HQ (max)	ΣHQ
Llobregat basin (main river)	LLO1	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
	LLO2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	LLO3	<0.1	0	0	0	0	0	0	0	0	333	0	98	333	431
	LLO4	<0.1	0	0	0	0	0	0	0.1	0	0	0	0	0.1	0.2
	LLO5	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
	LLO6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	LLO7	<0.1	0	0	0	0	0	0	0.5	0.4	5.4	53	0	0	53
Ebro basin (main river)	EBRO1	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
	EBRO2	<0.1	0	0	<0.1	0	0	0	0	0	0	0	0	<0.1	<0.1
	EBRO3	<0.1	0	0	0	0	0	0	<0.1	0.7	0	0	0	0.7	0.9
	EBRO4	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
	EBRO5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EBRO6	0.3	0	0	0	0	0	0	0.9	19	0	0	0	19	21
	EBRO7	0.2	0	0	0	0	0	0	0.2	0	0	0	0	0.2	0.4
	EBRO8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EBRO9	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
Jucar basin (main river)	JUC1	0	0	0	0	0	0	0	0.1	0	0	0	0	0.1	0.1
	JUC2	<0.1	0	0	0	0	0	0	0.1	2.2	0	0	0	2.2	2.5
	JUC3	0.1	0	0	0	0	0	0	0.7	3.4	0	0	0	3.4	4.2
	JUC4	0.3	0	0	0	0	0	0	0.1	0	0	0	0	0.3	0.5
	JUC5	<0.1	0	0	0	0	0	0	0.1	0	0	0	0	0.1	0.2
	JUC6	<0.1	0	0	0	0	0	0	0.1	0	36	0	0	36	36
	JUC7	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	0.2	0.3
	JUC8	<0.1	0	0	0	0	0	0	0.2	2.8	0	0	0	2.8	3.1

461 **Table 9.** (continued)

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	HQ (max)	ΣHQ
Guadalquivir basin (main river)	GUA1	0.1	0	0	0	0	0	0	0	0	0	0	0	0.1	0.1
	GUA2	0.1	0	0	0	0	0	0	0	0	0	0	0	0.1	0.1
	GUA3	0.1	0	0	0	0	0	0	0	0	0	0	0	0.1	0.1
	GUA4	<0.1	0	0	0	0	0	0	0.2	2.8	0	0	0	2.8	3.1
	GUA5	0.2	0	0	0	0	0	0	0	0	0	0	0	0.2	0.2
	GUA6	<0.1	0	0	0	0	0	0	0.3	0	0	0	0	0.3	0.4
	GUA7	0.1	0	0	0	0	0	0	0.1	0	0	0	0	0.1	0.2
	GUA8	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	0.2	0.3
	GUA9	<0.1	0	0	0	0	0	0	0.0	0	0	0	0	<0.1	<0.1
Llobregat basin (tributaries)	CAR1	0	0	0	0	0	0	0	0.2	0	0	0	0	0.2	0.2
	CAR2	0.1	0	0	0	0	0	0.3	0.1	0	0	0	0	0.3	0.5
	CAR3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	CAR4	0.1	0	0	<0.1	0	0	0	0.4	5.6	0	0	0	5.6	6.1
	ANO1	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
	ANO2	0.1	0	0	<0.1	0	0	0	0.8	17.4	57	0	0	57	75
	ANO3	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
Ebro basin (tributaries)	OCA	<0.1	0	<0.1	0	0	0	0	0	0	0	0	0	<0.1	<0.1
	ZAD	0.1	0	0	<0.1	0	0	0.5	2.8	43	324	55	338	338	762
	NAJ	<0.1	0	0	0	0	0	0	0.1	0	0	0	0	0.1	0.2
	ARG	0.1	0	0	<0.1	0	0	0	1.0	13	0	0	0	13	14
	GAL1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	GAL2	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
	HUE	0.3	0	0	<0.1	0	0	0	0.5	27	55	0	0	55	83
	MAR	<0.1	0	0	<0.1	0	0	0	0	0	0	0	0	<0.1	<0.1

462 **Table 9.** (continued)

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	HQ (max)	ΣHQ
	ESE	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	CIN1	0	0	0	0	0	0	0	0.3	0.0	40	0	0	40	40
	CIN2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	RS	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
	SEG	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MAT	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ALG	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	CAB1	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	0.2	0.3
	CAB2	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	0.2	0.2
Jucar basin (tributaries)	CAB3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CAB4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CAB5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MAG1	<0.1	0	0	0	0	0	0.4	0.8	3.1	0	0	0	3.1	4.4
	MAG2	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
	BOR	0.2	0	0	0	0	0	0	0.1	0	0	0	0	0.2	0.3
	GUA-M	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
	MAG	<0.1	0	0	0	0	0	0	0.3	0	0	0	0	0.3	0.4
	GUA-N	0.1	0	0	0	0	0	0	0.2	3.3	0	0	0	3.3	3.6
Guadalquivir basin (tributaries)	YEG	<0.1	0	0	0	0	0	0	0.1	0	0	0	0	0.2	0.2
	GUA-L	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	0.2	0.3
	PIC	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
	BEM	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	0.2	0.3
	CAC	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	0.2	0.3
	GEN1	0.1	0	0	0	0	0	0	0.1	4.8	0	0	0	4.8	5.1

463 **Table 9.** (continued)

	COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	<i>HQ</i> <i>(max)</i>	Σ <i>HQ</i>
GEN2	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	0.2	0.3
COR	<0.1	0	0	0	0	0	0	0.3	0	0	0	0	0.3	0.3
HER	<0.1	0	0	0	0	0	0	0.1	0.9	0	0	0	0.9	1.1
GUA-A	<0.1	0	0	0	0	0	0	1.2	26	0	0	0	26	28
GUA-R	<0.1	0	0	0	0	0	0	0.1	0	0	0		0.1	0.2
<i>HQ (max)</i>	0.3	0	0	0	0	0	0.5	2.8	43	333	55	338		

464 Σ HQ values between 1 and 10 are indicated in bold, and Σ HQ>10 in red. - Sampling stations where sediments could not be collected

465

466 **Table 10.** Hazard Quotients (HQ) values calculated for each compound at each sampling point during the 2011 sampling campaign.

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	HQ (max)	ΣHQ
Llobregat basin (main river)	LLO1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	LLO2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	LLO3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	LLO4	0	0	0	0	0	0	0	0.1	3.1	0	0	0	3.1	3.2
	LLO5	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
	LLO6	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
	LLO7	0.1	0.2	<0.1	0	0	0	2.8	0.7	17	127	0	0	127	148
Ebro basin (main river)	EBRO1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	EBRO2	<0.1	0	0	<0.1	0	0	0	0	0	0	0	0	<0.1	<0.1
	EBRO3	0	0	0	0	0	0	0	0.3	6.3	0	0	0	6.3	6.5
	EBRO4	0.1	0	0	0	0	0	0	0.1	0	0	0	0	0.1	0.2
	EBRO5	0.1	0	0	0	<0.1	0	0	0	0	0	0	0	0.1	0.1
	EBRO6	<0.1	0	0	0	0	0	0	0.5	11	0	0	0	11	12
	EBRO7	<0.1	0	0	0	0	0	0	0.1	3.6	0	0	0	3.6	3.8
	EBRO8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EBRO9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jucar basin (main river)	JUC1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	JUC2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	JUC3	<0.1	0	0	0	0	0	0	0.4	8.7	0	0	0	8.7	9.1
	JUC4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	JUC5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	JUC6	0.1	0	0	0	0	0	0	0	0	0	0	0	0.1	0.1
	JUC7	0	0	0	0	0	0	0	0	0	0	0	0	0	0

468 **Table 10.** (continued)

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	HQ (max)	ΣHQ
	JUC8	0.1	<0.1	0	0	0	0	0	0	4.1	0	0	0	4.1	4.3
Guadalquivir basin (main river)	GUA1	0.2	0	0	0	0	0	0	0	0	0	0	0	0.2	0.2
	GUA2	0.3	0	0	0	<0.1	0	0	0	0	0	0	0	0.3	0.3
	GUA3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	GUA4	1.3	0	0	0	<0.1	0	0	24	0	0	0	0	24	26
	GUA5	0.6	0	0	0	<0.1	0	0	0	0	0	0	0	0.6	0.6
	GUA6	0.2	0	0	0	0	0	0	0	0	0	0	0	0.2	0.2
	GUA7	1.4	0	0	0	0	0	0	0	0	0	0	0	1.4	1.4
	GUA8	0.3	0	0	0	0	0	0	0.5	0	0	0	0	0.5	0.7
	GUA9	0.2	0	0	0	0	0	0	0	0	0	0	0	0.2	0.2
Llobregat basin (tributaries)	CAR1	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0
	CAR2	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0
	CAR3	0.0	0	0	0	0	0	0	0	2.9	0	0	0	2.9	2.9
	CAR4	<0.1	0	0	<0.1	0	0	0	0.7	15	0	0	0	15	16
	ANO1	0.1	0	0	0	0	0	0	0	0	0	0	0	0.1	0.1
	ANO2	0.1	0	0	<0.1	0	0	0.6	4.3	72	0	0	0	72	77
ANO3	0.2	0	0	<0.1	0	0	0	0.2	12	0	0	0	12	12	
Ebro basin (tributaries)	OCA	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ZAD	0.1	0	<0.1	<0.1	0	0	0	2.0	35	0	0	0	35	38
	NAJ	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ARG	0	0	0	0	0	0	0	0.7	11	0	0	0	11	12
	GAL1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	GAL2	0	0	0	0	0	0	0	0.2	3.9	0	0	0	3.9	4.2
	HUE	0.1	0	0	<0.1	0	0	0	0.5	31	0	0	0	31	32

469 **Table 10.** (continued)

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	HQ (max)	Σ HQ
	MAR	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ESE	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	CIN1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	CIN2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	RS	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	SEG	<0.1	0	0	0	0	0	0	1.0	16	0	0	0	16	18
	MAT	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ALG	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	CAB1	1.2	0.1	0	0	0	0	0	0	0	0	0	0	1.2	1.4
	CAB2	0.2	0	0	0	0	0	0	0	0	0	0	0	0.2	0.2
	CAB3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	CAB4	<0.1	0	0	0	0	0	0	0.3	0	0	0	0	0.3	0.3
	CAB5	0	0	0	0	0	0	0	0.0	0	0	0	0	<0.1	<0.1
	MAG1	0	0	0	0	0	0	0.3	0.4	2.9	0	0	0	2.9	3.6
	MAG2	0.1	0	0	0	0	0	0	0	0	0	0	0	0.1	0.1
	BOR	0.2	0	0	0	0	0	0	0	0	0	0	0	0.2	0.2
	GUA-M	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	MAG	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	GUA-N	0.2	0	0	0	0	0	0	0.1	0	0	0	0	0.2	0.3
	YEG	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	GUA-L	0.1	0	0	0	0	0	0	0	0	0	0	0	0.1	0.1
	PIC	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BEM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CAC	0	0	0	0	0	0	0	0	0	0	0	0	0	0

470 **Table 10.** (continued)

	COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	<i>HQ (max)</i>	Σ HQ
GEN1	0.1	0	0	0	0	0	0	0.3	4.4	0	0	0	4.4	4.8
GEN2	0.3	0	0	0	0	0	0	0	0	0	0	0	0.3	0.3
COR	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	0.2	0.3
HER	0.2	0	0	0	0	0	0	0.1	0	0	0	0	0.2	0.4
GUA-A	0.4	0	0	0	0	0	0	2.9	44	0	0	0	44	47
GUA-R	<0.1	0	0	0	0	0	0	0	0	0	0	0	<0.1	<0.1
<i>HQ (max)</i>	1.4	0.2	0	0	0	0	2.8	24	72	127	0	0		

471 Σ HQ values between 1 and 10 are indicated in bold, and Σ HQ>10 in red. - Sampling stations where sediments could not be collected

472

473

474 **4. Conclusions**

475 An analytical methodology based on PLE extraction and SPE clean up followed by LC-
476 MS/MS determination has been validated and applied to assess the occurrence of 20
477 drugs of abuse and their metabolites in 144 sediment samples collected in four Spanish
478 river basins. Overall, concentrations in river sediment samples were in the low ng/g d.w,
479 being the most polluted samples those collected in tributary rivers and locations
480 downstream urban areas or impacted by WWTP effluents. Statistically significant
481 different distributions of some drugs of abuse and metabolites were observed between
482 sampling campaigns and among river basins. However, the observed changes could not
483 be related to a single factor, but a mixture of them (e.g., hydrological conditions, storm
484 events and consumption patterns of drugs of abuse in the investigated areas). Only in
485 the case of EDDP, which is mainly formed after methadone consumption, its significant
486 different distribution among river basins may be more solidly associated with different
487 consumption patterns of methadone in those areas.

488 The sediment-water distribution coefficient (K_D) of EDDP, methadone, MDMA,
489 diazepam, and cocaine were experimentally calculated by studying the relationship
490 between their concentrations in water and sediment in each investigated location. EDDP
491 and methadone were the drugs that showed the greatest tendency to become adsorbed
492 onto the sediments ($\text{Log } K_D \geq 2.68$).

493 Finally, the risk assessment study showed that the drugs present in some sampling sites
494 may pose a high risk for the aquatic organisms living or feeding on/in their sediments.
495 However, this assessment is based on grab samples. Further studies including composite
496 samples and extended in time would be required to assess the long-term exposure of
497 sediment-dwelling organisms to drugs of abuse.

498

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506

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