

SUPPLEMENTARY MATERIAL

Identification of Aquifer Recharge Sources as the Origin of Emerging Contaminants in Intensive Agricultural Areas. La Plana de Castellón, Spain

Arianna Renau-Pruñonosa ¹; Olga García-Menéndez ²; María Ibáñez ¹; Enric Vázquez-Suñé ^{3,*}; Clara Boix ¹; Bruno B. Ballesteros ⁴; Marta Hernández García ⁵; Ignacio Morell ¹; Félix Hernández ¹

¹ Research Institute for Pesticides and Water, University Jaume I, Avda. Sos Baynat, 12071 Castellón, Spain.

² Geological Survey of Spain, C/ Río Rosas 23, 28003, Madrid, Spain.

³ Institute of Environmental Assessment and Water Research (IDAEA), CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain.

⁴ Geological Survey of Spain, C/ Cirilo Amorós 42, 46004, Valencia, Spain.

⁵ CETAQUA, Centro Tecnológico del Agua, Carretera de Esplugues 75, 08940, Cornellà de Llobregat, Spain.

* Correspondence: enric.vazquez@idaea.csic.es; Tel.: +34-934 006-100

Supplementary Material

1.1. Reagents and Chemicals

Reference standards of 15 pharmaceuticals, 1 illegal drug (metabolite), and 4 pesticides and metabolites were purchased from Sigma–Aldrich (St Louis, MO, USA), LGC Promochem (London, UK), Toronto Research Chemicals (Ontario, Canada), Across Organics (Geel, Belgium), Bayer Hispania (Barcelona, Spain), Fort Dodge Veterinaria (Gerona, Spain), Vetoquinol Industrial (Madrid, Spain), and Aventis Pharma (Madrid, Spain). The main metabolite of cocaine, benzoylecgonine (BE), was obtained from Cerilliant (Round Rock, TX, USA).

Isotopically-labeled internal standards (ILIS) of acetaminophen-d₄, ibuprofen-d₃, diclofenac-d₄, valsartan-d₈, irbesartan-d₆, venlafaxine-d₆, and carbamazepine 10,11-epoxide-d₁₀ were acquired from CDN Isotopes (Quebec, Canada). Sulfamethoxazole-13C₆ and trimethoprim-13C₃ were from Cambridge Isotope Laboratories (Andover, MA, USA), and terbuthylazine-d₅ from Dr. Ehrenstorfer (Augsburg, Germany). Benzoylecgonine-d₃ was purchased from Cerilliant as a solution in methanol.

Formic acid (HCOOH, content >98%), ammonium acetate (NH₄Ac, reagent grade), sodium hydroxide (NaOH, >99%), and HPLC-grade methanol (MeOH) were purchased from Scharlab (Barcelona, Spain). HPLC grade water was obtained from distilled water passed through a Milli-Q water purification system (Millipore, Bedford, MA, USA).

1.2. Instrumentation

A Waters Acquity ultra-performance liquid chromatography (UPLC) system (Waters, Milford, MA, USA) equipped with a binary solvent manager and a sample manager was interfaced to a triple quadrupole mass spectrometer Xevo TQS (Waters) equipped with an orthogonal Z-spray electrospray ionization interface (ESI). Capillary voltages were 3.5 kV and 3.0 kV in positive and negative ionization modes, respectively. Positive/negative switching mode was applied within the same run. Chromatography separation of the compounds was carried out with an Acquity UPLC BEH C18 analytical column (100 mm × 2.1 mm, 1.7 μm, Waters). An optimized gradient was used at a constant flow rate of 0.4 mL/min using mobile phases H₂O (A) and MeOH (B), both with 0.01% HCOOH and 1mM NH₄Ac. The percentage of organic modifier (B) was changed linearly as follows: 0 min, 5%; 7 min, 90%; 8 min, 90%; 8.1 min, 5%; 10 min, 5%. The analysis run time was 10 min. The sample injection volume was 100 μL.

All data were acquired and processed using MassLynx v 4.1 software (Waters) (For further details, see Boix et al 2015).

Table SI-1. MS/MS parameters for the selected compounds. [ESI, Electrospray ionization mode; C.V., Cone voltage; Rt, Retention time; Q, Quantification transition; q, Confirmation transition; C.E., Collision energy; ILIS, isotopically labeled internal standards.]

Compounds	ESI	Rt (min)	C.V. (V)	Q	C.E. (eV)	q ¹	C.E. (eV)	q ²	C.E. (eV)
<i>Pharmaceuticals</i>									
Acetaminophen	+	1.99	10	152>110	15	152>65	25	152>93	20
Bezafibrate	-	6.01	10	360>274	20	360>154	25	360>85	15
Carbamazepine	+	5.24	10	237>194	20	237>192	20	237>179	25
Diclofenac	-	6.77	10	294>250	10	294>214	20	294>178	20
Gemfibrozil	-	7.39	10	249>121	20	249>127	10		
Iopromide	+	2.25	10	791.6>558.7	25	791.6>572.5	25	791.6>300	40
Irbesartan	+	6.19	10	429>207	25	429>195	20	429>180	25
Metoprolol	+	3.61	10	268.3>74.2	20	268.3>116.2	15	268.3>98.3	15
Pantoprazole	+	5.1	10	384>200	10	384>138	25	384>153	15
Phenazone	+	3.36	10	189.3>58.3	15	189.3>131.2	15	189.3>104.3	20
Primidone	+	3.75	10	219.2>162.3	10	219.2>91.3	20	219.2>119.2	10
Sulfamethoxazole	+	3.21	10	254>92	25	254>156	15	254>108	20
Trimethoprim	+	2.81	10	291>123	25	291>230	20	291>261	25
Valsartan	+	6.18	10	436>207	25	436>235	15	436>261	15
Venlafaxine	+	4.47	10	278>58	15	278>260	10	278>121	25
<i>Drug of abuse</i>									
Benzoylcegonine	+	3.27	10	290>168	15	290>105	25	290>92	25
<i>Pesticides</i>									
Atrazine desethyl (DEA)	+	3.99	10	188.3>146.3	15	188.3>104.2	25	188.3>79.3	25
Atrazine desisopropyl (DIA)	+	3.14	10	174.3>132.2	15	174.3>96.3	15	174.3>79.2	15
Simazine	+	4.88	10	202.3>132.1	15	202.3>124.2	15	202.3>104	20
Terbuthylazine	+	6.15	10	230.3>174.2	15	230.3>104.2	25	230.3>96.2	25
ILIS	ESI	Rt (min)	C.V. (V)	Q	C.E. (eV)				
Acetaminophen-d ₄	+	1.99	10	156>114	15				
Benzoylcegonine-d ₃	+	3.27	10	293>171	20				
Carbamazepine 10,11- epoxide-d ₁₀	+	4.41	10	263>190	25				
Diclofenac-d ₄	-	6.77	10	298>254	10				
Ibuprofen-d ₃	-	6.94	10	208.1>164.1	10				
Irbesartan-d ₆	+	6.19	10	435.1>213.3	25				
Sulfamethoxazole- ¹³ C ₆	+	3.21	10	260>162	15				
Terbuthylazine-d ₅	+	6.16	10	235.2>179.2	15				
Trimethoprim- ¹³ C ₃	+	2.81	10	294>264	18				
Valsartan-d ₈	+	6.18	10	444>207	15				
Venlafaxine-d ₆	+	4.47	10	284.3>64.1	25				

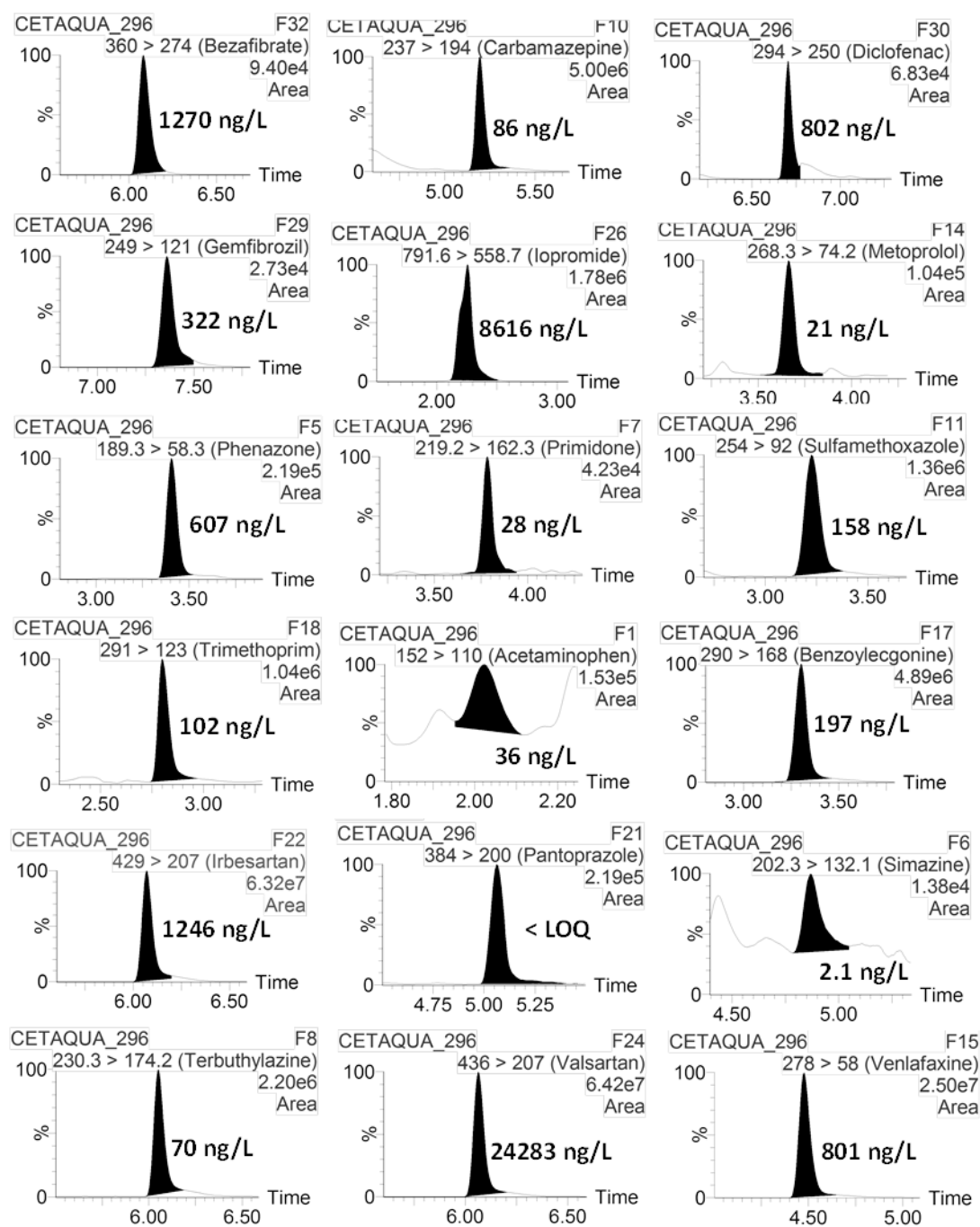


Figure SI-1. UHPLC-MS/MS chromatograms (Q transition) for an effluent wastewater sample (EWW-2*(A-B), see Table 3), where 20 target compounds were found.

Table SI-2. QC recoveries obtained for the selected compounds at the three concentration levels (10, 100, and 1000 ng/L) in six different water samples (3 GW and 3 EWW). [GW, Groundwater; EWW, effluent of the wastewater treatment plant]

Compound	QC (10 ng/L)		QC (100 ng/L)		QC (1000 ng/L)		ILIS
	G W	EWW	GW	EWW	GW	EWW	
<i>Pharmaceuticals</i>							
Acetaminophen	123 (4)	99 (2)	82 (6)	115 (5)	87 (6)	116 (5)	Acetaminophen-d ₄
Bezafibrate	83 (5)	102 (2)	111 (6)	125 (2)	89 (6)	100 (6)	-
Carbamazepine	98 (6)	89 (2)	89 (6)	117 (6)	90 (6)	120 (6)	Carbamazepine 10,11-epoxide- d ₁₀
Diclofenac	102 (5)	123 (2)	106 (2)	126 (2)	90 (6)	83 (6)	Diclofenac-d ₄
Gemfibrozil	95 (2)	94 (1)	112 (6)	123 (3)	100 (6)	88 (6)	Ibuprofen-d ₃
Iopromide	103 (6)	100 (2)	94 (6)	100 (2)	94 (6)	115 (2)	-
Irbesartan	89 (6)	74 (2)	82 (6)	91 (3)	94 (6)	94 (5)	Irbesartan-d ₆
Metoprolol	123 (6)	110 (2)	85 (6)	82 (5)	96 (6)	86 (6)	Venlafaxine-d ₆
Pantoprazole	128 (5)	103 (6)	108 (6)	85 (6)	127 (6)	101 (6)	-
Phenazone	113 (6)	81 (2)	74 (6)	69 (2)	80 (6)	105 (4)	Benzoylcegonine-d ₃
Primidone	111 (5)	102 (2)	87 (6)	71 (4)	87 (6)	84 (6)	Terbutylazine-d ₅
Sulfamethoxazole	95 (6)	89 (3)	74 (6)	74 (5)	73 (6)	68 (4)	Sulfamethoxazole- ¹³ C ₆
Trimethoprim	110 (6)	84 (2)	79 (6)	84 (6)	89 (6)	88 (6)	Trimethoprim- ¹³ C ₃
Valsartan	109 (6)	114 (2)	92 (6)	96 (2)	92 (6)	102 (2)	Valsartan-d ₈
Venlafaxine	92 (6)	83 (2)	87 (6)	95 (2)	96 (6)	91 (6)	Venlafaxine-d ₆
<i>Drug of abuse</i>							
Benzoylcegonine	125 (6)	111 (2)	100 (6)	113 (4)	100 (6)	106 (4)	Benzoylcegonine-d ₃
<i>Pesticides</i>							
DEA	128 (4)	118 (4)	63 (6)	106 (6)	66 (6)	111 (6)	Acetaminophen-d ₄
DIA	111 (6)	96 (2)	69 (6)	99 (3)	73 (6)	100 (3)	Acetaminophen-d ₄
Simazine	121 (6)	118 (6)	81 (6)	85 (6)	93 (6)	107 (6)	Carbamazepine 10,11-epoxide- d ₁₀
Terbutylazine	107 (5)	95 (6)	79 (6)	84 (6)	82 (6)	83 (6)	Terbutylazine-d ₅

Table SI-3. Minimum and maximum concentration levels of EOCs analyzed in the EWW of La Vall de Uxó and ranges found in the literature for different EWWs around the world. *A review of global publications from the last 10 years, Stankiewicz et al. 2015. [**Maximum value; ***Maximum and minimum, n=4; n.d: Not detected].

EOCs	La Vall de Uxó Min.- Max.(ng/L)***	Sampling Site	Min-Max (ng/L)	References
Acetaminophen	36-106	Greece (Central sector)	n.d.- 305	Papageorgiou et al 2016
		Portugal	1347.7-48,878	Pereira et al 2016
		U.K.	<20-11,733	Petrie et al 2015
		Spain (Castellón) *	20-390 0.2-2400	Gracia-Lor et al 2016 Stankiewicz et al 2015
Bezafibrate	780-1270	Greece (Central sector)	n.d.- <LOQ	Papageorgiou et al 2016
		Portugal	35.6-152.8	Pereira et al 2016
		U.K.	177-418	Petrie et al 2015
Carbamazepine	73-97	Greece (Central sector)	n.d.-125	Papageorgiou et al 2016
		U.K.	826-3117	Petrie et al 2015
		Germany	49 (µg/L)**	Zietzschmann et al 2016
		Spain (Castellón)	60-740	Gracia-Lor et al 2016
Diclofenac	408-802	Greece (Central sector)	n.d.- 2668	Papageorgiou et al 2016
		Portugal	n.d.-232.7	Pereira et al 2016
		U.K.	58-599	Petrie et al 2015
		Spain (Castellón)	0.15-1024 (µg/L)	Gracia-Lor et al 2016
Gemfibrozil	322-416	Greece (Central sector)	n.d.	Papageorgiou et al 2016
		*	<1-58,300	Stankiewicz et al 2015
		Portugal	49.4-187.9	Pereira et al 2016
Irbesartan	1123-1547	*	1570	Stankiewicz et al 2015
Metropolol	dic-30	Greece (Central sector)	n.d.-780	Papageorgiou et al 2016
		*	19-4470	Stankiewicz et al 2015
		U.K.	41-69	Petrie et al 2015
Pantoprazole	4.9-8.5	Spain (Castellón)	50-180	Gracia-Lor et al 2016
Phenazone	145-612	Greece (Central sector)	n.d.-18	Papageorgiou et al 2016
Primidone	28-151	Germany	47 (µg/L)**	Zietzschmann et al 2016
		Greece (Central sector)	n.d.-80	Papageorgiou et al 2016
		Germany	38 (µg/L)**	Zietzschmann et al 2016
Sulfamethoxazole	115-158	U.K.	10-19	Petrie et al 2015
		Greece (Central sector)	n.d.-95.8	Papageorgiou et al 2016
		U.K.	128-1152	Petrie et al 2015
Valsartan	13,509-24,283	*	15,700	Stankiewicz et al 2015
		U.K.	192-344	Petrie et al 2015
		Spain (Castellón)	60-300	Gracia-Lor et al 2016
Venlafaxine	619-962	U.K.	95-188	Petrie et al 2015
		Greece (Central sector)	n.d.-125	Papageorgiou et al 2016
Iopromide	1244-8616	Vietnam (Hanoi)	16-1600	Watanabe et al 2016
Benzoylecgonine	197-898	U.K	13-1597	Petrie et al 2015

Table SI-4. Location and characteristics of sources of contamination in the study area and surroundings. [WWTP, Wastewater treatment plant; IP, Irrigation pond].

ID	Pollution		UTM		Characteristics	Origin
	Diffused	Specific	X	Y		
1-1		X	738950	4410300	IP	WWTP
1-2		x	740422	4410983	IP	WWTP
1-3		x	739444	4412281	IP	WWTP
2-1		X	738720	4410230	Historical discharge of industrial wastewater to the Belcaire River	WWTP
2-2		X	737670	4410800	Historical discharge of industrial wastewater to the Belcaire River	Tannery
2-3		X	743264	4410153	Historical discharge of industrial wastewater to the Belcaire River	Tannery
2-3		X	742969	4410947	Historical discharge of industrial wastewater to the Belcaire River	Factory of cardboard packaging
3-1		X	737653	4410813	Historical brine discharge (rejection of reverse osmosis plants)	Reverse osmosis plant of La Vall d'Uxó
3-2		X	736000	4412000	Historical brine discharge (rejection of reverse osmosis plants)	Reverse osmosis plant of La Vall d'Uxó
3-3		X	742875	4410630	Historical brine discharge (rejection of reverse osmosis plants)	Reverse osmosis plant of Moncofar
4-1		X	745096	4410786	Uncontrolled closed landfill	Former municipal landfill of solid urban and industrial waste
4-2		X	734517	4410630	Uncontrolled closed landfill	Former municipal landfill of solid urban and industrial waste
4-3		X	741600	4413275	Uncontrolled closed landfill	Domestic material and industrial ceramic rejection (broken tiles)
4-4		X	741800	4413125	Uncontrolled closed landfill	Industrial ceramic rejection (broken tiles)
4-5		X	742327	4414173	Uncontrolled closed landfill	Former municipal landfill of solid urban and industrial waste
4-6		X	744239	4412208	Uncontrolled closed landfill	Former municipal landfill of solid urban
4-7		X	741821	4415512	Uncontrolled closed landfill	Former municipal landfill
4-9		X	743100	4410600	Uncontrolled closed landfill	Domestic material
5-1		X	741588	4412900	Uncontrolled landfill	Domestic material
6-1		X	741950	4413850	Landfill of inert industrial waste	Industrial ceramic rejection (broken tiles)
7-1		X	738850	4414100	Mining	Mining of Co-Hg-Cu
8-1	X				Crops irrigated with EWW (not continuously)	Irrigation
10-1	X		739160	4410032		Bullfighting ranching
10-2	X		738900	4409548		Sheep ranching
10-3	X		743571	4410533		Sheep ranching

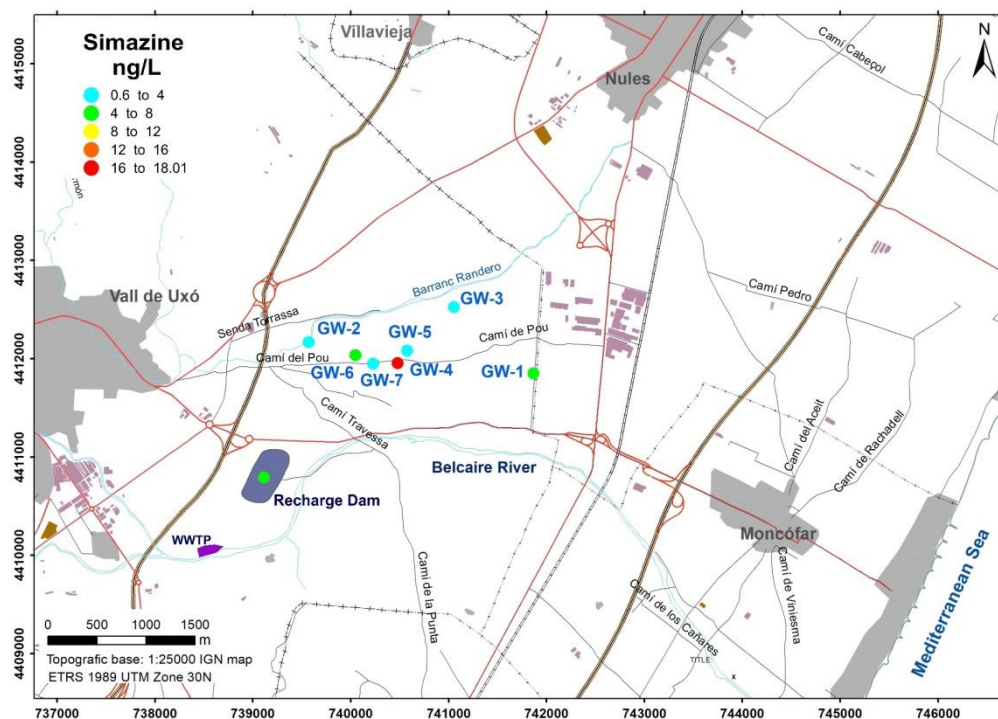


Figure SI-2. The location map of the groundwater (GW) samples and the detected maximum concentrations of simazine.

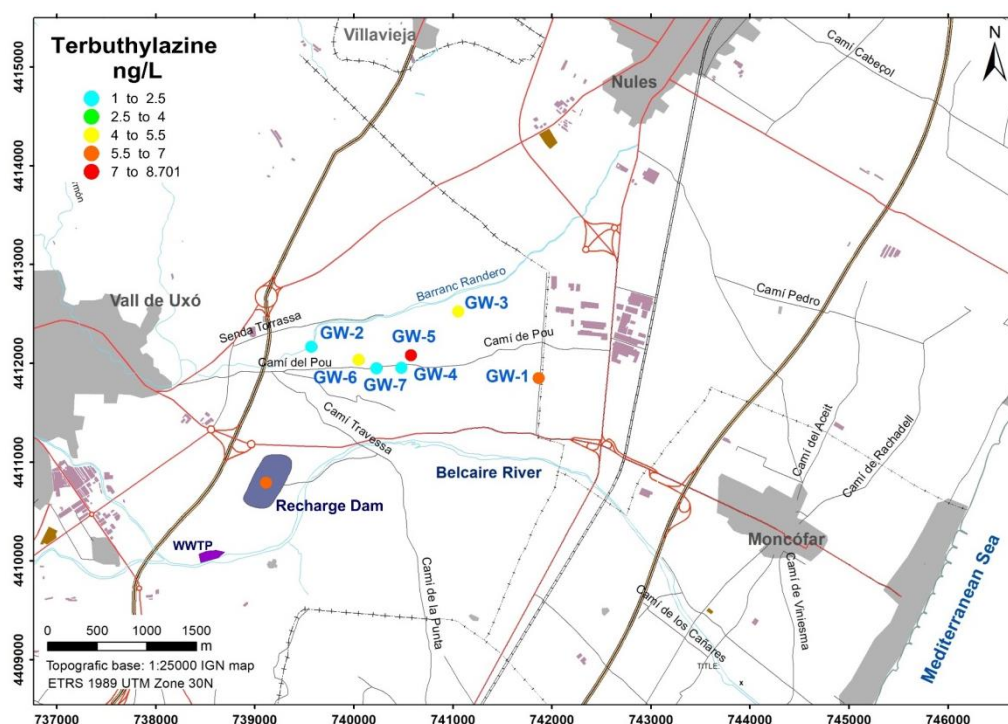


Figure SI-3. The location map of GW samples and the detected maximum concentrations of and terbutylazine.

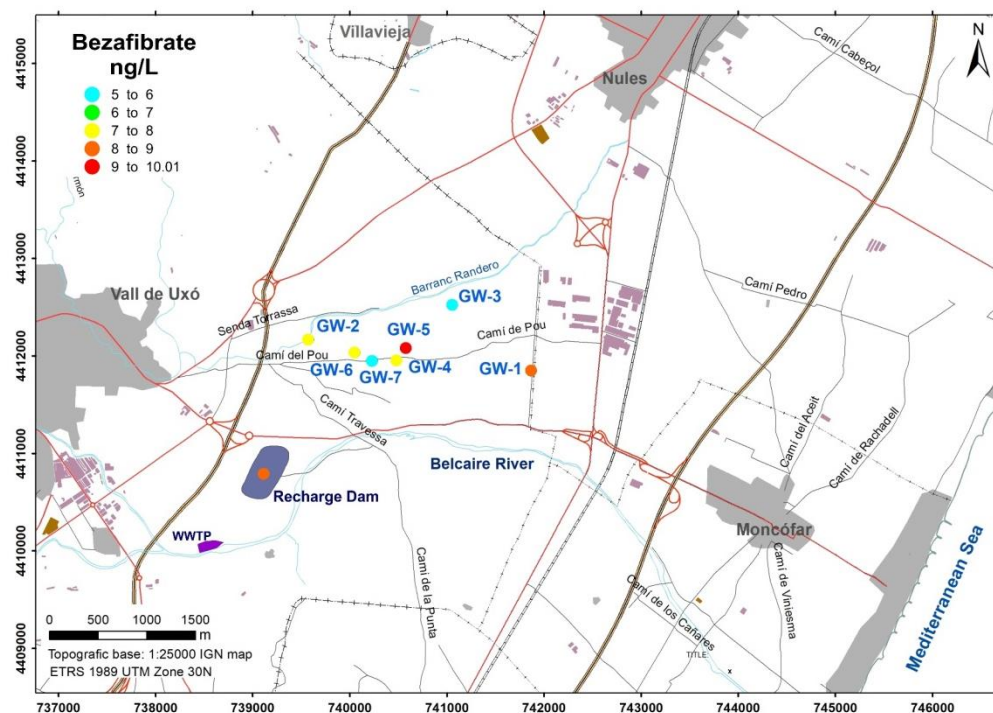


Figure SI-4. The location map of GW samples and the detected maximum concentrations of bezaifibrate.

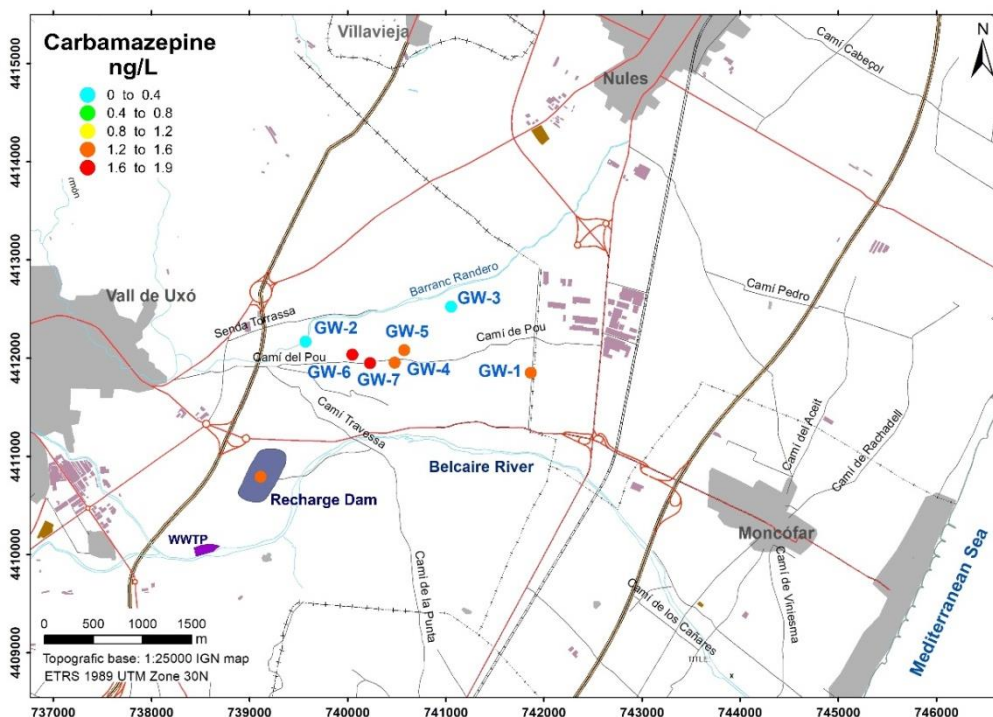


Figure SI-5. The location map of GW samples and the detected maximum concentrations of sulfamethoxazole.

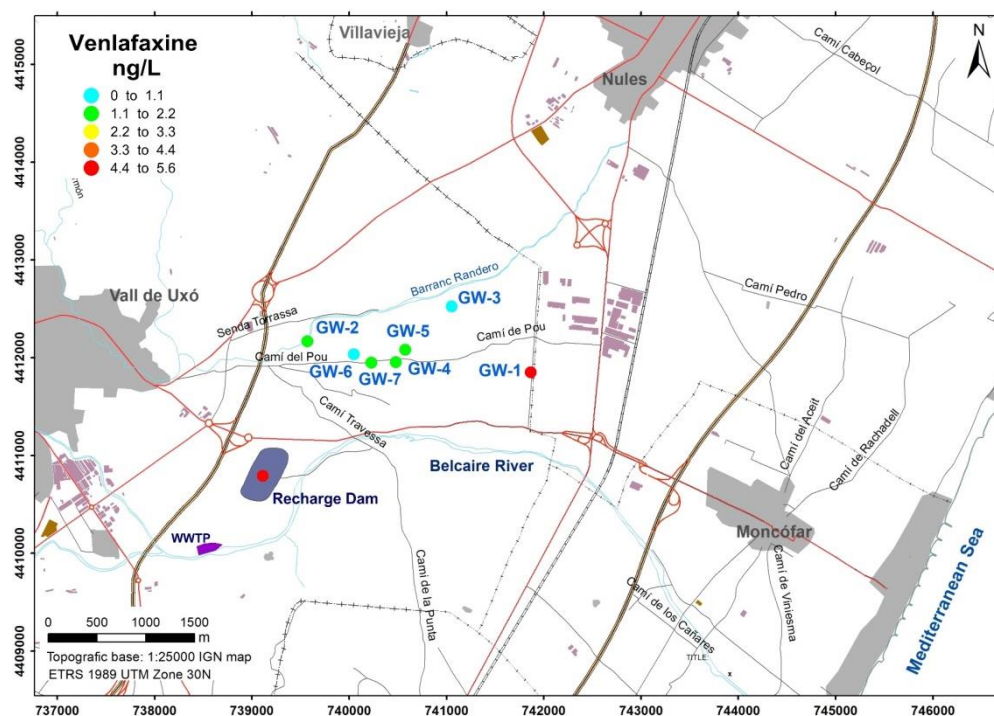


Figure SI-6. The location map of GW samples and the detected maximum concentrations of venlafaxine.



© 2019 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).