



Suspected-screening assessment of the occurrence of organic compounds in sewage sludge

Alejandro Cuñat^a, Rodrigo Álvarez-Ruiz^a, María M. Morales Suarez-Varela^{b,c}, Yolanda Pico^{a,c,*}

^a Environmental and Food Safety Research Group (SAMA-UV), Desertification Research Centre (CIDE), Universitat de València-CSIC-GV, Moncada-Naquera Road Km 4.5, 46113, Moncada, Valencia, Spain

^b Unit of Public Health and Environmental Care, Department of Preventive Medicine, University of Valencia, Valencia, Spain

^c CIBER Epidemiología y Salud Pública (CIBERESP), Institute of Health Carlos III, Madrid, Spain

ARTICLE INFO

Keywords:

High resolution mass spectrometry (HRMS)

Emerging contaminants

Tentative identification

Contaminants

Pharmaceuticals

Wastewater treatment plants (WWTP)

ABSTRACT

The profiling of emerging organic pollutants present in sludge and generated during wastewater treatment is much more limited than in water. This is mainly due to the difficulty of sludge analysis because of its high content of organic matter and interfering compounds. In this study, a generic extraction method using a mixture of buffered water (pH 4.1) and solid phase extraction (SPE) clean-up was applied to samples of sludge obtained in different treatment plants. This extraction was followed by determination of the contaminants by ultra-high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS), using suspected screening to detect the most relevant organic compounds that access the environment through sludge application. This screening (including >3000 substances, such as, pharmaceuticals, pesticides, metabolites and industrial chemicals) tentatively identified 122 compound and assigned most probable structure to 39. The set of compounds assigned to a probable structure was increased in 14 compounds by searching in a free database of metabolites. Fifteen compounds were unequivocally confirmed against the analytical standard. Pharmaceuticals and personal care products (PPCPs), with 31 substances identified and 8 confirmed were the main group of compounds. Compounds frequently detected in all sludge samples include nucleotides such as adenosine triphosphate, amino acids such as phenylalanine, or peptides such as leu-phe. Altogether, the results of this work highlight the interest of HRMS to draw the profile of organic compounds in complex matrices.

1. Introduction

Anthropogenic activities like agriculture, industry, living style or health care generate thousands of chemicals that reach the environment. Most of these organic compounds, due to its recent concern, are classified as emerging contaminants (ECs) (Murray et al., 2010). The main sources of their release to the environment are wastewater treatment plants (WWTPs). Their treatments remove only partly these organic compounds (Yap et al., 2019) that can enter to aquatic systems and soil through the effluents and the dry sludge, respectively (Andreu et al., 2009; Buta et al., 2021; García Valverde et al., 2021; Vazquez-Roig et al., 2011). During wastewater treatment, the sludge is obtained mainly by filtration of influent waters (primary treatment), and by biological treatments (secondary treatments) after anaerobic digestion to obtain biogas and a digestate that is dewatered (Clarke and Smith, 2011).

The amount of sewage sludge produced in the European Union (EU)

was 10.9 million tons in 2016 (<http://epp.eurostat.ec.europa.eu>), of which Spain produces 1 million tons, mostly disposed for agricultural use (0.941 million tons). Produced sewage sludge is called “biosolids” if they meet regulatory requirements for land application and surface disposal. This product is of interest in soils and agriculture, due to its nutrients percentage (up to 50%) that could restore overexploited soils, promoting growth of plants and improving soil’s physicochemical properties (Black et al., 2021; Buta et al., 2021; Cucina et al., 2019). However, the partial removal of compounds in WWTPs makes necessary a correct management for biosolids. The EU and many other countries have established limit levels and adequate control practices to prevent adverse effects of chemicals and pathogens in humans, animals, vegetation and soils. Currently, directive 86/278/ECC (CEC, 1986) and directive 1999/31/CE (CEC, 1999) aim to regulate the application of sludge to agricultural soil and introduce strict technical requirements, but the control of the presence of organic pollutants is still very limited.

* Corresponding author.

E-mail address: Yolanda.Pico@uv.es (Y. Pico).

<https://doi.org/10.1016/j.jenvman.2022.114587>

Received 13 August 2021; Received in revised form 16 January 2022; Accepted 21 January 2022

Available online 2 February 2022

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There is increasing concern about potential risks of the compounds present in biosolids. Different physicochemical properties, external soil properties and environmental conditions can influence the accumulation of organic compounds in soils, their transport, lixiviation (Pulkrabová et al., 2019; Verlicchi and Zambello, 2015), or bioaccumulation in different species through which they enter to food chain (Mohapatra, 2016). Recently, new methods have attained an accurate identification of organic compounds, such as liquid chromatography-high resolution mass spectrometry (LC-HRMS) (Bader et al., 2016; Osorio et al., 2018). In this way, the screening method (known as “wide target screening” or “suspected screening”) involves the use of extensive databases containing information of thousands of these compounds. The chromatographic extraction of target and/or suspect compounds, is performed with a suitable algorithm, such that, the exact masses of the appropriate target or suspect adduct are searched within a mass and retention time error. The accurate mass provides information about the most probable empirical formula and then, this empirical formula should be assigned to a structure. In recent years, studies that apply this type of analysis in samples of wastewater, or in the effluents of WWTPs, have been increasingly reported as indicated in several reviews (Bader et al., 2016; Česen et al., 2019; Kumar et al., 2019; Masiá et al., 2014) but studies in solid matrices are fewer (Black et al.; Buta et al., 2021; Castro et al., 2021; Gravert et al., 2021; Malvar et al., 2021; Martínez-Piernas et al., 2021; Riva et al., 2021; Silva et al., 2021). There is a higher complexity due to the presence of humic acids and biomolecules in this matrix that interfere with the extraction or the identification of compounds, making all these steps more complicated. LC-HMRS using either, time-of-flight (TOF) or orbitrap mass analyzers is increasingly considered as a suitable and beneficial alternative for the analysis of these challenging matrices, due to its high resolution power, mass accuracy and sensitivity (Martínez-Piernas et al., 2021; Picó and Barceló, 2015).

The present study performs a suspected or wide screening analysis by LC-HRMS using a quadrupole time-of-flight (QqTOF) and a database that contains >3000 compounds, including contaminants and natural compounds classified as pesticides, pharmaceuticals and personal care products, veterinary drugs, mycotoxins and illicit drugs on different sludge samples from several WWTPs located in the area of the Valencia province (Spain), to provide an approximation about the compounds that may access to soil through the application of the biosolids. The goal of this study is to establish a framework for the identification of the full spectrum of contaminants and natural organic compounds (comprising

parent compounds, metabolites and transformation products), including known and unknowns, to improve our understanding of the fate and impact of organic compounds present in sewage sludge.

2. Materials and methods

2.1. Location and characterization of sewage treatment plants

Eight WWTPs located in the populations close to the city of Valencia were chosen to collect and analyze the sludge samples (Fig. 1). The dehydrated sludge was collected and facilitated by the WWTPs in one sampling campaign (September 25th of 2016 to January 12th of 2017). In November 2016, the sludge samples were collected for the WWTPs of Albufera-Sur, Perelló-Sueca, Sueca and Pinedo I. Samples from Palmar and Quart were collected in December 2016. Finally, samples from Perellonet and Saler were collected in January 2017. The WWTPs selected for this study treat urban waters except for Quart-Benager that has 30% of industrial water but cover different population sizes, and consequently follows different processes for the treatment of sewage and sludge, as shown in Table 1. All WWTPs have removal of N and P and secondary treatment by activated sludge and prolonged aeration, detailed information about the treatments is available in Table S-1. Samples consist of 500 g of sludge dehydrated by centrifugation, except for Palmar WWTPs that dehydrates the sludge with a filter press.

2.2. Preparation and extraction of samples

Samples were transported to the laboratory in a portable refrigerator at 4 °C to avoid degradation. In the laboratory they were freeze-dried for 48 h in a Virtis SP Scientific lyophilizer (Gardiner, NY, USA) and stored at −20 °C until further analysis. Then, the samples were sieved to a particle size <125 µm. The sludge samples were extracted, in triplicate, using the method developed by Álvarez-Ruiz et al. (2015) and modified by Carmona et al. (2017), with some adjustments. Briefly, 1 g of lyophilized sludge was placed into a 50 mL centrifuge tube and mixed with 5 mL of H₂O, 5 mL of methanol (MeOH) and 5 mL of McIlvaine-EDTA solution (pH 4.1). This solution was prepared mixing 100 mL of 0.1 M citric acid (C₆H₈O₇), 62.5 mL of disodium hydrogen phosphate (Na₂HPO₄) 0.2 M and 6.05 g of Na₂EDTA (all reagents provided by Sigma Aldrich, Steinheim, Germany). The tube was shaken for 5 min, sonicated for 10 min (Elmasonic 120 H ultrasonic cleaner provided by ELMA, Singen, Germany) and centrifuged at 1811 rcf for 6 min.

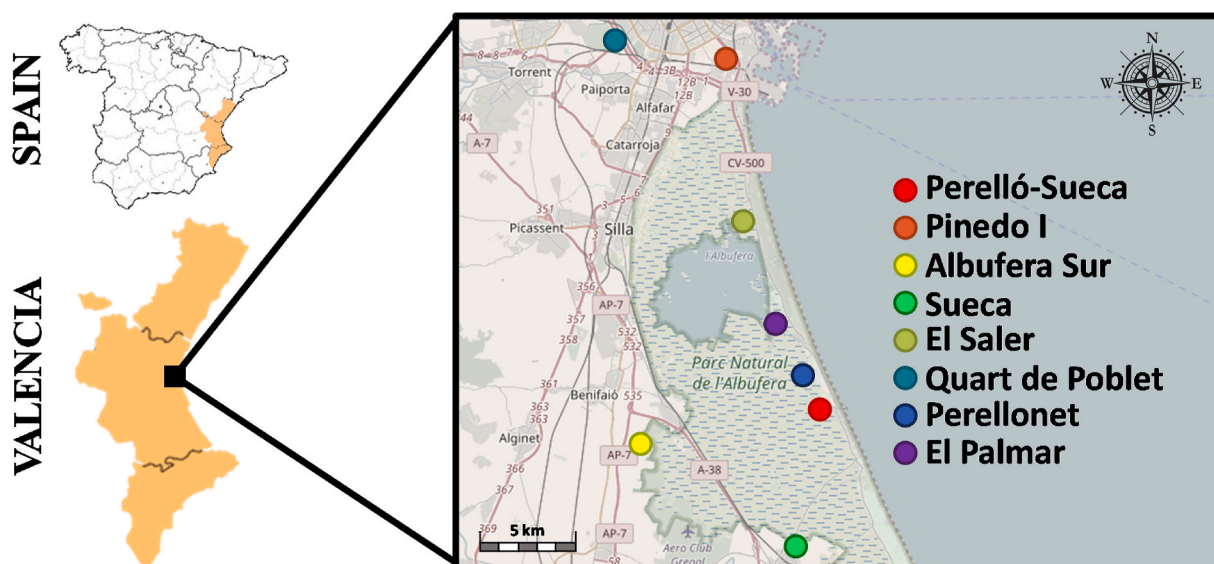


Fig. 1. Location of the studied WWTPs surrounding Albufera's Natural Park.

Table 1

General characteristics of wastewater treatment plants in 2016 (Source: EPSAR (EPSAR, Last accessed March 08, 2017)).

	Albufera Sur	Palmar	Perellonet	Perelló-Sueca	Pinedo I	Quart	Saler	Sueca
Population Served (he)	51.097	1.095	2.229	4.758	339.241	164.171	5.408	21.073
Flow (m ³ /d)	18.528	349	864	1.942	94.979	30.318	1.824	9.354
Location	La Ribera Baixa	Valencia	Valencia	La Ribera Baixa	Valencia	L'Horta Oest	Valencia	La Ribera Baixa
Wastewater Treatments	Pretreatment Primary treatment Secondary treatment	Pretreatment Secondary treatment	Pretreatment Secondary treatment	Pretreatment Secondary treatment Tertiary treatment	Pretreatment Primary treatment Secondary treatment Tertiary treatment	Pretreatment Primary treatment Secondary treatment Tertiary treatment	Pretreatment Secondary treatment	Pretreatment Secondary treatment Tertiary treatment
Sludge treatments	Chlorination GBT Anaerobic stabilization Centrifuge	Ultraviolet GBT Filter Press	Ultraviolet Mechanical thickening Centrifuge	Chlorination GBT Centrifuge	Ultraviolet GBT Anaerobic stabilization Centrifuge	Ultraviolet GBT Anaerobic stabilization Centrifuge	Ultraviolet GBT Centrifuge	Ultraviolet GBT Centrifuge

GBT: Gravity Belt Thickeners.

The supernatant was transferred into a 200 mL volumetric flask and filled with distilled water.

A solid-phase extraction (SPE) clean-up was performed using Strata-X 33U Polymeric Reversed Phase (200mg/6 mL). The 200 mL extract was passed through the cartridge, previously conditioned with 6 mL of MeOH and 6 mL of deionized water, using vacuum. Then, the cartridge was dried with vacuum for 15 min and the analytes were eluted with 6 mL of MeOH. The extract was evaporated to dryness with a gentle stream of nitrogen at 42 °C (Sample concentrator, SBHCONC/1, combined with a heating plate, SBH130D/3, both provided by Stuart®, Stafford, United Kingdom). Finally, the sample was reconstituted with 1 mL of MeOH-water (30:70, v/v), filtered using 0.22 µm syringe filters and stored in amber vials at -20 °C until analysis. The samples were analyzed in quintuplicate.

2.3. UHPLC-QqTOF-MS/MS

Ultra high-performance chromatography (UHPLC) was performed on an Agilent 1260 infinity (Agilent, Waldbronn, Germany) using a Poroshell 12 D EC-C18 column 50 mm × 30 mm i.d., 2.7 µm (Agilent). Flow rate was 0.4 ml min⁻¹ and injection volume 5 µL. Mobile phases consisted of 0.1% formic acid (positive ionization) and 10 mM ammonium formate (negative ionization) in Milli-Q-water (A) and 0.1% formic acid (positive ionization) and 10 mM ammonium formate (negative ionization) in MeOH (B). Separation was carried out in 20 min using the mobile phase gradient: 0 min, 30% B; 10 min, 85% B; 15 min, 98% B. The column was equilibrated for 15 min prior to each analysis with both ionization modes. The UHPLC system was coupled to a hybrid QqTOF ABSciex TripleTOF® 5600 calibrated as recommended by the manufacturer for MS and MS/MS in high sensitivity mode. The MS acquisition was performed using information-dependent acquisition (IDA), the survey scan type was a full scan mass spectrum between m/z 100–950. MS parameters were ion spray voltage, 5500 V; declustering potential (DP) 80 V; collision energy (CE) 10 V; at 450 °C with curtain gas (CUR) 30 (arbitrary units); ion source gas 1 (GS1) 35 and ion source gas 2 (GS2) 35. IDA MS/MS was performed on the 10 more intense ions (at least exceeding 10,000 cps) for each cycle of the instrument (ca. 250 ms) (to give an idea each chromatographic peak has between 10 and 50 cycles), with ion tolerance 50 mDa. Used as this, only one collision energy per chromatographic run can be selected but the system has the collision energy spread (CES) to improve the MS/MS spectra and make them more comparable to those of libraries and database. This feature step the across a range of energies, ensuring that an information rich spectrum is obtained during every MS/MS. To perform MS/MS CE at 35 V with CES ±35 and dynamic background subtract was activated as well as an exclusion list of the common interferences already well-known due to

the system.

Results were interpreted using the PeakView Software (Ver. 1.2) with eXtracted Ion Chromatogram (XIC) to carry out “suspected screening”. The different libraries used had more than 2300 compounds, including: 144 illicit drugs, 233 mycotoxins, 561 pesticides, 1219 pharmaceuticals and personal care products (PPCPs) and 169 veterinary antibiotics. Compounds were positively identified if the mass error was <5 ppm and the experimental isotope ratio was within 20% of the theoretical value. For some compounds, the retention time was available and was also taken into account (error <3%). These databases contain a MS/MS library, the matching score to consider a compound identified was >70%

3. Results and discussion

3.1. Extraction method performance

Numerous reports indicate that the number of organic compounds identified can vary with the extraction conditions (Buta et al., 2021; Masiá et al., 2015; Silva et al., 2021). The extraction method is very important since only compounds that pass from the sample to the extract can be further identified by LC-MS. The method used in this study will not be suitable to extract non-polar organic contaminants already widely reported in sludge, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, polychlorinated dioxins and furans (Chiaia-Hernández et al., 2020). These groups of contaminants are also not amenable by liquid chromatography but by gas chromatography and are therefore not considered in this study.

A mixture of 10 organic compounds from each group included in the libraries (pharmaceuticals, mycotoxins, pesticides, veterinary drugs and illicit drugs) were selected to perform a validation of the extraction method (Table S-2 shows the limits of detection and quantification, recoveries, precision and matrix effects for each compound). A summary of the results obtained is shown in Fig. 2, where can be observed that quantitative results were obtained for most of the pharmaceuticals, illicit drugs and veterinary drugs but not for pesticides and mycotoxins provided non-quantitative results (recoveries <70% and >25%). Since in this study compounds are identified but not quantified, this range of recoveries was considered acceptable.

These results were supported by previous studies wherein MeOH was found to be an efficient solvent compared to others to extract compounds determinable by liquid chromatography in non-target analysis of soils (Gravert et al., 2021) and sewage sludge (Black et al., 2019). According to Boix et al. (2016) and Riva et al. (2021), MeOH-water mixtures (50:50 v/v) at several pH values can be successfully used as extractants of a wide range of ECs in sewage sludge. In this study, the

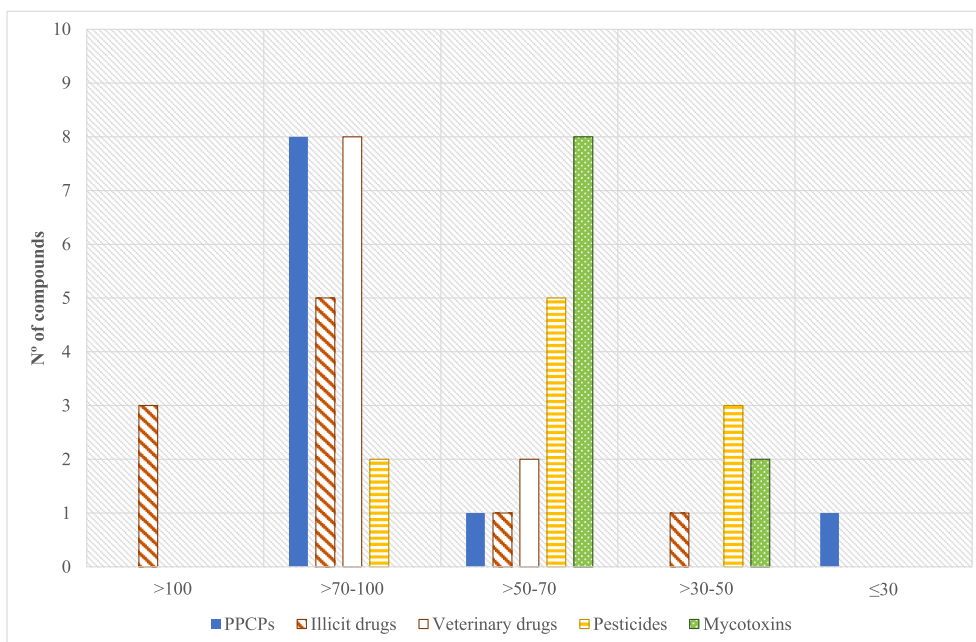


Fig. 2. Summary of the recoveries according to the type and number of compounds.

same approach was extended to a wider range of organic compounds polarities showing appropriate performance for the purpose.

3.2. Identification of organic compound present in sewage sludge

The identification scheme proposed by Schymanski et al. (2015) was followed (Fig. 3).

3.2.1. Identification of tentative candidates

According to this scheme the results of the full MS provided a list of compounds based on most probable empirical formula (those that fit theoretical mass with a mass error <5 ppm), retention times (if they are included) and isotopic pattern (<20%) match of those compounds showing a peak intensity >10,000 that were identified as “tentative candidates” (Level 3). Table 2 shows the total of 122 different compounds that were identified as tentative candidates in the sludge of the 8

WWTPs including information on the average experimental mass, its error regarding the theoretical mass and the retention times. These candidates included 19 pesticides, 71 PPCPs, 9 illicit drugs, 10 mycotoxins and 12 veterinary drugs (see Fig. S-1 for those assigned to a probable structure within each group). The high occurrence of PPCPs and their transformation products have been widely reported in other studies (Aalizadeh et al., 2019; Black et al., 2019; Boix et al., 2016; Chiaia-Hernández et al., 2020; Gravert et al., 2021; Martínez-Piernas et al., 2021).

The compounds were not detected in the sludge from all WWTPs (Table S-3). Sludge samples of Perelló-Sueca and Sueca WWTPs were those with more “tentative candidates” detected (57 and 46, respectively). These WWTPs differ in the disinfection process applied. UV in WWTP of Sueca and chlorination in WWTP of Perelló-Sueca. Most compounds detected in Perelló-Sueca (but not in Sueca) were PPCPs, suggesting either that treatment by chlorination degrade PPCPs less than

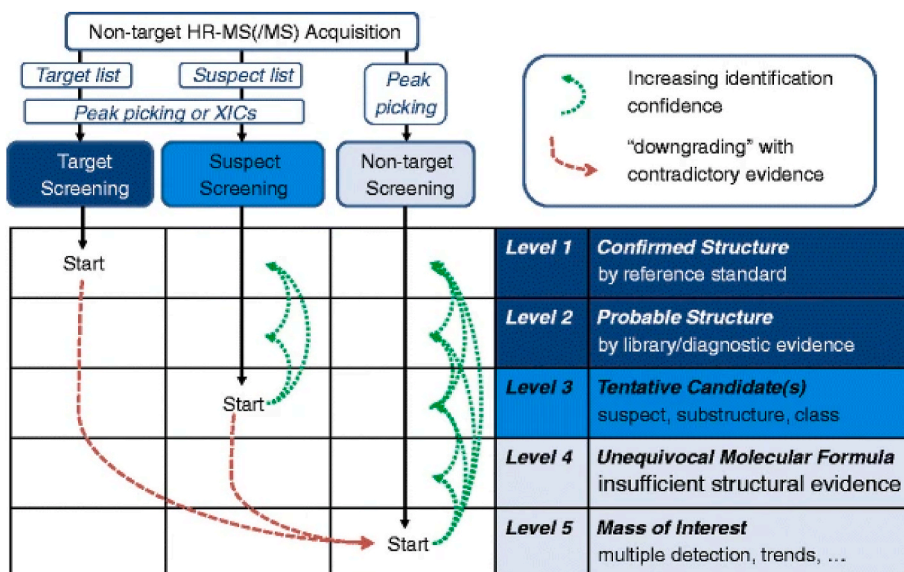


Fig. 3. Matrix of identification in target screening, suspect screening and non-target screening Schymanski et al. (2015). Reproduced with permission.

Table 2
Screening parameters of the tentatively identified compounds and assignment of the probable structure.

Tentative compound (Level 3)	Probable structure (Level 2)	Empirical formula	Mass (Da)	Extraction mass (Da) Average	Error (ppm) Average \pm SD	Retention time (min) Average \pm SD
PESTICIDES						
Acequinocyl	Acequinocyl	C ₂₄ H ₃₂ O ₄	384.2300	385.2370	-3.9 \pm 0.1	9.15 \pm 0.001
Benomyl	U.C.	C ₁₄ H ₁₈ N ₄ O ₃	290.1378	291.1449	-2.7 \pm 1.0	3.667 \pm 0.046
Carbofuran	Carbofuran	C ₁₂ H ₁₅ NO ₃	221.1052	222.1124	-0.3 \pm 0.1	2.435 \pm 0.340
Diphenamid	U.C.	C ₁₆ H ₁₇ NO	239.1310	240.1381	-0.7 \pm 0.1	6.659 \pm 0.001
Diphenylamine	Diphenylamine	C ₁₂ H ₁₁ N	169.0891	170.0963	-0.6 \pm 0.1	0.985 \pm 0.001
Dodemorph	Oleamide	C ₁₈ H ₃₅ NO	281.2718	282.2790	-0.7 \pm 0.2	13.033 \pm 0.026
Fenpropimorph	U.C.	C ₂₀ H ₃₃ NO	303.2562	304.2637	0.7 \pm 0.1	4.673 \pm 0.001
Hexazinone	U.C.	C ₁₂ H ₂₀ N ₄ O ₂	252.1586	253.1648	-3.5 \pm 0.2	0.786 \pm 0.001
Kresoxim-methyl	U.C.	C ₁₈ H ₁₉ NO ₄	313.1314	314.1385	-0.6 \pm 0.1	8.180 \pm 0.001
Mefenacet	Mefenacet	C ₁₆ H ₁₄ NO ₂	298.0776	299.0850	0.6 \pm 0.2	1.175 \pm 0.020
Metolcarb	Phenylalanine	C ₉ H ₁₁ NO ₂	165.0789	166.0859	-2.1 \pm 1.0	0.541 \pm 0.008
Imazalil	Imazalil	C ₁₄ H ₁₄ C ₁₂ N ₂ O	296.0483	297.0554	-0.7 \pm 0.1	5.664 \pm 0.002
Isoproturon	U.C.	C ₁₂ H ₁₈ N ₂ O	206.1419	207.1488	-0.5 \pm 0.1	0.922 \pm 0.001
Oxadixyl	5,6-Dimethylbenzimidazole	C ₁₄ H ₁₈ N ₂ O ₄	278.1267	279.1342	1.1 \pm 0.1	0.701 \pm 0.001
Pirimicarb	U.C.	C ₁₁ H ₁₈ N ₄ O ₂	238.1429	239.1489	-4.2 \pm 0.9	0.540 \pm 0.007
Promecarb	Ciclopirox	C ₁₂ H ₁₇ NO ₂	207.1259	208.1331	-0.6 \pm 0.1	2.557 \pm 0.001
Propham	Phenibut	C ₁₀ H ₁₃ NO ₂	179.0946	180.1015	-1.3 \pm 0.5	0.710 \pm 0.038
Tebutam	U.C.	C ₁₅ H ₂₃ NO	233.1779	234.1851	-0.8 \pm 0.1	9.347 \pm 0.001
Thiabendazole	Thiabendazole	C ₁₀ H ₁₇ N ₃ S	201.0360	202.0430	-0.9 \pm 0.1	0.794 \pm 0.001
PPCPs						
8-Hydroxyquinoline	N.D.	C ₉ H ₇ NO	145.0500	146.0600	-3.7 \pm 1.2	1.784 \pm 0.065
17-alpha-methyltestosterone	U.C.	C ₂₀ H ₃₀ O ₂	302.2245	303.2316	-0.9 \pm 0.1	10.355 \pm 0.001
Actinoquinol	U.C.	C ₁₁ H ₁₁ NO ₄ S	253.0408	254.0482	0.4 \pm 0.1	1.867 \pm 0.001
Adenine	Adenine	C ₅ H ₅ N ₅	135.0544	136.0607	-0.8 \pm 0.1	0.425 \pm 0.001
Adenosine	Adenosine	C ₁₀ H ₁₃ N ₅ O ₄	267.0967	268.1040	0.7 \pm 0.4	0.4183 \pm 0.006
Adenosine triphosphate	Adenosine triphosphate	C ₁₆ H ₁₆ N ₅ O ₁₃ P ₃	508.00457	508.00398	1.5 \pm 0.4	0.4829 \pm 0.007
Amisulpride	N.D.	C ₁₇ H ₂₇ NO ₄ S	369.1722	370.1797	0.5 \pm 0.1	0.560 \pm 0.001
Aminorex	Norcotinine	C ₉ H ₁₀ N ₂ O	162.0793	163.0860	-3.0 \pm 0.7	6.389 \pm 0.036
Amitriptyline	Amitriptyline	C ₂₀ H ₂₃ N	277.1830	278.1902	-0.4 \pm 0.2	6.389 \pm 0.046
Atenolol	Atenolol	C ₁₄ H ₂₂ N ₂ O ₃	266.1630	267.1713	3.9 \pm 0.6	10.284 \pm 0.001
Azelaic acid	Azelaic acid	C ₉ H ₁₆ O ₄	188.1048	187.0986	-1.2 \pm 0.3	3.565 \pm 0.032
Benzylamine	N.D.	C ₁₉ H ₂₃ N ₃ O	309.1841	310.1916	0.6 \pm 0.1	5.515 \pm 0.001
Benzocaine	Phenylalanine	C ₉ H ₁₁ NO ₂	165.0789	166.0862	-2.1 \pm 1.0	0.541 \pm 0.007
Betaine	Betaine	C ₅ H ₁₁ NO ₂	117.0789	118.0860	-2.0 \pm 0.1	19.302 \pm 0.001
Bevonium	N.D.	C ₂₂ H ₂₈ NO ₃	354.2069	355.2150	1.6 \pm 0.9	11.343 \pm 0.021
Bisoprolol	Bisoprolol	C ₁₈ H ₃₁ NO ₄	325.2253	326.2327	-1.4 \pm 0.8	3.008 \pm 0.007
Bupirofen	U.C.	C ₂₁ H ₃₁ N ₅ O ₂	385.2477	386.2567	3.8 \pm 0.2	4.464 \pm 0.045
Butalamine	N.D.	C ₁₈ H ₂₈ N ₄ O	316.2200	317.2328	-2.2 \pm 0.2	9.261 \pm 0.032
Butetamate	Desvenlafaxine	C ₁₆ H ₂₅ NO ₂	263.1885	264.1958	-1 \pm 0.4	1.188 \pm 0.039
Capdodiame	N.D.	C ₂₁ H ₂₉ N ₂ S	359.5940	360.1831	4.7 \pm 0.1	8.407 \pm 0.021
Carteolol	N.D.	C ₁₆ H ₂₄ N ₂ O ₃	292.1786	293.1857	-0.4 \pm 0.1	3.589 \pm 0.001
Ciprofloxacin	Ciprofloxacin	C ₁₇ H ₁₈ N ₃ O ₃	331.1332	332.1404	-0.2 \pm 0.1	0.777 \pm 0.001
Citalopram	Citalopram	C ₂₀ H ₂₁ FN ₂ O	324.1600	325.1700	0.3 \pm 0.1	4.005 \pm 0.036
Dapiprazole	U.C.	C ₁₅ H ₂₇ N ₅	325.2266	326.2331	-2.3 \pm 0.1	3.006 \pm 0.001
Dibenzepin	N.D.	C ₁₈ H ₂₁ N ₃ O	295.3788	296.1759	0.5 \pm 0.1	2.547 \pm 0.001
Dibutyl adipate	Dibutyl adipate	C ₁₄ H ₂₆ O ₄	258.3538	259.1904	-0.2 \pm 0.1	9.671 \pm 0.025
Dicycloverine	N.D.	C ₁₉ H ₃₅ NO ₂	309.4867	310.2738	-0.9 \pm 0.1	13.536 \pm 0.001
Dihydroergotomine	N.D.	C ₃₃ H ₃₇ N ₅ O	583.2794	584.2894	4.5 \pm 0.1	7.550 \pm 0.001
Doxapram	U.C.	C ₂₄ H ₃₀ N ₂ O ₂	378.5072	379.2382	0.7 \pm 0.3	11.938 \pm 0.025
Embutramide	N.D.	C ₁₇ H ₂₇ NO ₃	293.1990	294.2063	0.9 \pm 0.5	9.226 \pm 0.028
Emodin	Emodin	C ₁₅ H ₁₀ O ₅	270.0528	271.0599	-0.9 \pm 0.1	3.381 \pm 0.001
Esmolol	Esmolol	C ₁₆ H ₂₅ NO ₄	295.3742	296.1856	0.2 \pm 0.1	10.270 \pm 0.009
Eucatropine	U.C.	C ₁₇ H ₂₅ NO ₃	291.1834	292.1910	0.9 \pm 0.1	6.800 \pm 0.001
Etomidate	Etomidate	C ₁₄ H ₁₆ N ₂ O ₂	244.1211	245.1284	-0.5 \pm 0.2	1.917 \pm 0.001
Flavoxate	U.C.	C ₂₄ H ₂₅ NO ₄	391.4596	392.1839	3.4 \pm 0.1	1.183 \pm 0.001
Flecainide	Flecainide	C ₁₇ H ₂₀ F ₆ N ₂ O ₃	414.3427	415.1459	0.7 \pm 0.1	4.004 \pm 0.001
Flutamide	N.D.	C ₁₁ H ₁₁ F ₃ N ₂ O ₃	276.2118	277.0775	-4.8 \pm 0.1	6.759 \pm 0.001
Fluocinolone	Fluocinolone	C ₂₄ H ₃₀ F ₂ O ₆	452.4883	453.2105	4.4 \pm 0.1	1.052 \pm 0.001
Gemfibrozil	Gemfibrozil	C ₁₅ H ₂₂ O ₃	250.3334	251.1640	-0.8 \pm 0.1	9.384 \pm 0.001
Hexamidine	N.D.	C ₂₀ H ₂₆ N ₄ O ₂	354.4460	355.2140	3.3 \pm 0.1	11.357 \pm 0.001
Hydrocortisone	U.C.	C ₂₁ H ₃₀ O ₅	362.4599	363.2174	0.4 \pm 0.1	4.057 \pm 0.001
Hydrocortisone 21-acetate	Hydrocortisone 21-acetate	C ₂₃ H ₃₂ O ₆	404.2198	405.2271	1.5 \pm 0.4	7.059 \pm 0.021
Hydromorphone	Hydromorphone	C ₁₇ H ₁₉ NO ₃	285.3377	286.1438	0.3 \pm 0.1	8.330 \pm 0.001
Hymecromone	N.D.	C ₁₀ H ₈ O ₃	176.0473	177.0544	-2.0 \pm 0.9	6.270 \pm 0.046
Ibuprofen	Ibuprofen	C ₁₃ H ₁₈ O ₂	206.2808	205.1241	1.8 \pm 0.8	13.746 \pm 0.031
Imiquimod	N.D.	C ₁₄ H ₁₆ N ₄	240.3042	241.1440	-3.4 \pm 0.1	5.905 \pm 0.001
Imolamine	N.D.	C ₁₄ H ₂₀ N ₄ O	260.3348	261.1698	-4.4 \pm 0.1	6.607 \pm 0.001
Irbesartan	U.C.	C ₂₅ H ₂₈ N ₂ O	428.2324	429.2397	-1.2 \pm 0.5	14.014 \pm 0.026
Meptazinol	N.D.	C ₁₅ H ₂₃ NO	233.1779	234.1852	-0.4 \pm 0.2	9.401 \pm 0.045
Methenolone acetate	N.D.	C ₂₂ H ₃₂ O ₃	344.2351	345.2424	-0.4 \pm 0.3	9.975 \pm 0.020
Methohexital	U.C.	C ₁₄ H ₁₈ N ₂ O ₃	262.1317	263.1390	0.5 \pm 0.4	1.161 \pm 0.014

(continued on next page)

Table 2 (continued)

Tentative compound (Level 3)	Probable structure (Level 2)	Empirical formula	Mass (Da)	Extraction mass (Da) Average	Error (ppm) Average \pm SD	Retention time (min) Average \pm SD
Metoprolol	U.C.	C ₁₅ H ₂₅ NO ₃	267.1834	268.1909	0.9 \pm 0.1	9.421 \pm 0.004
Mexiletine	Mexiletine	C ₁₁ H ₁₇ NO	179.1310	180.1382	-2.4 \pm 0.4	0.468 \pm 0.026
Nitrendipine	Nitrendipine	C ₁₈ H ₂₀ N ₂ O ₆	360.3612	361.1401	1.1 \pm 0.4	3.981 \pm 0.012
Norfloxacin	N.D.	C ₁₆ H ₁₈ FN ₃ O ₃	319.3308	320.1406	0.4 \pm 0.1	0.723 \pm 0.001
Ofloxacin	Ofloxacin	C ₁₈ H ₂₀ FN ₃ O ₄	361.1437	362.1507	0.5 \pm 0.1	0.679 \pm 0.001
Penbutolol	U.C.	C ₁₈ H ₂₉ NO ₂	291.4284	292.2270	-0.4 \pm 0.1	2.598 \pm 0.006
Phenazone	Phenazone	C ₁₁ H ₁₂ N ₂ O	188.2258	189.1013	-3.2 \pm 0.1	1.085 \pm 0.001
Phenethylamine	Phenethylamine	C ₈ H ₁₁ N	121.0796	122.0959	-4.1 \pm 1.0	0.571 \pm 0.062
Prajalium	N.D.	C ₂₃ H ₃₃ N ₂ O ₂	369.5294	370.2619	0.6 \pm 0.1	11.623 \pm 0.068
Progesterone	Progesterone	C ₂₁ H ₃₀ O ₂	314.4617	315.2316	0.3 \pm 0.1	9.294 \pm 0.001
Propafenone	U.C.	C ₂₁ H ₂₇ NO ₃	341.4440	342.2066	0.7 \pm 0.1	2.101 \pm 0.001
Trapidil	Trapidil	C ₁₀ H ₁₅ N ₅	205.2654	206.1405	-3.3 \pm 0.1	3.562 \pm 0.001
Telmisartan	Telmisartan	C ₃₃ H ₃₀ N ₄ O ₂	514.6173	515.2445	-0.5 \pm 0.2	7.533 \pm 0.054
Thonzylamine	N.D.	C ₁₆ H ₂₂ N ₄ O	286.3723	287.1859	-2.7 \pm 0.1	6.790 \pm 0.055
Thymopentin	N.D.	C ₃₈ H ₄₉ N ₉ O ₉	679.3653	680.3726	-2.6 \pm 1.2	2.960 \pm 0.054
Tolycaine	Leu-Phe	C ₁₅ H ₂₂ N ₂ O ₃	278.1630	279.1704	0.6 \pm 0.2	1.590 \pm 0.065
Trimethoprim	U.C.	C ₁₄ H ₁₈ N ₄ O ₃	290.1300	291.1449	2.7 \pm 1.0	3.674 \pm 0.047
Tropium	N.D.	C ₂₃ H ₃₀ NO ₃	392.2100	393.2200	-2.4 \pm 1.1	3.609 \pm 0.044
Valdecoxib	U.C.	C ₁₆ H ₁₄ N ₂ O ₃ S	314.0725	315.0800	1.2 \pm 0.5	0.4121 \pm 0.008
Valsartan	Valsartan	C ₂₄ H ₂₉ N ₅ O ₃	435.2270	436.2327	0.3 \pm 0.1	8.308 \pm 0.001
Venlafaxine	U.C.	C ₁₇ H ₂₇ NO ₂	277.2041	278.2117	0.6 \pm 0.2	3.097 \pm 0.024
ILLICIT DRUGS						
bk-MMBDB	N.D.	C ₁₃ H ₁₇ NO ₃	235.1208	236.1281	-0.9 \pm 0.1	4.041 \pm 0.032
EDDP	Amitriptyline	C ₂₀ H ₂₃ N	277.1830	278.1902	-0.4 \pm 0.2	6.370 \pm 0.045
JWH-250	N.D.	C ₂₂ H ₂₅ NO	319.1936	320.2000	-2.1 \pm 0.5	2.468 \pm 0.020
MDA	Phenibut	C ₁₀ H ₁₃ NO ₂	179.0946	180.1019	-1.4 \pm 0.6	0.720 \pm 0.048
MDMA	Salsoline	C ₁₁ H ₁₅ NO ₂	193.1102	194.1174	-0.8 \pm 0.1	0.513 \pm 0.001
MDPV	U.C.	C ₁₆ H ₂₁ NO ₃	275.1521	274.1447	0.4 \pm 0.1	11.212 \pm 0.001
Mephedrone	U.C.	C ₁₁ H ₁₅ NO	177.1153	178.1226	-1.9 \pm 0.9	3.156 \pm 0.002
Metamphetamine	3-Pentylpyridine	C ₁₄ H ₁₅ N	149.1204	150.1270	-4.1 \pm 0.1	0.466 \pm 0.001
Methcathinone	U.C.	C ₁₀ H ₁₃ NO	163.0997	164.1067	-1.9 \pm 0.9	2.261 \pm 0.060
MYCOTOXINS						
Agroclavine	N.D.	C ₁₆ H ₁₈ N ₂	238.1469	239.1540	-1.3 \pm 0.1	0.909 \pm 0.001
Anisomycin	Anisomycin	C ₁₄ H ₁₉ NO ₄	265.1314	266.1388	0.3 \pm 0.1	2.792 \pm 0.001
Aspinonene	N.D.	C ₉ H ₁₆ O ₄	188.1049	189.1118	-1.1 \pm 0.3	3.532 \pm 0.028
Aurofusarin	N.D.	C ₃₀ H ₁₈ O ₁₂	570.0798	571.0898	4.8 \pm 0.1	14.242 \pm 0.001
Brefeldin A	Brefeldin A	C ₁₆ H ₂₄ O ₄	280.1674	281.1748	0.2 \pm 0.1	8.123 \pm 0.001
Cytochalasin J	N.D.	C ₂₈ H ₃₇ NO ₄	451.2722	452.2795	-2.6 \pm 1.2	4.395 \pm 0.056
Enniatin B1	N.D.	C ₉ H ₁₆ O ₄	188.1015	187.0986	1.7 \pm 0.9	1.672 \pm 0.068
HC-Toxin	N.D.	C ₂₁ H ₃₂ N ₄ O ₆	436.5092	437.2366	-3.65 \pm 0.8	1.032 \pm 0.001
Ionomycin	U.C.	C ₄₁ H ₇₂ O ₉	708.5176	709.5236	-1.8 \pm 0.1	13.373 \pm 0.001
Ophiobolin A	U.C.	C ₂₅ H ₃₆ O ₄	400.2613	401.2668	-4.6 \pm 0.1	3.045 \pm 0.001
VETERINARY DRUGS						
Amprolium	Amprolium	C ₁₄ H ₁₉ N ₄	242.1531	265.1423	1.8 \pm 0.7	9.522 \pm 0.028
Brilliant Green	N.D.	C ₂₇ H ₃₃ N ₂	385.2643	408.2529	-3.5 \pm 1.0	3.853 \pm 0.001
Enrofloxacin	U.C.	C ₁₉ H ₂₂ FN ₃ O ₃	359.1645	377.1970	0.6 \pm 0.1	3.855 \pm 0.040
Leucogentian Violet	N.D.	C ₂₃ H ₃₁ N ₃	373.2518	391.2856	-3.2 \pm 1.3	13.994 \pm 0.033
Malachite Green	N.D.	C ₂₃ H ₂₅ N ₂	364.1706	330.2090	1.1 \pm 0.5	11.228 \pm 0.017
Medroxyprogesterone 17-acetate	U.C.	C ₂₄ H ₃₄ O ₄	386.2457	387.2530	-1.8 \pm 0.3	9.144 \pm 0.045
Megestrol acetate	Megestrol acetate	C ₂₄ H ₃₂ O ₄	384.2300	383.2227	-2.4 \pm 0.4	12.847 \pm 0.020
Naproxen	U.C.	C ₁₄ H ₁₄ O ₃	228.0700	229.0877	-0.9 \pm 0.3	2.478 \pm 0.005
Orbifloxacin	N.D.	C ₁₉ H ₂₀ F ₃ N ₃ O ₃	395.1457	413.1779	-3.9 \pm 0.1	6.477 \pm 0.001
Sulfacetamide	U.C.	C ₈ H ₁₀ N ₂ O ₃ S	214.0412	232.0750	1.4 \pm 0.5	11.942 \pm 0.024
Sulfameter	U.C.	C ₁₁ H ₁₂ N ₄ O ₃ S	280.0630	298.0968	1.6 \pm 0.7	0.578 \pm 0.002
Tetracycline	Tetracycline	C ₂₂ H ₂₄ N ₂ O ₈	444.1500	445.1600	3.6 \pm 0.7	5.432 \pm 0.042

U.C.: Unidentified compound because no MS/MS that matched the experimental one was found in the library of the instrument of in the Metlin.

N.D.: No MS/MS data obtained that allow to assign a probable structure.

UV treatment, or the less plausible hypothesis that there is a higher consumption of these compounds in Perelló-Sueca.

On the other hand, Perellonet has the samples with fewer compounds detected (37). This is the only WWTP with mechanical thickener (first process in sludge line to reduce and concentrate sludge volume). The mechanical thickeners are like decanters, and particles in suspension are decanted individually or in flocs, they also use centrifugal force to sediment solid particles. However, thickener processes are not sufficient to remove organic compounds. The most probable explanation is the low population served (around 2000 inhabitants).

3.2.2. Assignment of probable structures

The tentative candidates of the list could be misinterpreted because the same empirical formula could correspond to several structures. The next step was to study the MS/MS spectra to establish whether the "probable structure" of the compounds (Level 2) match the initial identification. First, MS/MS spectra of the compounds were compared with those of the database, if the purity score is >70%, it was considered that the compound matched the probably structure identification. From the 122 candidates list, only 39 compounds were identified at the probably structure level using the MS/MS library of the database. These structures included 6 pesticides, 28 pharmaceuticals, 2 mycotoxins and

3 veterinary drugs (see Fig S-2 for imazalil). Of these, 21 pharmaceuticals, 2 mycotoxins and 2 veterinary drugs remains as probable structures. As an example, Fig. 4 illustrates the probable structure assignment of flecainide -an antiarrhythmic pharmaceutical-by UHPLC-QqTOF-MS in a sludge extract. The MS/MS in addition to the protonated molecule shows two fragments: one at m/z 398.11976 due to the loss of NH_3 and other at m/z 301.02998 corresponding to the neutral loss of piperidin-2-ylmethanamine and the formation of (2,5-bis(2,2,2-trifluoroethoxy)benzylidene) oxonium. Thus, the high purity score as well as the product ions obtained match well the flecainide. Interestingly, since the compounds phenylalanine, benzocaine, metolcarb have the same empirical formula ($\text{C}_9\text{H}_{11}\text{NO}_2$) and the same theoretical mass (m/z 165.0789). Fig. 5 displays the MS/MS that shows two products ions at m/z 120.07683 and 103.95468, that correspond the loss of CO_2 and formation of the 2-amino-1-phenylethen-1-ylum and to the subsequent loss of ammonia and the formation of phenylethen-1-ylum. These products ions only match with the L-phenylalanine. It is indispensable the verification using a reference spectrum from a database (or AS) to perform a correct identification.

Of the 10 tentative mycotoxins, 2 were identified by the MS/MS. The

compounds detected are the anisomycin (a compound produced by *Streptomyces griseolus* used in some cases as antibiotic) and the brefeldin A (a compound produced by *Eupenicillium brefeldianum*).

Among the 12 tentatively detected veterinary antibiotics, only amprolium, megestrol acetate and tetracycline were assigned by the MS/MS to their probable structures (Table 3). In the case of the amprolium, some fragments that do not match with the referent spectra were also detected (Fig. S-3).

However, 83 tentatively identified compounds could not be associated to any probable structure and remain as tentative identified compounds in the list. Of them, 35 do not provide MS/MS because they were not detected between the 10 more intense ions in any cycle. However, there are 48 compounds whose MS/MS spectrum does not match that of the proposed compound in the database.

To enlarge the number of compounds that can be assigned to a probable structure. Then, the possible structures were searched in the METLIN database (METLIN, <https://metlin.scripps.edu/>). The MS/MS spectra of this database were free available until December 31, 2021. Now, the database has increased their capabilities but also has a fee to be used for identification. The METLIN database provided free

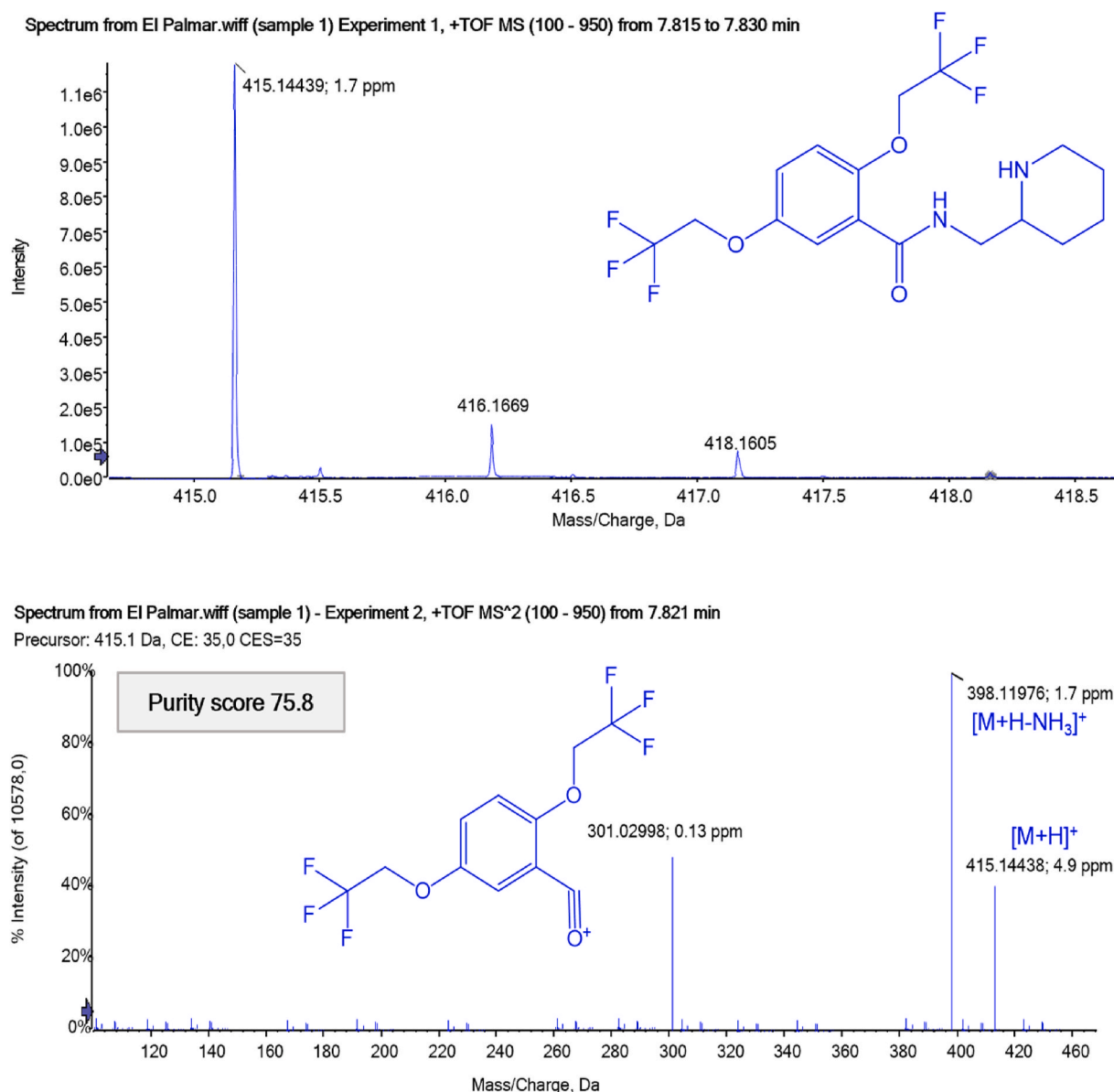
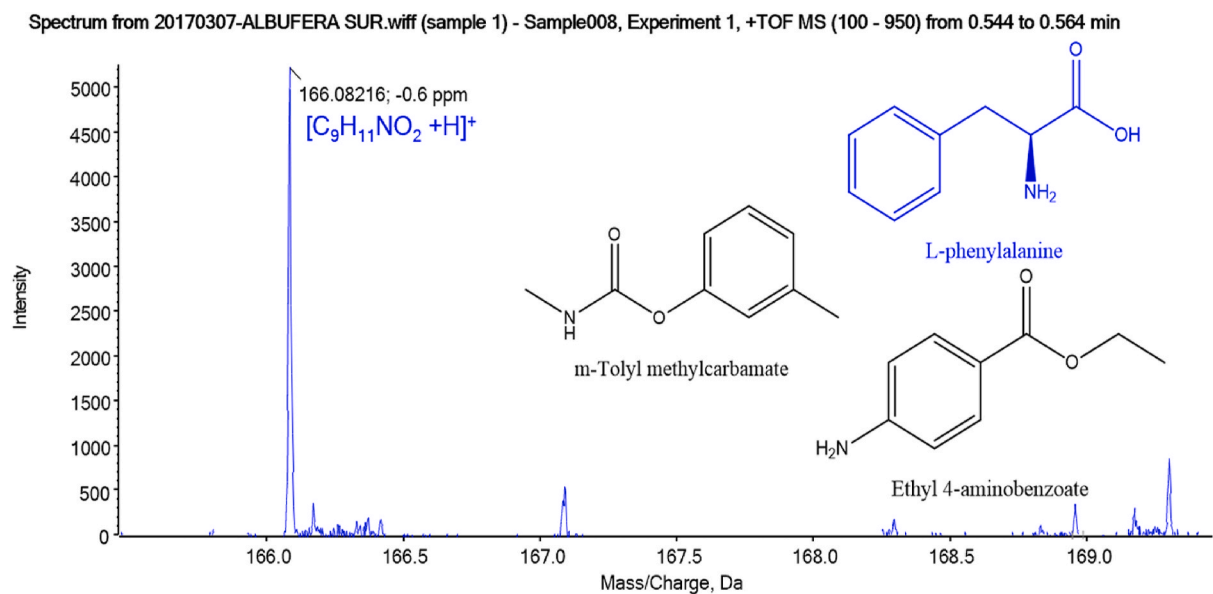


Fig. 4. MS and MS/MS of the peak assigned to the structure of Flecainide.



Spectrum from 20170307-ALBUFERA SUR.wiff (sample 1) - Sample008, Experiment 3, +TOF MS² (100 - 950) from 0,554 min

Precursor: 166,1 Da, CE: 35,0 CES=35

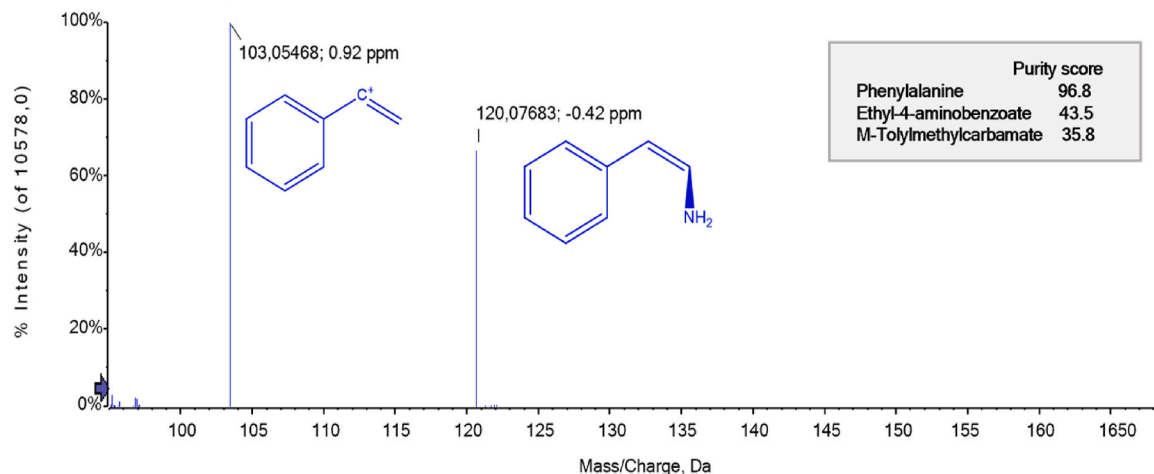


Fig. 5. MS and MS/MS of the peak tentatively identified as phenylalanine/benzocaine (ethyl 4-aminobenzoate)/metolcarb (m-tolyl methylcarbamate) and assigned to the phenyl alanine as possible structure.

experimental MS/MS data for ca. 15,000 molecules. The criteria to identify a compound is that all product ions with an intensity >30% match and that the intensity of these ions were within an error of 30% of the standard. Furthermore, a feature of the Peak View software, the formula finder, was also used because once there is a possible structure, its algorithm estimates the most favored product ions. Hence, it can provide an additional support to the identification.

Thirty-four compounds that provided MS/MS remained as unknowns because their MS/MS spectra could not be matched according to the criteria with any of the Metlin spectra. The other pesticides tentatively identified (**level 3**) could not be assigned to a probable structure (**level 2**). One relevant example is the compound tentatively identified as benomyl or trimethoprim (same empirical formula), but that showed a MS/MS spectrum that does not match any of these compounds (Fig. S-4). Since there are approximately 6500 commercial compounds with the same empirical formula (C₁₄H₁₈N₄O₃), the difficulty for screening and identification is enormous and, indeed, this compound could not be identified.

It was possible to assign a probable structure for 14 compounds. Regarding pesticides, comparison of the MS/MS spectrum with those of the compounds with the same empirical formula contained in the Metlin database, shows that tentatively identified dodemorph, actually matched the oleamide spectrum, which is an amide derived from the oleic acid, used in the therapy of sleep disorders and depression produced by cannabis, but also used as additive in different plastics and is a human metabolite). Fig. 6 shows the MS/MS attributed to oleamide as well the basic data of the fragments observed. The QqTOF-MS of the oleamide in the database showed several abundant fragments between *m/z* 50 and 100, a mass range that were not cover in our spectra, but the fragments in the range covered in our spectra and that of the database are coincident. Furthermore, as can be observed exists a good match between the proposed structure and the fragmentation observed. The pesticides oxadixyl, promecarb and propham were according to the MS/MS assigned, respectively, as 5,6-dimethylbenzimidazole (amino acid), ciclopirox (fungicide) and phenibut (anxiolytic). Adenosine triphosphate (ATP) was also assigned to its probably structure thanks to the

Table 3

Compounds with a possible structure assigned at level 2 and confirmed at level 1 in the sludge samples.

Compound group	Compound name	Albufera Sur	Palmar	Perellonet	Perelló-Sueca	Pinedo	Quart	Saler	Sueca
PESTICIDES									
	Acequinocyl	n.d.	n.d.	n.d.	n.d.	C	n.d.	n.d.	n.d.
	Carbofuran	n.d.	C	n.d.	C	n.d.	n.d.	n.d.	n.d.
	Diphenylamine	C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Oleamide	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.
	Mefenacet	n.d.	C	n.d.	C	n.d.	C	n.d.	C
	Imazalil	n.d.	n.d.	n.d.	C	C	n.d.	n.d.	n.d.
	Ciclopirox	n.d.	n.d.	n.d.	P.S.	n.d.	n.d.	n.d.	n.d.
	Thiabendazole	n.d.	n.d.	n.d.	n.d.	C	n.d.	n.d.	n.d.
PPCPs									
Analgesic	Fluocinolone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	P.S.	n.d.
	Hydrocortisone 21-acetate	P.S.	P.S.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Hydromorphone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	P.S.
	Ibuprofen	C	n.d.	C	C	n.d.	n.d.	C	C
	Phenazone	n.d.	n.d.	n.d.	n.d.	n.d.	P.S.	n.d.	n.d.
Anesthetic	Phenylalanine	C	C	C	C	C	C	C	C
	Etomidate	P.S.	P.S.	n.d.	n.d.	n.d.	n.d.	P.S.	n.d.
Stimulant	Norcotinine	P.S.	n.d.	n.d.	n.d.	P.S.	P.S.	n.d.	P.S.
Antiarrhythmic	Flecainide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	P.S.
	Mexiletine	P.S.	P.S.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antibiotic	Ciprofloxacin	n.d.	n.d.	C	n.d.	n.d.	n.d.	n.d.	n.d.
	Ofloxacin	n.d.	n.d.	C	n.d.	n.d.	n.d.	n.d.	n.d.
Antidepressant	Amitriptyline	n.d.	n.d.	n.d.	P.S.	n.d.	n.d.	P.S.	P.S.
	Citalopram	n.d.	n.d.	P.S.	P.S.	n.d.	n.d.	P.S.	P.S.
	Desvenlafaxine	n.d.	n.d.	P.S.	n.d.	P.S.	P.S.	n.d.	n.d.
	Telmisartan	n.d.	n.d.	n.d.	n.d.	C	C	n.d.	C
	Valsartan	n.d.	n.d.	n.d.	n.d.	C	n.d.	n.d.	n.d.
Antiviral	Adenine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	P.S.	n.d.
B-Bloquer	Atenolol	C	C	n.d.	n.d.	n.d.	C	n.d.	n.d.
	Bisoprolol	P.S.	P.S.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Esmolol	n.d.	n.d.	n.d.	P.S.	n.d.	n.d.	n.d.	P.S.
Channel bloquer	Nitrendipine	P.S.	P.S.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CNS stimulant	Phenethylamine	n.d.	n.d.	n.d.	P.S.	P.S.	n.d.	n.d.	P.S.
Laxative	Emodin	n.d.	n.d.	n.d.	P.S.	n.d.	n.d.	n.d.	n.d.
Lipid regulator	Adenosine triphosphate	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.
	Gemfibrozil	n.d.	n.d.	n.d.	C	n.d.	n.d.	n.d.	n.d.
PCP	Azelaic acid	n.d.	n.d.	P.S.	P.S.	n.d.	n.d.	P.S.	P.S.
	Betaine	n.d.	n.d.	n.d.	n.d.	n.d.	P.S.	n.d.	n.d.
	Dibutyl adipate	n.d.	n.d.	n.d.	P.S.	n.d.	n.d.	n.d.	P.S.
Steroid	Progesterone	n.d.	n.d.	n.d.	n.d.	n.d.	P.S.	n.d.	n.d.
Vasodilator	Trapidil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	P.S.	n.d.
ILICIT DRUGS									
	Phenibut	n.d.	P.S.	n.d.	P.S.	n.d.	n.d.	n.d.	P.S.
MYCOTOXINS									
	Anisomycin	n.d.	n.d.	n.d.	P.S.	n.d.	n.d.	n.d.	n.d.
	Brefeldin A	n.d.	n.d.	n.d.	P.S.	n.d.	n.d.	n.d.	n.d.
VETERINARY ANTIBIOTICS									
	Amprolium	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.
	Megestrol acetate	P.S.	n.d.	n.d.	n.d.	n.d.	P.S.	n.d.	n.d.
	Tetracycline	n.d.	C	C	n.d.	C	C	n.d.	n.d.
BIOLOGICAL COMPOUNDS									
	5,6 Dimethyl benzimidazole	n.d.	n.d.	n.d.	n.d.	n.d.	P.S.	n.d.	n.d.
	3-Pentylpyridine	P.S.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Leu-Phe	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.
	Salsoline	n.d.	n.d.	n.d.	n.d.	n.d.	P.S.	n.d.	n.d.
	Adenosine Triphosphate	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.	P.S.

P.S.: Possible Structure.

C: Confirmed.

n.d.: not detected.

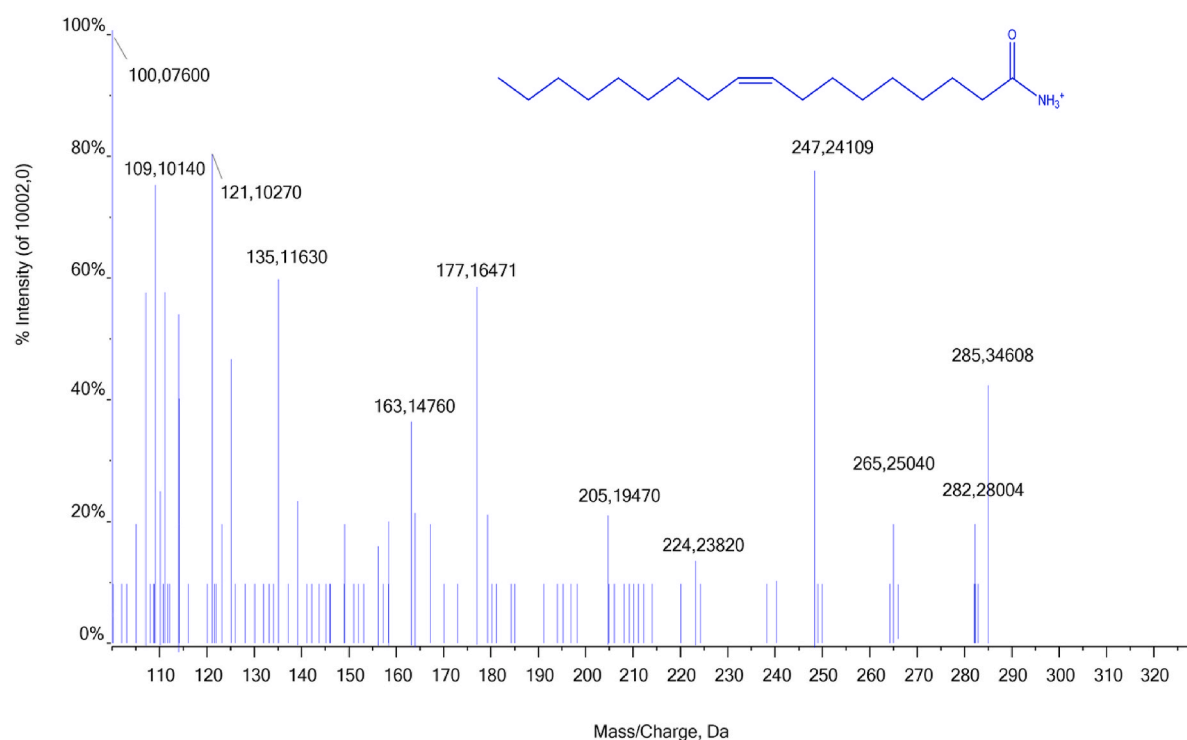
MS/MS reported in this database since it was not available in the instrumental database. This compound plays an important role in the metabolism of all living cells as a source of energy. ATP is used in the biological processes of wastewater treatment, to generate energy for the growth of microorganisms that metabolize the influent substrates (Nguyen and Chong, 2015).

On the other hand, 4 tentative identified as pharmaceuticals at level 3: butetamate, benzocaine, tolycaine and aminorex; were respectively assigned as probable structure to desvenlafaxine, phenylalanine, leu-phe and norcotinine (level 2), after MS/MS analysis against the Metlin

database.

None of the tentatively identified drugs of abuse could be assigned by its MS/MS to its structure. However, four compounds could be assigned as possible structures to other compounds, the pharmaceuticals amitriptyline, phenibut and salsoline (previously identified as 2-ethylidene-1,5-dimethyl-3,3-dihenylpyrrolidine [EDDP], 2,3-methylenedioxyamphetamine [MDA] and 3,4-methylenedioxyamphetamine [MDMA] respectively) and the pyridine 3-pentylpyridine, a compound present in animal food (tentatively identified as metamphetamine).

Spectrum from 20170307-ALBUFERA SUR.wiff (sample 1) - Sample008, Experiment 2, +TOF MS² (100 - 950) from 13,023 min
Precursor: 282,3 Da, CE: 35,0 CES=35



m/z experimental	m/z theoretical	Mass error ppm	Product ion	%Intensity
100.07600	100.07559	-4.1	C5H10NO+	100
107.08580	107.08530	-4.7	C8H11+	50
109.10140	109.10188	4.4	C8H13+	70
110.09690	110.09643	-4.3	C7H12N+	33
111.11800	111.11683	-10.5	C8H15+	47
114.09000	114.09034	3.0	C6H12NO+	57
121.10270	121.10118	-12.6	C9H13+	47
125.13214	125.13248	2.7	C9H16+	43
135.11630	135.11683	3.9	C10H15+	53
139.11100	139.11174	5.3	C9H14O+	17
156.17396	156.17468	4.6	C10H22N+	13
158.97010	-----	-----	-----	13
163.14760	163.14813	3.2	C12H19+	43
164.94320	-----	-----	-----	17
177.16471	177.16378	-5.2	C13H21+	63
179.18300	179.17943	-19.9	C13H23+	10
205.19470	205.19508	1.9	C15H25+	17
224.23820	224.23728	-4.1	C15H30N+	13
247.24109	247.24203	3.8	C18H31+	80
265.25040	265.25259	8.3	C18H33O+	33
281.27518	281.27132	-13.7	C18H35NO+	20
282.28004	282.27914	-3.2	C18H36NO+	17
285.30348	285.30316	-1.1	C18H38NO+	47

Fig. 6. MS/MS of the peak tentatively identified as dodemorph and assigned to oleamide as possible structure as well as table showing the characteristics of the MS/MS.

3.2.3. Compounds confirmed

The structure confirmation (level 1) was achieved only in those compounds whose analytical standards were available in the laboratory. However, achieving level 1 identification for all the organic compounds

identified at level 3 (and without further information) or level 2 was not possible due to the variety and high cost of the required analytical standards.

Six pesticides, acequinocyl, carbofuran, diphenylamine, mefenacet,

imazalil and thiabendazole were confirmed with the analytical standards. The presence of pesticides in urban WWTPs is mainly due to their non-agricultural uses, including application in green spaces, gardens, parks, industrial vegetation, public health, etc. but also by washing fruits and vegetables prior to consumption. It is interesting to note that imazalil, thiabendazole and diphenylamine are well known post-harvest fungicides used in the fruit processing. The presence of fungicides (specially imazalil and thiabendazole) in sludge has also been reported in previous studies (Aalizadeh et al., 2019; Black et al., 2019; Boix et al., 2016; Castro et al., 2021; Chiaia-Hernández et al., 2020). The removal of pesticides at the WWTPs is variable (Wang et al., 2019), but generally, it is poor and reported concentrations are even higher in the effluent than in the influent (Köck-Schulmeyer et al., 2013). Furthermore, other studies have not been able to establish a relation between the physico-chemical properties of pesticides and the removal efficiency (Campo et al., 2013). It is expected that hydrophobicity was one property dominating the presences of pesticides in sludge. However, our results, together with many previous studies, demonstrated that could not be the only one (Martínez-Piernas et al., 2021; Riva et al., 2021; Silva et al., 2021; Vazquez-Roig et al., 2011).

Nine compounds with pharmaceutical action (atenolol, ciprofloxacin, gemfibrozil, ibuprofen, ofloxacin, phenylalanine, telmisartan, tetracycline, valsartan) were also confirmed. This group is one of the most efficiently removed in WWTPs (Collado et al., 2014; Papageorgiou et al., 2016). The accumulation in sludge of analgesics (telmisartan, valsartan) and anti-inflammatories (ibuprofen) could be explained by their lipophilic properties, with K_{ow} between 3 and 5 and K_{oc} between 2 and 3 (Pal et al., 2010). In WWTPs, antibiotics (ciprofloxacin, ofloxacin, tetracycline) show a partial removal with conventional treatment (Papageorgiou et al., 2016). High concentrations in the influent could result in their accumulation at relevant concentration in the sludge (Collado et al., 2014). The presence of telmisartan in sludge has also been reported in the study of Castro et al. (2021). Finally, tetracycline (a broad-spectrum antibiotic), was also confirmed in sludge has been reported in several studies, although it is a compound influenced by factors like pH, temperature and the ionic strength, which makes difficult to predict its sorption processes (Gago-Ferrero et al., 2015). Moreover, it has been demonstrated that tetracyclines are degraded by photolysis and consequently in the UV treatment (Collado et al., 2014).

4. Conclusions

A total of 122 tentative compounds were detected in 8 WWTPs close to Valencia city. The town of Sueca has shown to be the place with more compounds detected in the sludge of their two WWTPs. On the other hand, WWTP of Perellonet showed the lowest number of compounds detected. These differences could be due to the different population size or differences in sludge treatment.

After searching the product ion mass spectrum in the free available databases and literature, 53 compounds were identified, being PPCPs the dominant group found. However, just 5 out of 18 tentative compounds present in all sludge samples were correctly identified: amprolium, adenosine triphosphate, the amino acid phenylalanine, the peptide leu-phe and oleamide. These results pointed out that sediment could be a reservoir of ECs, as well as the many difficulties related to the identification of organic compounds coming mostly from the human metabolism.

The suspected screening carried out by HRMS against a database is an effective tool to identify (quickly and easily) the compounds present in dehydrated sewage sludge. The compounds tentatively identified assigning the most probable empirical formula to their accurate mass (level 3), need additional MS/MS spectra to be properly assigned to a possible structure from different types of libraries and confirmed with the analytical standards. Due to the high consumption of resources and time that supposes the comparison with analytical standards, further research is needed to increase the number of compounds available in the

free databases. The present work just attempts to identify the most relevant compounds present in the biosolids. Further research is necessary to provide a more complete profile of the compounds accumulated in this matrix, that could be use as organic amendment in soils.

Sample credit author statement

Alejandro Cuñat: Conceptualization, Methodology, Software. **Rodrigo Alvarez-Ruiz:** Data curation, Writing – original draft preparation. **Maria Morales Suarez-Varela:** Visualization, Investigation. **Yolanda Picó:** Conceptualization, Methodology, Writing- Reviewing and Editing, Supervision, Project administration; Funding acquisition.

Funding resources

This work has been supported by Grant RTI2018-097158-B-C31 funded by MCIN/AEI/10.13039/501100011033 and by “ERDF A way of making Europe”, and project ANTROPOCEN@ (PROMETEO/2018/155) funded by Generalitat Valenciana.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

R. Alvarez-Ruiz also acknowledges her pre-doctoral contract by the grant BES-2016-078612 funded by MCIN/AEI/10.13039/501100011033 and by “ESF Investing in your future”.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2022.114587>.

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