



# Occurrence of the main metabolites of pharmaceuticals and personal care products in sludge stabilization treatments

José Luis Malvar, Juan Luis Santos\*, Julia Martín, Irene Aparicio, Esteban Alonso

Department of Analytical Chemistry, Escuela Politécnica Superior, University of Seville, C/ Virgen de África, 7, E-41011 Seville, Spain

## ARTICLE INFO

### Article history:

Received 21 May 2020

Revised 9 July 2020

Accepted 17 July 2020

Available online 8 August 2020

### Keywords:

Pharmaceutically active compounds

Personal care products

Metabolites

Distribution

Sludge stabilization technologies

## ABSTRACT

In Europe, approximately 40% of sludge yearly produced in wastewater treatment plants (WWTPs) is applied in agricultural lands as organic amendment, especially in arid regions. Sludge tends to concentrate wastewater pollutants. Many of them are not removed by sludge stabilization treatments and, as a result, they could originate adverse effects on soils, vegetation, animals, and humans. Although sludge stabilization treatments play an important role in removal contaminants from sludge and, therefore, in preventing contaminant discharges onto soils, there is scarce information about the occurrence of these compounds in these treatments. This fact is especially acute for emerging pollutants and, particularly, their metabolites. In this work, seven pharmaceuticals and personal care products, and their main metabolites, have been monitored in five different stabilization treatments: anaerobic and aerobic digestion, dehydration, composting, and lagooning. Sixteen compounds were measured in the analysed samples. Their distribution was similar in primary sludge, in spite of the different geographic locations of the WWTPs. The distribution was in accordance with the metabolic ratios of most of the studied compounds. Different behaviour was observed depending on the compound, for example, CBZ, 3-OH-CBZ, DIC, and 4-OH-DIC were highly persistent in all studied stabilization technologies whereas CAF, PX showed high degradability. Most of the studied compounds were measured in the final product of the sludge stabilization processes evaluated. This fact shows the necessity to improve the knowledge about the presence of these compounds in sludge intended to be applied onto soil and about the potential ecotoxicological risks of these compounds.

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## 1. Introduction

The Directive 86/278/EEC (EU, 1986), on the protection of the environment, and in particular of the soil, recommends the use of sewage sludge in agriculture and regulates its use to prevent harmful effects on soil, vegetation, animals, and humans. In Europe, it is estimated that almost 40% of sludge generated in 2008 by the 27 member states were applied onto agricultural lands (EC, 2008). This percentage is even higher in some countries as Denmark, France, Ireland, Spain, or the United Kingdom (EC, 2008). However, sludge is generated as a byproduct of wastewater treatment plants (WWTPs) and, consequently, tends to concentrate pollutants from wastewater. As a result, a large amount of pollutants can be spread into the environment by sludge application onto soils. Previously, sludge is stabilized by means of different technologies, which play an important role in the release of contaminants onto soil. Sludge digestion under anaerobic conditions

(in anaerobic treatment plants (AnTPs)) is the most applied sludge stabilization process. However, in some cases, only dehydration (in dehydration treatment plants (DTPs)) is applied. In many cases, sludge generated in AnTPs is composted under aerobic conditions in composting plants (CPs). Other sludge stabilization treatments are sludge digestion under aerobic conditions (in aerobic treatment plants (AeTPs)) and lagooning (AnSPs), in which sludge is pooled and stabilized under anaerobic conditions at the bottom of a lagoon. These treatments are usually applied in small communities.

Nowadays, there is an increasing concern about the discharge and occurrence of emerging pollutants in the environment, especially in the case of pharmaceuticals and personal care products (PPCPs) since these compounds, mainly pharmaceuticals, are designed to cause changes in living organisms. Some examples are fish feminization, what has been related to the exposition to the hormone 17 $\alpha$ -ethinylestradiol (Pereira et al., 2020) or the serious threat to vultures, aquatic animals, plants and mammals related to diclofenac (Sathishkumar et al., 2020). Moreover, some studies have reported the capacity of these compounds to affect

\* Corresponding author.

E-mail address: [jsantos@us.es](mailto:jsantos@us.es) (J. Luis Santos).

soil microorganisms (Thelusmond et al., 2018) and crop yield (Christou et al., 2018) or even to be uptaken by plants and, consequently, to enter into the food chain (Mordechay et al., 2018).

For example, Camotti Bastos et al. (2020) reported significant environmental risks associated to pharmaceuticals, such as carbamazepine, diclofenac or sulfamethoxazole, present in sludge and compost applied onto soil. Health effects of these compounds, especially those related to pattern compounds, are being evaluated in the last years in terms of environmental risks, toxicity to different living species and human exposure. These pollutants can entry into the food chain through sewage sludge application to agricultural lands. In the case of parabens, the dimension of the problem reaches ordinary citizens with the offer of personal care products free of these compounds and, therefore, the use of alternatives with the consequently associated cost. In the case of pharmaceuticals, citizen awareness campaigns on environmental impact have increased considerably. The elimination of these compounds in the sewage sludge would undoubtedly represent a very high economic investment in those countries that currently use biological technologies that are proving not to be completely effective in the degradation of persistent organic compounds.

To the date, most of the studies in the literature have been focused on the determination of PPCPs in the final product (digested or composted sludge) (Abril et al., 2018; Biel-Maeso et al., 2019; Luque-Muñoz et al., 2017; Peysson and Vulliet, 2013; Rossini et al., 2016). Moreover, only a few of them paid attention to the occurrence of the metabolites of these compounds in the final product of sludge stabilization treatments (Ferrando-Climent et al., 2012; García-Galán et al., 2013; Karthikaj et al., 2017; Malvar et al., 2020b; Wang and Kannan, 2016). As a result, there is scarce information about their distribution through different sludge stabilization treatments. Moreover, they are mainly focused on the parent compounds and on anaerobic digestion treatments (Abril et al., 2020; Jelic et al., 2012; Martín et al., 2012). Indeed, to the best of our knowledge, there is no study about the occurrence of metabolites of PPCPs in different sewage sludge stabilization treatments.

The aim of this work was to evaluate the occurrence of the main metabolites of a recurrent group of PPCPs in sludge stabilization treatments based on anaerobic and aerobic digestion, composting, dehydration, and anaerobic stabilization ponds.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Nineteen compounds were selected, including five pharmaceutically-active compounds (caffeine, carbamazepine, diclofenac, ibuprofen, and sulfamethoxazole), two personal care products (methylparaben and propylparaben) and twelve of their main metabolites (1,7-dimethylxanthine, 3-hydroxycarbamazepine, 10,11-dihydro-10-hydroxycarbamazepine, carbamazepine-10,11-epoxide, 4-hydroxydiclofenac, 1-hydroxyibuprofen, 2-hydroxyibuprofen, carboxyibuprofen, N<sup>4</sup>-acetylsulfamethoxazole, methylprotocatechuate, 3,4-dihydroxybenzoic acid, and 4-hydroxybenzoic acid).

High purity standards of 1,3,7-trimethylxanthine (caffeine) (CAF), 1,7-dimethylxanthine (PX), carbamazepine (CBZ), 3-hydroxycarbamazepine (3-OH-CBZ), 10,11-dihydro-10-hydroxycarbamazepine (10-OH-CBZ), carbamazepine-10,11-epoxide (EP-CBZ), diclofenac (DIC), 4-hydroxydiclofenac (4-OH-DIC), ibuprofen (IBU), 1-hydroxyibuprofen (1-OH-IBU), 2-hydroxyibuprofen (2-OH-IBU), carboxyibuprofen (CBX-IBU), sulfamethoxazole (SMX), N<sup>4</sup>-acetylsulfamethoxazole (AcSMX), methylparaben (MeP), methyl protocatechuate (OH-MeP), propylparaben (PrP),

3,4-dihydroxybenzoic acid (3,4-DHB) and 4-hydroxybenzoic acid (4-HB) were provided by Sigma-Aldrich (St. Louis MO, USA). Chemical structures and physical-chemical properties of each compound are summarized in Table S1 (supplementary material). HPLC-grade methanol and water were obtained from Romil (Barcelona, Spain). Analytical-grade formic acid (98%) was purchased from Panreac (Barcelona, Spain). Individual stock standard solutions were prepared at 1000 mg L<sup>-1</sup> in MeOH and stored at -18 °C. Working solutions were prepared by dilution of the stock standard solutions in MeOH.

### 2.2. Sludge stabilization treatments and sampling

Five sludge stabilization processes were evaluated (Figure S1): anaerobic digestion in AnTPs, aerobic digestion in AeTPs, composting in CP, dehydration in DTPs and anaerobic lagooning in AnSPs. In AnTPs, AeTPs, and DTP, sludge from primary and secondary settling tanks is concentrated by sedimentation (in the primary tank) and flotation (in the secondary tank). Primary and secondary sludge are mixed before the stabilization process. Then, mixed sludges are stabilized under anaerobic (in AnTPs) or aerobic (in AeTP) conditions. Subsequently, digested sludge is dehydrated. In DTP, only dehydration of mixed sludge is carried out. In CP, anaerobically-digested and dehydrated sludge is composted by thermally-controlled aeration by turning.

Anaerobic lagoons are a wastewater treatment technology, typically applied in small towns with a low population density. Sludge stabilization takes place at the bottom of the lagoon under anaerobic conditions.

Sludge samples were collected from eleven WWTPs and one CP located in the South of Spain (Figure S2) distributed as follows: four AnTPs sited in the cities of Granada, Huelva, Malaga and Seville; one CP sited in the city of Seville; one DTP sited in Cordoba; and three AeTPs and three AnSPs sited in small populations from the Cádiz area. The characteristics of each WWTP can be seen in Table S2. Selected compounds were analysed in primary and secondary sludge from AnTPs and DTP; anaerobically-digested and dehydrated sludge from AnTPs; composted sludge from CP; dehydrated sludge from DTP; mixed sludge and aerobically-digested and dehydrated sludge from AeTPs, and lagoon sludge from AnSPs. Sampling points are shown in Figure S2. Sampling was carried out during one year to cover seasonal variables, such as consumption patterns, ambient conditions and even social events that could affect pollutant concentration in influent wastewater and treatment processes. Samples were collected bimonthly, except in the case of AnSPs, whose samples were collected every six months, and CP whose samples were collected monthly.

Two litres of primary, secondary, mixed, and lagoon sludge and one kilogram of digested and dehydrated sludge, dehydrated sludge, and compost were collected using amber glass bottles. Primary, secondary and mixed sludge from AnTPs and AeTPs were collected directly from the sampling points sited at the outlet of the settling tanks and after the mixture of primary and secondary sludge. Lagoon sludges from AnSPs were collected by mixing four aliquots collected from four sampling points sited at the bottom of each lagoon. Digested and dehydrated sludge, from AnTPs and AeTPs, and dehydrated sludge, from DTP, were collected from the outlet of the sludge dehydration system. For this purpose, at least ten aliquots of approximately 0.5 kg of dehydrated sludge were collected from the outlet of the sludge dehydration system. The aliquots were mixed and one aliquot of 1 kg was collected in an amber glass bottle. Finally, combined compost samples were obtained by mixing the aliquots collected from at least five sampling sites approximately 30 cm deep in the compost battery. Sludge samples were frozen at -18 °C and freeze-dried in a Cryodos-50 lyophiliser (Telstar, Terrasa, Spain). Afterwards, the

samples were homogenized, crushed in a mortar, sieved (<1 mm), and stored in glass bottles at  $-18^{\circ}\text{C}$  until analysis.

### 2.3. Sample treatment and LC-MS/MS analysis

Target compounds were extracted by ultrasound-assisted extraction. The determination was carried out by liquid-chromatography tandem mass spectrometry according to Malvar et al. (2020c). As a summary, 0.25 g of lyophilized sludge samples were weighted in a centrifuge glass tube and 5 mL of MeOH (0.5% formic acid) were added. The tube was shaken in a vortex for 30 s, sonicated for 15 min in an ultrasonic bath, and centrifuged at 4000 rpm for 20 min. The solid extract was extracted twice more. Combined liquid extracts were cleaned up by dispersive solid-phase extraction (dSPE) using 0.4 g of PSA and 0.8 g of C18 as sorbents. The extract was dried to dryness by a gentle nitrogen stream and reconstituted using 250  $\mu\text{L}$  of MeOH:water solution (1:1, v/v), filtered through a 0.22  $\mu\text{m}$  cellulose syringe filter and transferred to automatic LC injector vials. Aliquots of 10  $\mu\text{L}$  were injected into LC-MS/MS system.

The chromatographic determination was carried out on an Agilent 1200 series HPLC (Agilent, USA) coupled to a 6410 triple quadrupole (QqQ) mass spectrometer (MS) with an electrospray ionization source (ESI) using conditions previously described by Malvar et al. (2020c). MS parameters were as follows: drying gas flow rate, 9  $\text{L min}^{-1}$ ; drying gas temperature,  $350^{\circ}\text{C}$ ; capillary voltage, 4000 V; and nebulizer pressure, 40 psi. Instrument control and data acquisition were carried out with MassHunter software (Agilent, USA). The MS/MS parameters for each compound are given in Table S3.

### 2.4. Quality assurance and quality control

Analytical performance was previously reported by Malvar et al. (2020c). A summary including recoveries and method detection and quantification limits of target compounds are summarized in Table S4.

For each batch of samples, samples, matrix-matched calibration standards, procedural blanks, and spiked samples were processed. Recovery and precision were determined and compared with those obtained in the validation of the analytical method. Moreover, three quality control standards (matrix-matched calibration standards) at three concentration levels were injected once every ten samples.

## 3. Results and discussion

### 3.1. Concentrations of PPCPs and metabolites in primary and secondary sludge

The distribution of the analysed compounds in primary sludge was similar in all studied WWTPs (Fig. 1), in spite of their different geographic locations. Moreover, the distribution for each parent compound and their metabolites was in accordance with their metabolic ratios in most of the cases (Ferrando-Climent et al., 2012; Sathishkumar et al., 2020). For example, 4-OH-DIC in primary sludge represented more than 80% of the sum of DIC and 4-OH-DIC. This distribution was in accordance with the high metabolization of DIC, which is widely transformed into 4-OH-DIC (Sathishkumar et al., 2020). In the case of IBU, only 15% of IBU administered is excreted unaltered whereas 85% of the total dose is excreted as metabolites. 2-OH-IBU and CBX-IBU correspond to 26% and 46% of the total dose, respectively, whereas 1-OH-IBU is

a minority metabolite (Ferrando-Climent et al., 2012). This distribution is similar to that in primary sludge in this work. The slight differences found could be explained considering the different sorption capacity of these compounds onto sludge (Wojaslawski et al., 2019) and their potential degradation after being excreted. In the case of CBZ, >30 metabolites have been identified (Bahlman et al., 2014). In this work, some of its major metabolites (Bahlman et al., 2014) were selected. Their distribution was similar in all the studied WWTPs. The percentages of each compound were in the ranges: 27–41% for CBZ, 4–16% for 3-OH-CBZ, and 49–59% for 10-OH-CBZ. Finally, CAF, PX, and parabens showed different distribution pattern in primary sludge in comparison to their metabolic ratios. This fact could be explained considering that CAF and 4-HB could be originated from other sources different from pharmaceutical ingestion. In summary, for most of the compounds, the results in this work show that PPCP consumption is the main source of their release to wastewater, except for CAF and PX for which other sources are supposed.

The concentrations of PPCPs and their metabolites in sludge from different sludge stabilization treatment plants are shown in Figures S3 to S7 as box-and-whisker plots. Lines in each box represent the lower ( $\leq 5\%$ ), medium ( $\leq 50\%$ ), and upper ( $\leq 95\%$ ) percentiles. The points sited into each box represent the mean concentration value whereas lines extending from each box indicate the maximum and minimum concentration values. The concentration ranges, mean concentration, and frequency of detection of each compound in each type of sludge are summarized in Tables 1 and 2. Sixteen of the nineteen selected compounds were detected in sludge samples. Only EP-CBZ ( $<0.05 \mu\text{g kg}^{-1} \text{ dm}$  (dry matter)), SMX ( $1.17 \mu\text{g kg}^{-1} \text{ dm}$ ), and AcSMX ( $0.53 \mu\text{g kg}^{-1} \text{ dm}$ ) were below the limits of detection in all the analysed sludge samples. Considering the concentrations of parent compounds in fresh sludge (sludge before stabilization treatment: primary and secondary in AnTPs and DTPs, and mixed in AeTPs), the highest mean concentrations were found for CAF ( $82.7 \mu\text{g kg}^{-1} \text{ dm}$  in primary sludge and  $128 \mu\text{g kg}^{-1} \text{ dm}$  in secondary sludge) and IBU ( $44.2 \mu\text{g kg}^{-1} \text{ dm}$  in primary sludge), in the case of pharmaceuticals. These results are in accordance with those previously measured in sludge from WWTPs sited in the same area (Martín et al., 2012; Martín et al., 2015). In the case of parabens, MeP ( $117.9 \mu\text{g kg}^{-1} \text{ dm}$  in primary sludge) was measured at the highest mean concentration. Regarding to the measured metabolites, 4-HB showed the highest mean concentrations in fresh sludge ( $409$ ,  $342$ , and  $366 \mu\text{g kg}^{-1} \text{ dm}$  in primary, secondary and mixed sludge, respectively). This fact could be explained considering other sources for 4-HB, in addition to paraben metabolization (Karthikraj et al., 2017). Additionally, other metabolites were detected at higher concentrations than their parent compounds, for example IBU, CBZ and DIC. This fact could be explained considering the extensive metabolization of these compounds (Jiang et al., 2019; Sathishkumar et al., 2020; Mazaleuskaya et al., 2015).

Considering fresh sludges from AnTPs, nine of the sixteen detected compounds showed similar concentrations in both primary and secondary sludge. Other compounds such as PX, 10-OH-CBZ, IBU, 2-OH-IBU, CBX-IBU, OH-MeP, and 3,4-DHB were detected at concentrations significantly higher in primary sludge than in secondary sludge. Moreover, the frequency of detection was higher in primary sludge than in secondary sludge. These results could be explained, first, considering the low  $\log K_{\text{OW}}$  and high  $\text{pK}_{\text{a}}$  values of these compounds, what explain their sorption onto sludge (Abril et al., 2020; Martín et al., 2012) and, second, considering that secondary sludge is produced in the secondary treatment where the removal of these compounds takes place mainly by biodegradation (Pereira et al., 2020).

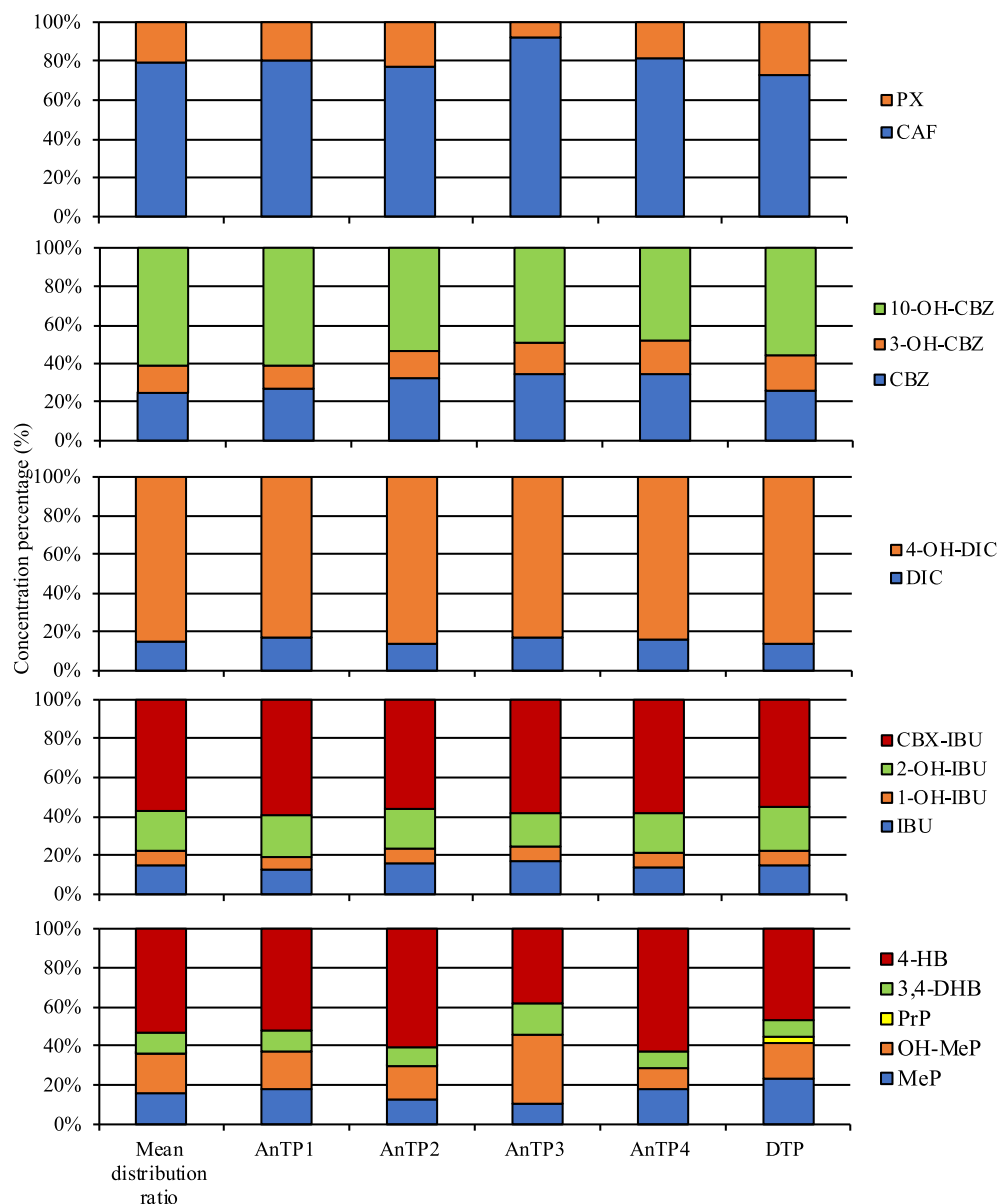


Fig. 1. Distribution of PPCPs and their metabolites in primary sludge from AnTPs.

### 3.2. Distribution of PPCPs and their main metabolites in the evaluated sludge stabilization technologies

For most of the studied compounds, concentrations measured after sludge stabilization treatments were similar or lower than in fresh sludge. An evaluation of the distribution of PPCPs and their metabolites along the studied sludge stabilization treatments is featured below.

#### 3.2.1. Caffeine and paraxanthine

Figure S3 shows the concentrations of CAF and PX in sludge samples collected alongside all studied sludge stabilization technologies. As it was described above, in contrast with the metabolic ratio of CAF, with more than 80% of the total dose excreted as PX (Alsabri et al., 2018; Lazenka et al., 2015), CAF was found at significantly higher concentrations than PX in all samples, except in compost, where were present at similar concentrations. This fact could be explained considering other sources of release CAF to wastewater. For example, Jagoda et al. (2015) showed the uncon-

sumed coffee, tea, and other drinks containing CAF, as well as coffee-making facilities, are the main source of CAF to wastewater. Both compounds showed the same distribution pattern what can be explained by their similar log Kow and pKa values (Table S1). The results showed a higher persistence of CAF and PX in AnTPs than in AeTPs. Concentrations in primary and secondary sludge were similar to those in anaerobically-digested and dehydrated sludge (Table 1). These results are in concordance with the results obtained previously by Martin et al. (2012) in sludge stabilization processes from the same geographic area. In the case of AeTPs, the concentration of CAF decreased significantly from 127.9  $\mu\text{g kg}^{-1}$ , in mixed sludge, to 25.8  $\mu\text{g kg}^{-1}$ , in aerobically-digested and dehydrated sludge. However, it was still quantified in the 21% of the treated sludge samples. After composting, CAF and PX were frequently found (frequencies of detection were 90 and 100%, respectively) at similar mean concentrations (9.8  $\mu\text{g kg}^{-1}$  and 11.3  $\mu\text{g kg}^{-1}$ , respectively). Moreover, the concentrations measured in AnSP were 26.1  $\mu\text{g kg}^{-1}$  for CAF and 17.7  $\mu\text{g kg}^{-1}$  for PX. The concentration ratios of both compounds remained constant alongside



**Table 1**  
Concentration ranges, frequency of detection (Freq.), mean concentrations in the positive samples and, relative standard deviation (RSD) of selected compounds in sewage sludge from anaerobic treatment plants and composting plant.

Compounds	Anaerobic treatment plants (AnTPs)					Secondary sludge (n = 30)					Anaerobically-digested and dehydrated sludge (n = 24)					Composting plant (CP)				
	Primary sludge (n = 30)															Compost (n = 10)				
	Range ( $\mu\text{g Kg}^{-1}$ )	Freq. (%)	Mean ( $\mu\text{g Kg}^{-1}$ )	RSD (%)		Range ( $\mu\text{g Kg}^{-1}$ )	Freq. (%)	Mean ( $\mu\text{g Kg}^{-1}$ )	RSD (%)		Range ( $\mu\text{g Kg}^{-1}$ )	Freq. (%)	Mean ( $\mu\text{g Kg}^{-1}$ )	RSD (%)		Range ( $\mu\text{g Kg}^{-1}$ )	Freq. (%)	Mean ( $\mu\text{g Kg}^{-1}$ )	RSD (%)	
<b>CAF</b>	<MDL-169	90	82.7	34		<MDL-87.0	9	68.5	22		<MDL-41.4	5	41.4	-		<MDL-14.7	78	9.80	3	
<b>PX</b>	<MDL-47.4	75	25.9	10		<MDL-16.9	13	9.90	6		<MDL-9.60	5	9.60	-		6.0-17.1	100	11.3	3	
<b>CBZ</b>	2.40-5.00	100	3.40	1		<MDL-5.90	91	3.40	1		2.00-4.20	100	2.70	1		<MDL-9.20	33	7.40	1	
<b>3-OH-CBZ</b>	<MDL-4.00	75	1.80	1		<MDL-3.30	43	2.00	1		<MDL-1.30	77	1.00	0.2		<MDL-5.10	22	3.70	2	
<b>10-OH-CBZ</b>	<MDL-42.1	85	8.20	9		<MDL-8.60	95	5.10	2		<MDL-3.00	64	2.10	0.4		<MDL-2.30	22	1.40	1	
<b>EP-CBZ</b>	<MDL	0	<MDL	-		<MDL	0	<MDL	-		<MDL	0	<MDL	-		<MDL	0	<MDL	-	
<b>DIC</b>	1.90-7.10	100	4.10	1		0.70-7.20	100	3.40	2		<MDL	0	<MDL	-		2.20-5.80	100	3.20	1	
<b>4-OH-DIC</b>	9.30-41.9	100	22.3	9		4.50-49.7	100	25.8	11		10.2-36.5	100	22.2	7		<MDL-3.10	22	2.70	1	
<b>IBU</b>	<MDL-80.2	90	44.2	19		<MDL-33.8	48	11.1	8		<MDL-28.0	100	16.5	5		<MDL	0	<MDL	-	
<b>1-OH-IBU</b>	<MDL-43.5	90	21.1	11		<MDL-38.4	57	19.0	7		<MDL	0	<MDL	-		<MDL	0	<MDL	-	
<b>2-OH-IBU</b>	<MDL-118	90	60.7	30		<MDL-43.1	52	21.9	11		<MDL-10.1	23	9.30	1		<MDL	0	<MDL	-	
<b>CBX-IBU</b>	<MDL-300	90	169	79		<MDL	0	<MDL	-		<MDL-32.6	55	18.3	6		<MDL	0	<MDL	-	
<b>SMX</b>	<MDL	0	<MDL	-		<MDL	0	<MDL	-		<MDL	0	<MDL	-		<MDL	0	<MDL	-	
<b>AcSMX</b>	<MDL	0	<MDL	-		<MDL	0	<MDL	-		<MDL	0	<MDL	-		<MDL	0	<MDL	-	
<b>MeP</b>	49.1-210	100	118	48		40.1-356	100	97.6	65		60.9-158	100	109	31		<MDL-12.1	78	6.80	3	
<b>OH-MeP</b>	<MDL-499	100	156	11		31.6-176	96	79.9	37		50.6-586	100	236	147		1.40-6.10	78	4.00	2	
<b>PrP</b>	<MDL-29.8	20	25.3	5		<MDL	0	<MDL	-		<MDL	0	<MDL	-		<MDL	0	<MDL	-	
<b>3,4-DHB</b>	23.9-171	100	79.9	34		18.4-73.7	100	45.2	16		42.0-317	100	124	65		28.8-252	100	73.8	72	
<b>4-HB</b>	70.0-1321	100	404	279		86.6-1336	100	342	273		168-2187	100	708	522		<MDL-330.2	78	88.1	70	

&lt;MDL: Lower than method detection limit.

the sludge stabilization processes (Fig. 2). This fact could indicate that no transformation from CAF to PX is produced during sludge stabilization treatments. Instead, a different degradation route has been proposed, the formation of theobromine, one of the main metabolites of CAF (Wu et al., 2020) that has been measured in wastewater at concentrations up to  $534 \mu\text{g L}^{-1}$  (Egea-Corbacho et al., 2019). Only compost and aerobically-digested and dehydrated sludge showed different distribution of CAF and PX. This fact could be due to different degradation rates of these compounds under aerobic conditions.

### 3.2.2. Carbamazepine and their main metabolites

Figure S4 shows the occurrence of CBZ and three of its main metabolites on the sludge stabilization treatments evaluated. 10-OH-CBZ showed the highest mean concentrations in primary ( $8.2 \mu\text{g kg}^{-1}$  dm), secondary ( $5.1 \mu\text{g kg}^{-1}$  dm), and mixed sludge ( $10.5 \mu\text{g kg}^{-1}$  dm). A strong decrease was observed after aerobic and anaerobic digestion and composting. This fact could indicate a high degradability for this compound. Additionally, complete removal was produced in DTP. In contrast, CBZ and 3-OH-CBZ only showed a slight decrease of their concentrations after aerobic and anaerobic digestion and a strong increase after composting. The concentration ratio of CBZ (Fig. 2) increased from primary and secondary sludge to anaerobically-digested and dehydrated sludge and compost; and from mixed to aerobically-digested and dehydrated sludge while the proportion of 10-OH-CBZ decreased. In the case of 3-OH-CBZ, its concentration ratio remained approximately constant through the different stages, except in composting. The degradation of 10-OH-CBZ is produced by its transformation into other metabolites such as 9-carboxy acridine, as it was reported by Brezina et al. (2017). Consequently, this behaviour cannot be explained considering a possible transformation of 10-OH-CBZ in CBZ during digestion processes by the loss of the hydroxyl group. Therefore, the increase of concentration ratio of CBZ in sludge stabilization processes was probably due to the high persistence of this compound together with the degradation of their metabolites, mainly 10-OH-CBZ. Moreover, the increase of concentration of CBZ and 3-OH-CBZ could be explained by the concentration effect due to the degradation of organic matter and, consequently, the loss of weight produced after composting. In DTP, only CBZ was found at a mean concentration of  $2.3 \mu\text{g kg}^{-1}$  dm while AnSPs, CBZ, 3-OH-CBZ, and 10-OH-CBZ were found at similar concentrations, in the range from  $1.8 \mu\text{g kg}^{-1}$  dm, for 3-OH-CBZ, to  $3.8 \mu\text{g kg}^{-1}$  dm, for 10-OH-CBZ.

### 3.2.3. Diclofenac and 4-hydroxy diclofenac

DIC and 4-OH-DIC showed high persistence in the studied stabilization treatments. They were detected in all the analysed sludge samples (Figure S5), except in compost samples, where 4-OH-DIC was detected only in 22% of the samples. DIC was found at lower mean concentrations than 4-OH-DIC in all sludge samples except in compost. Their proportion remained approximately constant in all stabilization processes evaluated (Fig. 2), between 9 and 17% in the case of DIC and from 83 to 91% in the case of 4-OH-DIC and even, in all WWTP studied (%RSD values were below 20% in all sludge samples) in spite of their different geographic location. The high persistence of these compounds contrast with the relatively high removal reported by other authors in wastewater treatments based on anaerobic (Kahl et al., 2017; Nivala et al., 2019) and aerobic (Kahl et al., 2017) conditions. This fact could be explained considering the sorption onto the sludge generated during wastewater treatment as the main removal mechanism, as it was previously described (Yan et al., 2019). For example, several authors (Kahl et al., 2017; Nivala et al., 2019; Larsson et al., 2014) have evaluated the removal rates of DIC in wastewater samples under aerobic and anaerobic conditions, achieving removals up to 100% in some cases

**Table 2**  
Concentration ranges, frequency detection (Freq.), mean concentrations in the positive samples, and relative standard deviation (RSD) of selected compounds in sewage sludge from chemical stabilization plant, aerobic treatment plants and anaerobic stabilization ponds.

Compounds	Dehydration treatment plant (DTP) Dehydrated sludge (n = 6)				Aerobic treatment plant (AeTPs) Mixed sludge (n = 18)				Aerobically-digested dehydrated sludge (n = 18)				Anaerobic stabilization ponds (AnSPs) Lagoon sludge (n = 6)			
	Range ( $\mu\text{g Kg}^{-1}$ )	Freq. (%)	Mean ( $\mu\text{g Kg}^{-1}$ )	RSD (%)	Range ( $\mu\text{g Kg}^{-1}$ )	Freq. (%)	Mean ( $\mu\text{g Kg}^{-1}$ )	RSD (%)	Range ( $\mu\text{g Kg}^{-1}$ )	Freq. (%)	Mean ( $\mu\text{g Kg}^{-1}$ )	RSD (%)	Range ( $\mu\text{g Kg}^{-1}$ )	Freq. (%)	Mean ( $\mu\text{g Kg}^{-1}$ )	RSD (%)
<b>CAF</b>	<MDL	0	<MDL	–	<MDL-163	50	128	50	<MDL-36.3	21	25.8	10	<MDL-35.1	75	26.1	12
<b>PX</b>	<MDL	0	<MDL	–	<MDL-55.0	75	37.8	25	<MDL	0	<MDL	–	<MDL-24.4	50	17.7	9
<b>CBZ</b>	1.60–2.60	100	2.3	0.4	3.30–5.50	100	4.40	1	1.40–3.90	100	2.70	1	2.70–4.00	100	3.30	1
<b>3-OH-CBZ</b>	<MDL	0	<MDL	–	<MDL-2.60	75	2.30	0.3	<MDL-2.90	36	1.8	1	1.50–2.10	100	1.80	0.2
<b>10-OH-CBZ</b>	<MDL	0	<MDL	–	6.30–15.5	100	10.5	4	<MDL-2.60	14	2.60	0.1	2.30–6.60	100	3.80	2
<b>EP-CBZ</b>	<MDL	0	<MDL	–	<MDL	0	<MDL	–	<MDL	0	<MDL	–	<MDL	0	<MDL	–
<b>DIC</b>	2.50–4.30	100	3.20	1	0.90–3.90	100	3.00	1	0.60–3.30	100	2.10	1	2.60–11.1	100	5.50	4
<b>4-OH-DIC</b>	14.8–30.7	100	23.4	7	20.3–35.0	100	28.8	6	6.30–36.1	100	21.7	8	12.4–66.1	100	29.5	25
<b>IBU</b>	<MDL-17.8	60	11.7	6	<MDL-38.2	50	28.1	14	<MDL-20.4	43	14.2	5	24.2–50.0	100	34.9	11
<b>1-OH-IBU</b>	<MDL	0	<MDL	–	31.5–40.6	100	37.4	4	<MDL	0	<MDL	–	20.5–42.1	100	31.2	12
<b>2-OH-IBU</b>	<MDL	0	<MDL	–	65.1–143	100	100	33	<MDL-21.2	36	12.4	6	38.1–96.4	100	65.2	31
<b>CBX-IBU</b>	<MDL	0	<MDL	–	<MDL-231	50	204	38	<MDL	0	<MDL	–	<MDL-279	100	177	108
<b>SMX</b>	<MDL	0	<MDL	–	<MDL	0	<MDL	–	<MDL	0	<MDL	–	<MDL	0	<MDL	–
<b>ACSMX</b>	<MDL	0	<MDL	–	<MDL	0	<MDL	–	<MDL	0	<MDL	–	<MDL	0	<MDL	–
<b>MeP</b>	71.7–163	100	93.2	39	45.9–88.5	100	71.8	18	56.1–128	100	84.9	21	72.9–255	100	141	79
<b>OH-MeP</b>	<MDL-47.2	20	47.2	–	67.8–195	100	149	59	59.4–447	100	159	114	270–998	100	480	348
<b>PrP</b>	<MDL-7.80	20	7.8	–	<MDL	0	<MDL	–	<MDL	0	<MDL	–	<MDL	0	<MDL	–
<b>3,4-DHB</b>	28.1–54.7	100	45.8	11	44.6–36.5	100	92.6	82	36.5–227	100	88.4	47	104–300	100	184	97

<MDL: Lower than method detection limit.

(Larsson et al., 2014). However, in this work, DIC showed high persistence in sludge samples under both anaerobic and aerobic conditions (Figure S3). Considering the biodegradation and adsorption onto sludge as the main removal mechanism of contaminants during wastewater treatments, the results obtained in this work reveals sorption onto sludge as the main removal mechanism in wastewater treatments as it has been proposed by other authors (Yan et al., 2019). The high persistence of these compounds to biodegradation processes could be due to chlorine atoms in their structure which provide them with high stability (Hatoum et al., 2019).

### 3.2.4. Ibuprofen and their main metabolites

The concentrations of IBU and their main metabolites were significantly lower than in fresh sludges, after sludge stabilization treatments, especially under aerobic conditions (Figure S6). For example, the concentrations of IBU in AnTPs decreased from 44.2 and 11.1  $\mu\text{g kg}^{-1}$  dm in primary and secondary sludge (mean 27.7  $\mu\text{g kg}^{-1}$  dm), respectively, to 16.5  $\mu\text{g kg}^{-1}$  dm in anaerobically-digested and dehydrated sludge (Student *t*-test:  $t_{\text{cal}} = 10.276$ ,  $t_{\text{tab}} = 2.080$ ,  $p > 0.05$ ). The concentrations of CBX-IBU were reduced from 169  $\mu\text{g kg}^{-1}$  dm in primary sludge (concentration in secondary sludge was lower than detection limit) to 18.1  $\mu\text{g kg}^{-1}$  dm (Student *t*-test:  $t_{\text{cal}} = 33.561$ ,  $t_{\text{tab}} = 2.080$ ,  $p > 0.05$ ). In spite of the concentration decrease, IBU could be quantified in all anaerobically-digested and dehydrated sludges. In the case of AeTPs, these concentrations decreased from 28.1 to 204  $\mu\text{g kg}^{-1}$  dm in mixed sludge to 12.4–14.2  $\mu\text{g kg}^{-1}$  dm in aerobically-digested and dehydrated sludge. Moreover, the frequencies of detection were significantly lower in treated sludges. The distribution of IBU and its metabolites in the studied stabilization treatments (Fig. 2) showed an increase of the percentage of IBU and a high persistence of CBX-IBU and 2OH-IBU, in AnTPs and AnSPs; and of IBU and 2-OH-IBU in aerobic treatments (Figure S6). These results are in accordance with previous studies where IBU hydroxylation was described as one of the main degradation routes of IBU under aerobic conditions. Ibuprofen carboxylation, and the consequent formation of CBX-IBU, has been described as the main degradation route under anaerobic conditions (Tiware et al., 2017). The results obtained in this work showed that the removal of IBU in AnTPs could be produced through the formation of its carboxylate metabolite and the further degradation of this metabolite. Under aerobic conditions, the degradation of IBU could take place through the formation of its hydroxylated metabolites.

### 3.2.5. Personal care products and their main metabolites

MeP and PrP are the most common parabens present in the environment (Chen et al., 2017). OH-MeP is formed by hydroxylation of MeP whereas 3,4-DHB and 4-HB are common metabolites of parabens such as MeP, EtP and PrP (Wang and Kannan, 2016). The occurrence of these compounds along the studied stabilization treatments can be seen in Figure S7. Despite their low log  $K_{\text{ow}}$  values (from 0.87 to 2.97), MeP, OH-MeP, 3,4-DHB, and 4-HB were detected in all types of sewage sludge with a frequency detection of 100%, in most cases. In contrast, PrP was only detected in primary sludge and in dehydrated sludge in the 20% of the samples with mean concentrations of 25.3 and 7.80  $\mu\text{g kg}^{-1}$  dm, respectively. The highest concentrations were obtained for 4-HB, with values up to 2187  $\mu\text{g kg}^{-1}$ , probably due to the fact that 4-HB can be released to the environment from other sources in addition to paraben metabolization (Karthikraj et al., 2017). Detected compounds followed a similar pattern in all cases. As can be seen in Table 1, mean concentration values increased after anaerobic digestion, especially in the case of OH-MeP, from 156 and 79.9  $\mu\text{g kg}^{-1}$  dm (mean 120  $\mu\text{g kg}^{-1}$  dm), in primary and secondary

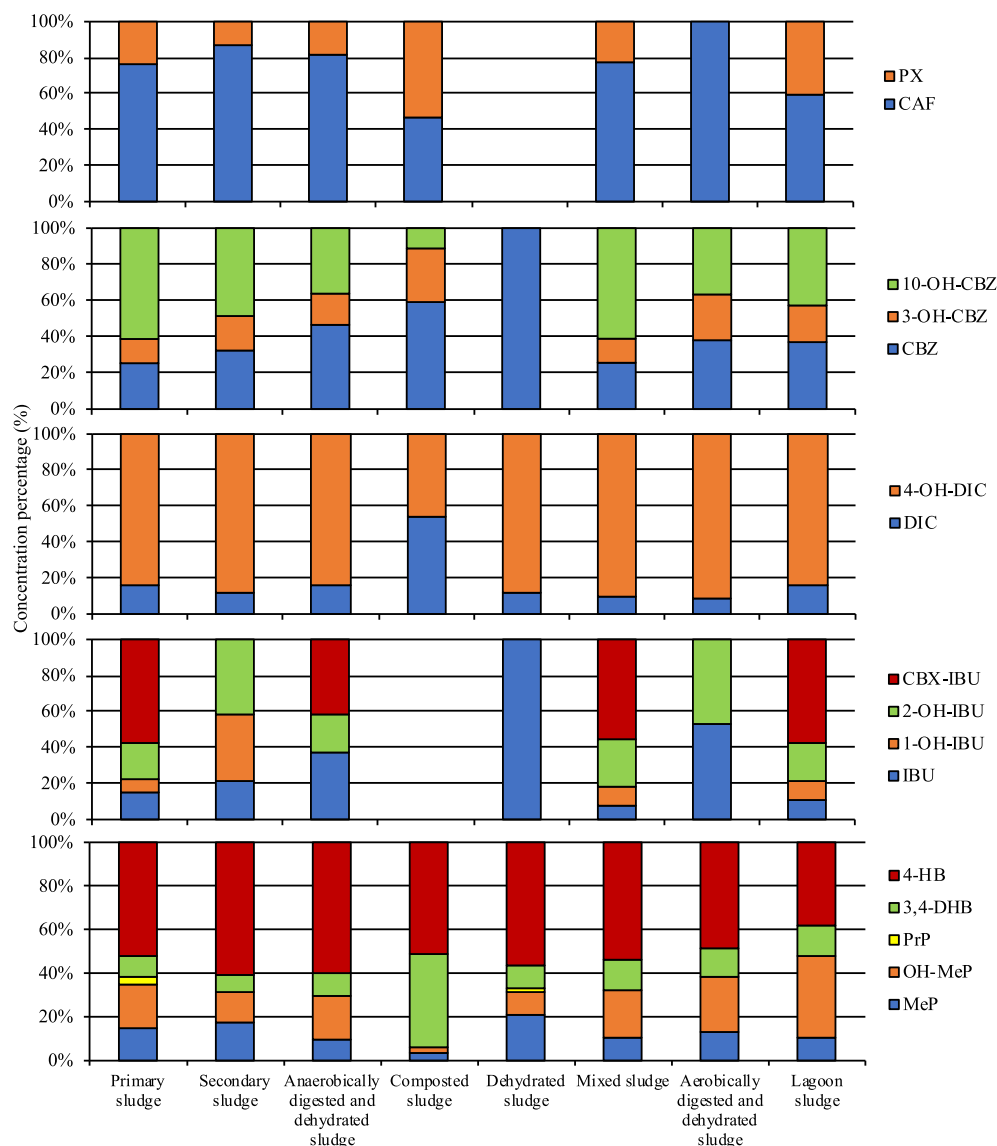


Fig. 2. Distribution of PPCPs and their metabolites alongside the sludge stabilization processes.

sludge, respectively, to  $236 \mu\text{g kg}^{-1} \text{ dm}$ , (Student's  $t$ -test:  $t_{\text{cal}} = 36.266$ ,  $t_{\text{tab}} = 2.080$ ,  $p > 0.05$ ) and 4-HB, from 404 and  $342 \mu\text{g kg}^{-1} \text{ dm}$  (mean  $373 \mu\text{g kg}^{-1} \text{ dm}$ ), in primary and secondary sludge, respectively, to  $708 \mu\text{g kg}^{-1} \text{ dm}$  (Student's  $t$ -test:  $t_{\text{cal}} = 2.667$ ,  $t_{\text{tab}} = 2.080$ ,  $p > 0.05$ ). In contrast to OH-MeP, a slight increase was observed for MeP indicating MeP hydroxylation to OH-MeP. Conversion of other organic pollutants to 4-HB is also expected (Karthikraj et al., 2017). After composting, concentrations of MeP, OH-MeP, 3,4-DHB, and 4-HB showed a substantial decrease to mean concentrations in the range from  $4 \mu\text{g kg}^{-1}$  for OH-MeP, to  $88.1 \mu\text{g kg}^{-1}$ , for 4-HB. However, as can be seen in Fig. 2, a strong concentration increase was observed for 3,4-DHB after composting, indicating a possible hydroxylation of 4-HB. The concentrations obtained in DTPs were in the range from  $45.8 \mu\text{g kg}^{-1}$ , for OH-MeP, to  $250 \mu\text{g kg}^{-1}$  for 4-HB. These concentrations were lower than those obtained for AnTPs, AeTPs, and AnSPs, indicating the best removal, especially in the case of OH-MeP. In AeTPs, concentrations of parabens remained similar after sludge stabilization (Table 2). The highest concentrations were obtained in AnSPs (mean values from

$141.4 \mu\text{g kg}^{-1}$  (MeP) to  $493.5 \mu\text{g kg}^{-1}$  for 4-HB) what could be due to the high residence time (normally higher than 1–2 years) of sludge, favouring the accumulation of these compounds. As can be seen in Fig. 2, a remarkable concentration increase of OH-MeP occurred from primary and secondary sludge to digested and dehydrated sludge in AnTPs. In addition, high concentrations were measured in AnSPs, reinforcing the hypothesis of the MeP conversion into OH-MeP. These results could indicate a high persistence of these compounds under anaerobic conditions while under aerobic conditions a higher degradation was measured. These results are in accordance with those reported by Wu et al. (2017).

The results obtained in this work show the persistence of most of the target compounds in the studied sludge stabilization technologies. Similar results have been reported for pharmaceutical compounds (Martín et al., 2015) and personal care products (Wu et al., 2017) whereas advanced oxidation treatments (Mohapatra et al., 2014), hydrothermal carbonization (Eyser et al., 2016) and sorption onto materials (Malvar et al., 2020a) have showed promising results on the removal of some of the studied compounds.

## 4. Conclusions

PPCPs and their main metabolites have been measured along different sludge stabilization treatments including anaerobic and aerobic digestion, dehydration, composting, and anaerobic stabilization ponds. MeP, CAF, and IBU were the parent compounds at the highest concentrations. However, some of their metabolites, such as OH-MeP, CBX-IBU and 4HB, were found at even higher concentrations.

The distribution of PPCPs and their metabolites in fresh sludge was in accordance with their metabolic ratios, except for CAF and parabens. In the case of CAF and parabens, the different distribution pattern could be attributed to the different sources of CAF and 4-HB, respectively. Moreover, all primary sludges showed a similar distribution for all studied compounds in spite of the different geographical locations of wastewater treatment plants.

The different physical–chemical properties of the studied compounds resulted in different behaviours in the sludge stabilization processes. CBZ, 3-OH-CBZ, DIC, and 4-OH DIC revealed a high persistence whereas CAF and PX showed high persistence only in anaerobic treatment. In the case of IBU, hydroxylation and carboxylation of the parent compound seemed to occur under aerobic and anaerobic digestion, respectively, to form its metabolites. Exceptionally, a similar distribution pattern was observed for both DIC and 4-OH DIC in all sludge samples, and in each wastewater treatment plant, including treated sludge samples such as anaerobically- and aerobically-digested and dehydrated sludge, except in compost.

The results obtained in this work show the necessity of monitoring not only PPCPs, but also their metabolites, in sludge stabilization processes and, especially, in sludge and compost intended to be applied onto soil. Moreover, the concentrations measured in the final products of sludge stabilization treatments reveal the necessity to improve the knowledge about their distribution in sludge-amended soil, especially in the case of metabolites, as well as the potential ecotoxicological risks associated to the presence of these compounds in sludge applied onto soil.

## 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.

## Acknowledgments

This work was financially supported by the Spanish Ministry of Economy and Competitiveness (Project No. CTM2017-82778-R). J. L. Malvar gratefully acknowledges FPU programme from Spanish Government for his pre-doctoral contract (FPU17/00368).

## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.wasman.2020.07.051>.

## References

Abril, C., Santos, J.L., Malvar, J.L., Martín, J., Aparicio, I., Alonso, E., 2018. Determination of perfluorinated compounds, bisphenol A and personal care products in digested sludge, compost and soil by liquid-chromatography-tandem mass spectrometry. *J. Chromatogr. A* 1576, 34–41. <https://doi.org/10.1016/j.chroma.2018.09.028>.

Abril, C., Santos, J.L., Martín, J., Aparicio, I., Alonso, E., 2020. Occurrence, fate and environmental risk of anionic surfactants, bisphenol A, perfluorinated compounds and personal care products in sludge stabilization treatments. *Sci. Total Environ.* 711. <https://doi.org/10.1016/j.scitotenv.2019.135048>.

Alsabri, S.G., Mari, W.O., Younes, S., Alsadawi, M.A., Oroszi, T.L., 2018. Kinetic and Dynamic Description of Caffeine. *J. Caffeine Adenosine*. 8 (1), 3–9. <https://doi.org/10.1089/caff.2017.0011>.

Bahlman, A., Brack, W., Schneider, R.J., Krauss, M., 2014. Carbamazepine and its metabolites in wastewater: Analytical pitfalls and occurrence in Germany and Portugal. *Water Res.* 57, 104–114. <https://doi.org/10.1016/j.watres.2014.03.022>.

Biel-Maeso, M., González-González, C., Lara-Martín, P.A., Corada-Fernández, C., 2019. Sorption and degradation of contaminants of emerging concern in soils under aerobic and anaerobic conditions. *Sci. Total Environ.* 666, 662–671. <https://doi.org/10.1016/j.scitotenv.2019.02.279>.

Brezina, E., Prasse, C., Meyer, J., Mückter, H., Ternes, T.A., 2017. Investigation and risk evaluation of the occurrence of carbamazepine, oxcarbazepine, their human metabolites and transformation products in the urban water cycle. *Environ. Pollut.* 225, 261–269. <https://doi.org/10.1016/j.envpol.2016.10.106>.

Chen, J., Pycke, B.F.G., Brownawell, B.J., Kinney, C.A., Furlong, E.T., Kolpin, D.W., Halden, R.U., 2017. Occurrence, temporal variation, and estrogenic burden of five parabens in sewage sludge collected across the United States. *Sci. Total Environ.* 593–594, 368–374. <https://doi.org/10.1016/j.scitotenv.2017.03.162>.

Christou, A., Michael, C., Fatta-Kassinos, D., Fotopoulos, V., 2018. Can the pharmaceutically active compounds released in agroecosystems be considered as emerging plant stressors? *Environ. Int.* 114, 360–364. <https://doi.org/10.1016/j.envint.2018.03.003>.

EC, 2008. Environmental, economic and social impacts of the use of sewage sludge on land; Final Report; Part I: Overview Report. In: W. A. R. f. t. E. C. Milieu Ltd, DG Environment under Study Contract DG ENV.G.4/ETU/2008/0076r, (Ed.). [http://ec.europa.eu/environment/archives/waste/sludge/pdf/part\\_i\\_report.pdf](http://ec.europa.eu/environment/archives/waste/sludge/pdf/part_i_report.pdf) (accessed on 11 May 2020).

Egea-Corbacho, A., Gutiérrez-Ruiz, S., Quiroga-Alonso, J.M., 2019. Removal of emerging contaminants from wastewater using reverse osmosis for its subsequent reuse: Pilot plant. *J. Water Process Eng.* 29. <https://doi.org/10.1016/j.jwpe.2019.100800>.

EU, 1986. Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. *Off. J. Eur. Commun.* 181, 0006-0012.

Eyser, C., Schmidt, T.C., Tuerk, J., 2016. Fate and behaviour of diclofenac during hydrothermal carbonization. *Chemosphere* 153, 280–286. <https://doi.org/10.1016/j.chemosphere.2016.03.051>.

Ferrando-Climent, L., Collado, N., Buttigliere, G., Gros, M., Rodríguez-Roda, I., Rodríguez-Mozaz, S., Barceló, D., 2012. Comprehensive study of ibuprofen and its metabolites in activated sludge batch experiments and aquatic environment. *Sci. Total Environ.* 438, 404–413. <https://doi.org/10.1016/j.scitotenv.2012.08.073>.

García-Galán, M.J., Díaz-Cruz, S., Barceló, D., 2013. Multiresidue trace analysis of sulfonamide antibiotics and their metabolites in soils and sewage sludge by pressurized liquid extraction followed by liquid chromatography–electrospray–quadrupole linear ion trap mass spectrometry. *J. Chromatogr. A* 1275, 32–40. <https://doi.org/10.1016/j.chroma.2012.12.004>.

Hatoum, R., Potier, O., Roques-Carmes, T., Lemaitre, C., Hamieh, T., Toufaily, J., Horn, H., Borowska, A., 2019. Elimination of micropollutants in activated sludge reactors with a special focus on the effect of biomass concentration. *Water* 11, 2217. <https://doi.org/10.3390/w11112217>.

Jagoda, A., Zukowski, W., Dabrowska, B., 2015. Investigations of the presence of caffeine in the Rudawa River, Kraków, Poland. *Environ. Monit. Assess.* 187 (9), 566. <https://doi.org/10.1007/s10661-015-4760-7>.

Jelic, A., Fatone, F., Di Fabio, S., Petrovic, M., Cecchi, F., Barceló, D., 2012. Tracing pharmaceuticals in a municipal plant for integrated wastewater and organic solid waste treatment. *Sci. Total Environ.* 433, 352–361. <https://doi.org/10.1016/j.scitotenv.2012.06.059>.

Jiang, W., Xia, T., Yun, Y., Li, M., Zhang, F., Gao, S., Chen, W., 2019. UHPLC-MS/MS method for simultaneous determination of carbamazepine and its seven major metabolites in serum of epileptic patients. *J. Chromatogr. B* 1108, 17–24. <https://doi.org/10.1016/j.jchromb.2018.12.016>.

Kahl, S., Nivala, J., Van Afferden, M., Müller, R.A., Reemtsma, T., 2017. Effect of design and operational conditions on the performance of subsurface flow treatment wetlands: emerging organic contaminants as indicators. *Water Res.* 15 (125), 490–500. <https://doi.org/10.1016/j.watres.2017.09.004>.

Karthikraj, R., Vasu, A.K., Balakrishna, K., Sinha, R.K., Kannan, K., 2017. Occurrence and fate of parabens and their metabolites in five sewage treatment plants in India. *Sci. Total Environ.* 593–594, 592–598. <https://doi.org/10.1016/j.scitotenv.2017.03.173>.

Larsson, E., al-Hamimi, S., Jönsson, J.A., 2014. Behaviour of nonsteroidal anti-inflammatory drugs and eight of their metabolites during wastewater treatment studied by hollow fibre liquid phase microextraction and liquid chromatography mass spectrometry. *Sci. Total Environ.* 485–486, 300–308. <https://doi.org/10.1016/j.scitotenv.2014.03.055>.

Lazenka, M.F., Moeller, F.G., Negus, S.S., 2015. Effects of caffeine and its metabolite paraxanthine on intracranial self-stimulation in male rats. *Exp. Clin. Psychopharmacol.* 23 (2) 71–80.

Luque-Muñoz, A., Vilchez, J.L., Zafra-Gómez, A., 2017. Multiclass method for the determination of pharmaceuticals and personal care products in compost from sewage sludge using ultrasound and salt-assisted liquid-liquid extraction followed by ultrahigh performance liquid chromatography–tandem mass spectrometry analysis. *J. Chromatogr. A* 1507, 72–83. <https://doi.org/10.1016/j.chroma.2017.05.051>.



- Malvar, J.L., Martín, J., Orta, M.M., Medina-Carrasco, S., Santos, J.L., Aparicio, I., Alonso, E., 2020a. Simultaneous and individual adsorption of ibuprofen metabolites by a modified montmorillonite. *Appl. Clay Sci.* 189. <https://doi.org/10.1016/j.clay.2020.105529> 105529.
- Malvar, J.L., Santos, J.L., Martín, J., Aparicio, I., Alonso, E., 2020b. Simultaneous pressurized liquid extraction and clean-up for the determination of metabolites in complex environmental solid matrices. *Microchem. J.* 152. <https://doi.org/10.1016/j.microc.2019.104370> 104370.
- Malvar, J.L., Santos, J.L., Martín, J., Aparicio, I., Alonso, E., 2020c. Comparison of ultrasound-assisted extraction, QuEChERS and selective pressurized liquid extraction for the determination of metabolites of parabens and pharmaceuticals in sludge. *Microchem. J.* 157, 104987. <https://doi.org/10.1016/j.microc.2020.104987>.
- Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2015. Pharmaceutically active compounds in sludge stabilization treatments: Anaerobic and aerobic digestion, wastewater stabilization ponds and composting. *Sci. Total Environ.* 503–504, 97–104. <https://doi.org/10.1016/j.scitotenv.2014.05.089>.
- Martín, J., Camacho-Muñoz D., Santos J.L., Aparicio I., Alonso E., 2012. Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal. *J. Hazard. Mat.* 239–240, 40–47. <https://doi.org/10.1016/j.jhazmat.2012.04.068>.
- Mazaleuskaya, L., Theken, K.N., Gong, L., Thorn, C.F., FitzGerald, G.A., Altman, R.B., Klein, T.E., 2015. PharmGKB summary: ibuprofen pathways. *Pharmacogenet. Genom.* 25 (2), 96–106.
- Mohapatra, D.P., Brar, S.K., Tyagi, R.D., Picard, P., Surampalli, R.Y., 2014. Analysis and advanced oxidation treatment of a persistent pharmaceutical compound in wastewater and wastewater sludge-carbamazepine. *Sci. Total Environ.* 470–471, 58–75. <https://doi.org/10.1016/j.scitotenv.2013.09.034>.
- Mordechay, E., Tarchitzky, J., Chen, Y., Shenker, M., Chefetz, B., 2018. Composted biosolids and treated wastewater as sources of pharmaceuticals and personal care products for plant uptake: a case study with carbamazepine. *Environ. Int.* 232, 164–172. <https://doi.org/10.1016/j.envpol.2017.09.029>.
- Nivala, J., Kahl, S., Boog, J., Afferden, M., Reemtsma, Müller, R., 2019. Dynamics of emerging organic contaminant removal in conventional and intensified subsurface flow treatment wetlands. *Sci. Total Environ.* 649, 1144–1156. <https://doi.org/10.1016/j.scitotenv.2018.08.339>.
- Pereira, A., Silva, L., Laranjeiro, C., Lino, C., Pena, A., 2020. Selected pharmaceuticals in different aquatic compartments: Part I—Source, fate and occurrence. *Molecules* 25, 1026. <https://doi.org/10.3390/molecules25051026>.
- Peysson, W., Vulliet, E., 2013. Determination of 136 pharmaceuticals and hormones in sewage sludge using quick, easy, cheap, effective, rugged and safe extraction followed by analysis with liquid chromatography-time-of-flight-mass spectrometry. *J. Chromatogr. A* 1290, 46–61. <https://doi.org/10.1016/j.chroma.2013.03.057>.
- Rossini, D., Ciofi, L., Ancilotti, C., Checchini, L., Bruzzoniti, M.C., Rivoira, L., Fibbi, D., Orlandini, S., Del Bubba, M., 2016. Innovative combination of QuEChERS extraction with on-line solid-phase extract purification and pre-concentration, followed by liquid chromatography-tandem mass spectrometry for the determination of non-steroidal anti-inflammatory drugs and their metabolites in sewage sludge. *Anal. Chim. Acta* 935, 269–281. <https://doi.org/10.1016/j.aca.2016.06.023>.
- Sathishkumar, P., Meena, R.A.A., Palanisami, T., Asshokkumar, V., Palvannan, T., Gu, F.L., 2020. Occurrence, interactive effects and ecological risk of diclofenac in environmental compartments and biota – a review. *Sci. Total Environ.* 698. <https://doi.org/10.1016/j.scitotenv.2019.134057>.
- Thelusmond, J., Kawka, E., Strathmann, T.J., Cupples, A.M., 2018. Diclofenac, carbamazepine and triclocarban biodegradation in agricultural soils and the microorganisms and metabolic pathways affected. *Sci. Total Environ.* 640–641, 1393–1410. <https://doi.org/10.1016/j.scitotenv.2018.05.403>.
- Tiwari, B., Sellamuthu, B., Ouada, Y., Drogui, P., Tyagi, R.D., Buelna, G., 2017. Review on fate and mechanism of removal of pharmaceutical pollutants from wastewater using biological approach. *Bioresour. Technol.* 224, 1–12. <https://doi.org/10.1016/j.biortech.2016.11.042>.
- Wang, W., Kannan, K., 2016. Fate of parabens and their metabolites in two wastewater treatment plants in New York State, United States. *Environ. Sci. Technol.* 50 (3), 1174–1181. <https://doi.org/10.1021/acs.est.5b05516>.
- Wojslawski, J., Bialk-Bielinska, A., Stepnowski, P., Dolzonek, J., 2019. Leaching behavior of pharmaceuticals and their metabolites in the soil environment. *Chemosphere* 231, 269–275. <https://doi.org/10.1016/j.chemosphere.2019.05.031>.
- Wu, Y., Sun, Q., Wang, Y., Deng, C., Yu, C., 2017. Comparative studies of aerobic and anaerobic biodegradation of methylparaben and propylparaben in activated sludge. *Ecotox. Environ. Safe* 138, 25–31. <https://doi.org/10.1016/j.ecoenv.2016.12.017>.
- Wu, L., Yuan, Z.-C., Li, Z.-M., Huang, Z., Hu, B., 2020. In vivo solid-phase microextraction swab sampling of environmental pollutants and drugs in human body for nano-electrospray ionization mass spectrometry analysis. *Anal. Chim. Acta* 1124, 71–77. <https://doi.org/10.1016/j.aca.2020.05.022>.
- Yan, J., Zhang, X., Lin, W., Yang, C., Ren, Y., 2019. Adsorption behavior of diclofenac-containing wastewater on three kinds of sewage sludge. *Water Sci. Technol.* 80 (4), 717–726. <https://doi.org/10.2166/wst.2019.315>.