Pharmaceutical biological degradation, sorption and mass balance determination in a conventional activated-sludge wastewater treatment plant from Murcia, Spain

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HIGHLIGHTS

- Five pharmaceuticals were evaluated through the stages of a wastewater treatment plant.
- Low levels of pharmaceuticals were detected in waters in comparison to sewage sludge.
- The K_{biol} and K_d were estimated for the pharmaceutical compounds studied.
- The highest degradation was produced through the biological treatment for the bulk of the pharmaceutical.

GRAPHICAL ABSTRACT

ABSTRACT

Pharmaceutical compounds are being detected widely in the aquatic environment due to their global consumption. Some ecotoxicological studies have revealed their implication in different toxic effects and the only mechanism available nowadays to combat with this problem are the wastewater treatment plants, which in function of the system employed seem to be more successful in the pharmaceuticals degradation. The contribution of adsorption and bio-degradation to the overall removal was estimated to be the main reason for their elimination from the environment. For that reason, in this paper the biological degradation, sorption and mass balance in a conventional activate sludge (CAS) WWTP are evaluated. Among of the pharmaceutical studied (carbamazepine, diclofenac, ibuprofen, ketoprofen and naproxen), the most of them (except carbamazepine), had an extraordinary degradation (>80%). The percentages of the elimination due to microorganism degradation in the secondary treatment, was estimated for all of the pharmaceutical and it was observed that it was very important for ketoprofen and ibuprofen, while for the others pharmaceutical the sorption onto sludge was most predominant that biodegradation in order to eliminate them from the water.

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1. Introduction

Pharmaceutical compounds are continuously being used by humans and animals for different medical cares. The consumption of pharmaceuticals per capita and per year is estimated to be about 15 g and in industrialized countries the value is expected to be between 50 and 150 g [1]. For example, the globally consumed volumes of pharmaceutical compounds like carbamazepine and diclofenac are estimated to be 1014 tons and 940 tons per year, respectively [2].

Since some years ago, the pharmaceutically active compounds (PhACs) become an increased reason of concern due to their occurrence in the environment as a contaminant and the discover of some eco-toxicological effects [3]. There are numerous ways for environmental entry of these compounds [4]. For them, the main way of contamination is via the unaltered excretion in urine and faeces, although, other anthropogenic mechanisms should be assumed like: post-consumption metabolism; diagnostic compounds (X-ray contrast media); household disposal of unused and expired pharmaceuticals; the use of WWTPs sludge in agriculture; veterinary and aquaculture medicines; pharmaceutical manufacturing facilities that can provoke discharges into the environment.

Even though low concentrations, effects of these substances on the human health and the environment cannot be despised. For example, the non-steroidal anti-inflammatory drug diclofenac (DCF) belongs to the group of Chemicals of Emerging Concern (CECs) and has an important environmental relevance. Because in addition to the well-known toxic effects of DCF to vultures (*Gyps bengalensis*), especially in the Indian subcontinent [5], renal and hepatic toxicity has been documented in some aquatic organism like fish species at concentrations in the low $\mu g\,L^{-1}$ range [6]. Since there has been demonstrated several hard injuries to some species due to the presence of DCF, it has been included in the Watch List of the European Water Framework Directive (WFD) [7], which contains the candidates for a revised list of priority substances.

WWTPs are not designed for this purpose and are able to efficiently remove very well some of these PhACs, but not all of them, especially those with more recalcitrant characteristics [8]. The most WWTPs employ activated sludge processes where microorganisms are used to mineralize the contaminants to water and carbon dioxide, or degrade them to less dangerous forms [2]. Several studies about degradation of many PhACs have been carried out in the literature [9,10]. As biological degradation is a key mechanism for the removal of many PhACs, some numerical parameters are necessary to understand and estimate its efficiency. The biological degradation rate constant (K_{biol}) has been suggested to be a strong indicator of the removal efficiency of PhACs due to biological transformation [11–14]. However, the K_{biol} values for many PhACs are not available [15]. Some K_{biol} values have been determined for a limited number of compounds using pseudo-first-order kinetics [16,17]. At this time, the majority of K_{biol} values used in modelling the removal of PhACs are estimated using their chemical composition and characteristics [15]. Other mechanism to remove these pollutants from the WWTPs is the sorption into the sludge [18]. The sorption coefficient (K_d) is defined for equilibrium conditions in the WWTPs [17,19].

The main aim of this study is evaluate the biological degradation of the target compounds carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBP), ketoprofen (KTF) and naproxen (NPX) in the secondary treatment of a conventional activated sludge WWTPs and estimate their sorption into the sludge. For finally evaluate their mass balance and their degradation into the WWTPs. The sampling campaign was in February of 2016 and the selection of the PhACs was based on their abroad consumption, prevalence in biosolids as well as their environmental importance.

2. Materials and methods

2.1. Chemicals and materials

Label pharmaceutical standards, Carbamazepine-D_{10} solution 100 $\mu g\,mL^{-1}$ in methanol, Ibuprofen-d₃, from Sigma-Aldrich, (Schnelldorf, Germany) and diclofenac-(acetophenyl ring $^{-13}C_6$) sodium salt 4.5-hydrate VETRANAL from Fluka with purity degree >98%, were purchased from Sigma-Aldrich, (Schnelldorf, Germany). Pharmaceutical standards of carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBP) and Ketoprofen (KTF) were provided by Sigma-Aldrich, while naproxen (NPX) was provided by Fluka. Individual stock solutions were prepared in methanol at 500 $\mu g\,mL^{-1}$ and stored at $-20\,°C$ in the dark. An intermediate solution was prepared, in mixture, at a concentration of 2.5 $\mu g\,mL^{-1}$, in methanol.

Ultrapure water (gradient HPLC) from Scharlau (Sachalab, Barcelona, Spain) was used for the blanks and ongoing precision and recovery standard samples. Methanol and acetone multisolvent (HPLC grade), ter-butil methyl ether (HPLC grade) were obtained from Sacharlau (Barcelona, Spain). Formic acid (<95%) was obtained from Sigma-Aldrich (Spain). Oasis HLB (60 mg, 3 mL) extraction cartridges, from Waters Corporation (Dublin, Ireland), were used for solid phase extractions (SPE). Nylon filters (45 μm pore size, 25 mm diameter) were acquired from Análisis vinílicos S.A. (Tomelloso, Spain).

2.2. Sampling site and collection

Monitoring was performed at the Wastewater treatment plant of the Roldán, Lo Ferro-Balsicas ((UTM) X: 679615.7525, Y: 4185244.9214), where a conventional activated-sludge (CAS) treatment plant is run in parallel with prolonged aeration (PA). This WWTP handles 8910 population equivalents (PE), is equipped for nutrient removal. Primary treatment consists of a screen, an aerated grit-removal tank and a primary clarifier. Then, the biologically treated wastewater from the conventional activated-sludge is filtered through a layer of a continuously operated sand filter before being disinfected by ultraviolet radiation. The effluents are used mainly for irrigation purposes in agriculture.

The sampling campaign was carried out in 2016 and from 09th to 11th February – winter, one sample day for the raw urban wastewater and first part of the biological reactor ((B.R. 1) anoxic phase) and depending of the hydraulic retention time, other day for the other two parts of the biological reactor (anaerobic (B.R. 2) and aerobic (B.R. 3)) and the effluent. The characterization of the conditions of the WWTP of Roldan, is shown in Table S1 (Supporting information). Samples were collected in high-density polyethylene plastic containers previously rinsed with bi-distilled water, as time proportional 24-h composite samples and were kept refrigerated (4 °C) during the transport to the laboratory, and then they were process immediately.

2.3. Sample preparation and analysis

2.3.1. Waters

Samples were collected in 1000 mL silanized, amber glass bottles. Sample bottles were kept on ice and brought back to the laboratory within 4 h of collection. Immediately, they were preserved by adjusting to pH 2 with formic acid and stored at 4 °C until extraction. Samples were extracted within 4 days of collection.

Pharmaceutical extraction was performed according to described method of Vanderford [20] with slight modifications. The pharmaceutical compounds were extracted using solid phase extraction (SPE). Due to the high organic content of raw urban wastewater, only 500 mL of influent samples were used to avoid the blockage of the cartridges. For the others samples, 1000 mL samples were used. The first of the three replicates samples were spiked with 2.5 $\mu g\,mL^{-1}$ of surrogate standards ([D_{10}]-carbamazepine, [$^{13}C_6$]-diclofenac, [D-₃]-ibuprofen). The samples were then loaded onto the cartridges, rinsed with reagent water and eluted with methanol/MTBE followed by methanol. The resulting extract was evaporated to dryness under vacuum at 40–50 °C using a TurboVap LV concentrator. Then the second of the three replicates samples were spiked with 2.5 $\mu g\,mL^{-1}$ of surrogate stan-

dards ([D$_{10}$]-carbamazepine, [^{13}C$_6$]-diclofenac, [D-$_3$]-ibuprofen), and the all the extracts were brought to a final volume of 1 mL using methanol. Internal standards were added to the samples extracts in order to obtain a final concentration of 100 ng mL^{-1}.

All samples were finally passed through 0.45 μm nylon filter before instrumental analysis in UPLC Acquity I-Class System (Milford, MA, USA, Waters).

2.3.2. Sludge

Sludge samples were collected from the same wastewater treatment plant during the same sampling period. The sludge was obtained by flotation of fresh sludge from the biological reactor in the different sample points of the water. Samples of dry sludge are dehydrated by centrifugation.

Pharmaceutical extraction was performed following the method of Martín [21] with slight modifications. Lyophilized solid phase (Freeze Dryer Christ alpha 1–2/LD plus) was homogenized using a glass mortar and a 0.1 g of homogenized solid residue were accurately weighted directly in Eppendorf tubes and the first of the three replicates samples were spiked with 2.5 μg mL^{-1} of surrogate standards ([D$_{10}$]-carbamazepine, [^{13}C$_6$]-diclofenac, [D-$_3$]-ibuprofen) to a final concentration of 100 ng mL^{-1}. Afterwards, samples were successively extracted with methanol and acetone. The supernatants obtained were combined and evaporated using a TurboVap LV concentrator, and finally the second of the three replicates samples were spiked with 2.5 μg mL^{-1} of surrogate standards ([D$_{10}$]-carbamazepine, [13C6]-diclofenac, [D-3]-ibuprofen). Internal standards were added to the samples extracts in order to obtain a final concentration of 100 ng mL^{-1}. All the samples were and transferred into a LC vial for its injection in the UPLC Acquity I-Class System.

2.3.3. Liquid chromatography analysis

An UPLC Acquity I-Class System and HR-QTOF-MS maXis Series (Daltonik GmbH, German, Bruker) were used for all analyses. All analytes were separated using a (50 × 2.1 mm) ACQUITY UPLC BEH C$_{18}$ column with 1.7 μm particle size (Milford, MA, USA, Waters). A binary gradient consisting of 0.1% formic acid (v/v) in water (A) and 100% methanol (B) at a flow rate of 700 μL min^{-1} was used. The gradient was as follows: 5% B held for 3.5 min, increased linearly to 80% by 10 min and held for 3 min, and stepped to 100% and held for 8 min. A 9-min equilibration step at 5% B was used at the beginning of each run to bring the total run time per sample to 30 min. An injection volume of 10 μL was used for all analyses.

2.3.4. Quality control

Extraction recoveries for target compounds were determined for different matrices (water and sludge) by spiking samples (n = 3) at 100 ng mL^{-1}. For each type of water and sludge samples, recoveries were determined by comparing one replicate of the samples spiked before of the SPE procedure and other sample spiked after the SPE procedure, calculated by internal standard calibration. Non-spiked samples replicates were analysed in order to determine also their concentrations.

Three-reference method blank were analysed with each sample batch to demonstrate freedom of contamination. In addition, three samples with water and standards were used on an ongoing basis to reveal through the analysis of the ongoing precision and recovery standard (OPR) that the analytical system was robust and was reproducible.

Precision of the method was determined by calculating the relative standard deviation (% RSD) of the triplicate spiked samples. Quantification of target analytes, based on peak area, was achieved by the internal standard approach, and the results were corrected for the recovery. Calibration curves were produced using linear

regression analysis. Limit of detection (LOD) and limit of quantification (LOQ) were determined (Table S2).

2.4. Kinetic models

2.4.1. K$_{biol}$ calculation

The biological rate degradation constant (K$_{biol}$) of CBZ, DCF, IBP, KTF and NPX (parental compounds), were estimated following a pseudo first-order equation [17] values were found by regression using the negative of the slope of the natural log of the concentration divided by the initial concentration over time, with the intercept set at zero. The biological rate degradation constant equation was:

$$\frac{dS_t}{d_t} = -K_{biol} \cdot MLSS \cdot S_t \tag{1}$$

Where St is the soluble compound concentration at time t (ng L^{-1}), t is hydraulic retention time (h), K$_{biol}$ is the intrinsic biological rate constant (L g$_{ss}^{-1}$ h^{-1}), MLSS is the concentration of suspended solids (average g L^{-1}), and S$_t$ is the soluble compound concentration in the raw urban wastewater (ng L^{-1}).

This equation allows predicting the elimination rate of these compounds, depending on the configuration of the biological reactor. In WWTPs with CAS systems like that used in this experiment, this constant allows form three groups [17]:

a) Compounds with K$_{biol}$ < 0.1 L g$_{ss}^{-1}$ h^{-1} are not degraded in a significant grade (<20%);

b) Compounds with 0.1 < K$_{biol}$ < 10 L g$_{ss}^{-1}$ h^{-1} have a partial degradation (between 20% and 90%);

c) Compounds with K$_{biol}$ > 10 L g$_{ss}^{-1}$ h^{-1} have a high degradation (>90%).

2.4.2. Kd calculation

The sorption coefficient (K$_d$) of the PhACs is typically defined for equilibrium conditions in a batch reactor [17,18]. The following equation was used to evaluate the extent of sorption:

$$K_d = \frac{X}{MLSS \cdot S} \tag{2}$$

Where K$_d$ is the sorption coefficient of activated sludge (L g$_{ss}^{-1}$), X is the sorbed compound concentration expressed per unit of volume (ng L^{-1}), S is the soluble compound concentration (ng L^{-1}), and MLSS is the mixed liquor suspended solids concentration (kg L^{-1}).

2.5. Mass balances and degradation percentages

The mass balance was calculated following the method of Gao [22]. The average mass flow of each compound was calculated by multiplying the sum of the concentrations in the aqueous and sludge phases with the corresponding average flows in the influent, different parts of the biologic reactor and the effluent of the wastewater treatment plant. The equation can be written as:

$$m_{aq} = Q_{aq} \cdot C_{aq} \tag{3}$$

$$m_s = Q_s \cdot C_s \tag{4}$$

Where m$_{aq}$ and m$_s$ (mg d^{-1}) respectively, are the mass flux of PhACs calculated in the aqueous and sludge phases, respectively. Q$_{aq}$ (L d^{-1}) and Q$_s$ (kg d^{-1}) are wastewater and sludge flow, respectively. C$_{aq}$ (mg L^{-1}) and C$_s$ (mg kg^{-1}) are the average concentrations of pharmaceutical measured in the wastewater and sludge, respectively.

Usually, the PhACs degradation in the wastewater treatment plants are due mainly to their microorganism bio-transformation and to the sorption into the sludge. For that reason, we have

estimated the loss of pharmaceutical compounds due to the sludge sorption like the sludge remove from the WWTP (m_{sor}). The mass from the influent (m_{inf}) is considered as the corresponded to the water and the recirculation sludge. The mass of the effluent (m_{efl}) is the loss in the water from the effluent.

For the estimation of the PhACs mass that is lost due to the microorganism action the following equation is used:

$$m_{bio} = m_{inf} - m_{efl} - m_{sor} \tag{5}$$

With data of the mass of the respective pharmaceuticals, an estimation of the degradation percentages due to the biological degradation (Eq. (6)) and sludge sorption (Eq. (7)) was made.

$$R_{bio} = \frac{m_{bio}}{m_{inf}} \cdot 100 \tag{6}$$

$$R_{sor} = \frac{m_{sor}}{m_{inf}} \cdot 100 \tag{7}$$

A calculation of the total loss of PhACs in the water was made evaluating the loss of pharmaceutical compounds in the effluent with respect to data in the influent.

3. Results and discussion

3.1. PhACs in liquids and sludge

Water samples from the raw urban wastewater, several parts of the biological reactor, and from the effluents and sludge samples from the different parts of the secondary treatment and dry sludge, were analysed. Since these PhACs are retained in the WWTP sludge, knowing their concentration on it, give us a better understanding of the degradation processes that occur in a biological reactor.

3.1.1. Antiepileptic/psychiatric drug
3.1.1.1. Carbamazepine.
Carbamazepine is an anticonvulsant that is used to treat partial seizures, pain of neurologic origin, and also psychiatric disorders, between others. In the water samples, CBZ was detected in values from below the detection limit in the influent and the anoxic part (B.R 1) of the secondary treatment, to 10.1 ng L^{-1} in the effluent (Fig. 1A). The results are in agreements with the obtained by Behera [23], where in the effluent of a WWTP were found values of 40 ng L^{-1}. In a work of Blair [19], they found a maximum soluble concentration of CBZ of 220 ng L^{-1} but they do not mention the minimum concentration obtained in their analyses.

This increase of CBZ though the WWTP has been seen before in other studies. In some WWTPs, are circumstances where the effluent concentrations of some micropollutants exceed their influent concentrations like in the CBZ case. Two potential theories exist to explain this: PhACs are enclosed in faecal particles and are released from them when the faecal are broken down by the microbes [24]; and/or the undetected PhACs metabolites are being retransformed into the parent compound through microbial activity [12,25].

If we observed the CBZ concentrations found in the sludge samples (Fig. 1B), it can be appreciated that it showed a similar behaviour to found in waters, since no concentration was observed in the influent and the anoxic phase of the WWTP, but the CBZ increased heavily in the aerobic phase with 24.0 ng g^{-1} D.W. Nevertheless, the concentration found in the dry sludge was lower than in the aerobic phase of the secondary treatment with a value of 7.68 ng g^{-1} DW. This results are in agreement with other samples analysed in sludge [21,26] while the results obtained by Chenxi [27] showed concentrations of CBZ quite higher, with values of approximately 2250 ng g^{-1}.

3.1.2. Analgesic/anti-inflammatories
3.1.2.1. Diclofenac.
Diclofenac is an important nonsteroidal anti-inflammatory drugs (NSAIDs), with also analgesic and antipyretic properties. They act by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase, which converts arachidonic acid to cyclic endoperoxides, precursors of prostaglandins. The water concentrations along the WWTP (Fig. 2A) varied from 77.3 ng L^{-1} in the raw urban wastewater to 6.64 ng L^{-1} in the effluent. These values of concentrations are similar to other found by Behera [23] in influents with values of 59 ng L^{-1} and in effluents with values of 13 ng L^{-1} and by Reyes-Contreras [28] in raw urban wastewaters, with concentrations of 200 ng L^{-1}. Nevertheless, in a [25] review of several European wastewater studies, an average concentration in raw wastewater of 700 ng L^{-1} with a maximum concentration of 11.000 ng L^{-1} was reported. A tentative reason of the low concentration for this pharmaceutical compound is that the population that is served by this WWTP is young (data not shown) and also this WWTP does not receive waters from any hospital.

The toxic effects of DCF in the environment are well known at it is mentioned in the introduction. Especial attention is put on vultures from the Indian subcontinent [5], as at fish species [6]. Due to the demonstrated hard injuries to some species, it has been
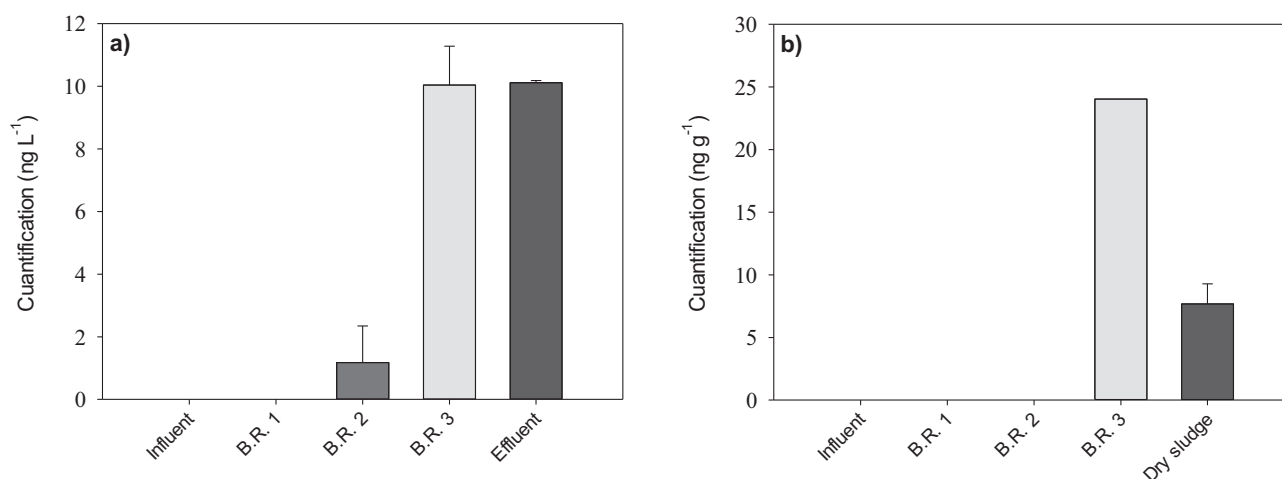

Fig. 1. Carbamazepine concentration in a) water and b) sludge samples obtained from different parts of the WWTPs. Medium ± error standard (n = 3).
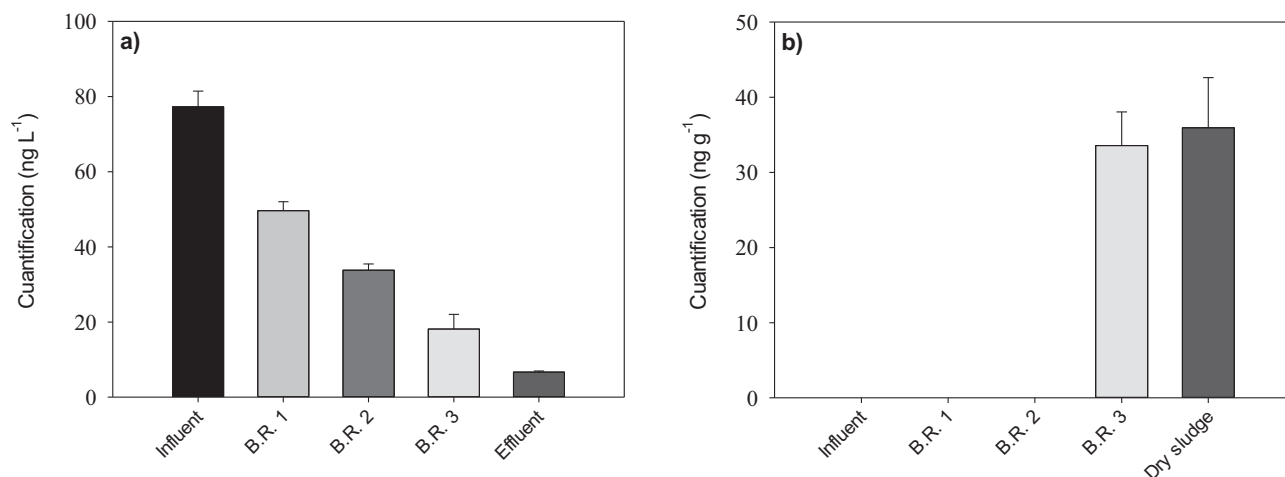
Fig. 2. Diclofenac concentration in a) water and b) sludge samples obtained from different parts of the WWTPs. Medium ± error standard (n = 3).

included in the list of priority substances of the Watch List of the European Water Framework Directive [7]. Some authors affirm that DCF is mainly discharged into the aquatic environment via WWTPs [29], but the main reason for the affection to some species such as the vultures was the use of DCF in the livestock. Fortunately, the eco-toxicological effects of DCF have resulted in the proposal of a relatively low Environmental Quality Standard (EQS) of 100 ng L^{-1} as an annual average for inland surface waters [7]. Consequently, an increase of the EQS in surface waters is likely if the percentage of treated wastewater is higher than 10%. This concentration has been observed in European surface waters by several authors [30,31]. For that reason, an improvement of municipal wastewater treatment would be fundamental to achieve the requests of the revised WFD. At the same time, it is noted that this applies not only to DCF but a complete range of CECs, both known and unknowns, which are discharged into receiving waters due to insufficient removal from the water cycle.

The analyses of the sludge samples (Fig. 2B) revealed that DCF was not detected in the first parts (influent, anoxic and anaerobic phase of the secondary treatment) of the WWTP, but they were found in the aerobic phase of the biological reactor and in the dry sludge at concentrations of 33.6 ng g^{-1} and 35.9 ng g^{-1} on dry weight (DW), respectively. These results are in agreement with the values obtained by Gago-Ferrero [26] that obtained values of 40 ng g^{-1}.

3.1.2.2. Ibuprofen. Ibuprofen is also a NSAIDs, and the most popular analgesic in the world. It is used by millions of people every day as a headache remedy, to reduce fever symptoms, for chronic bone and joint pains with analgesic and antipyretic properties. IBP concentrations along the WWTP (Fig. 3A) varied from 734 ng L^{-1} in the raw urban wastewater to below the detection limit in the final effluent. Behera [23], found values in the influent of 1599 ng L^{-1} while in the effluent were of 15 ng L^{-1}. Other authors [28], found concentrations of 4000 ng L^{-1} in the raw urban wastewater. It is important highlight the degradation of IBP thought the different parts of the WWTP, showing an almost complete depletion in the effluent waters (1.80 ng L^{-1}). This decrease of IBP thought the WWTPs is the typical behaviour detected by other authors for this pharmaceutical compound [24].

The concentration in sludge of IBP in the WWTP (Fig. 3B) was 30.1 ng g^{-1} in the anaerobic phase of the biological reactor, 71.1 ng g^{-1} in the aerobic phase, and 63.8 ng g^{-1} in the dry sludge. The results are generally in good agreement with data found in the literature that were unable to find IBP in the sludge samples due to

that the concentrations were below the limit of quantification of 38.6 ng g^{-1} [26]. However, some studies differ significantly from our IBP results [21] where the concentration obtained in the secondary sludge was 1322 ng g^{-1} and in the digested sludge was 5096 ng g^{-1}.

3.1.2.3. Ketoprofen. Ketoprofen is also a NSAID, used also to treat different painful conditions such as arthritis. The analysis of KTP in waters (Fig. 4A) revealed that in the influent, the concentration was 39.1 ng L^{-1} while in the effluent was 18.9 ng L^{-1}. Therefore, KTP concentration showed a significant decrease during the water treatment, especially in the first part (anoxic phase) of the biological reactor. Behera [23], found values in the influent of 81 ng L^{-1} while no concentration was obtained in the effluent. However, Reyes-Contreras [28] in a winter experiment found concentrations in influent of 300 ng L^{-1}.

The KTP concentrations in the sludge (Fig. 4B) were 7.43 ng g^{-1} in the aerobic phase of the biological reactor and 17.7 ng g^{-1} in the dry sludge. Martín [21] found values of KTP in the secondary sludge below 14.4 ng g^{-1} and in the digested sludge of 10.5 ng g^{-1}.

3.1.2.4. Naproxen. Naproxen is other NSAID that is also used to treat pain or inflammation caused by arthritis, ankylosing spondylitis and tendinitis between others. The NPX concentrations found in the waters of the WWTP (Fig. 5A) varied from 444 ng L^{-1} in the influent and 11.5 ng L^{-1} in the effluent. So it was produced an extraordinary NPX reduction of the concentration thought the WWTP. However, the values obtained in the biological reactor were heterogeneous as it can be observed an increase in the concentration in the anaerobic phase of the reactor. Behera [23], found values in the influent of 1360 ng L^{-1} while in the effluent were of 37 ng L^{-1} Reyes-Contreras [28] found concentrations in influent of 600 ng L^{-1}.

With respect to the NPX concentration found in the WWTP sludge (Fig. 5B), it can be deduced that this pharmaceutical compound also showed heterogeneous values in the WWTP, since it was only detected in the anaerobic phase of the biological reactor and in the dry sludge with concentrations of 3.96 ng g^{-1} and 75.5 ng g^{-1}, respectively. The results are generally in good agreement with data found in the literature [21] with concentrations of NPX in the secondary sludge of 29.1 ng g^{-1} and in the digested sludge of 14.9 ng g^{-1}.

It is necessary clarify that all the pharmaceutical compounds studied in the waters had concentrations below PNEC (Predicted No Effect Concentrations) values that are found in the literature
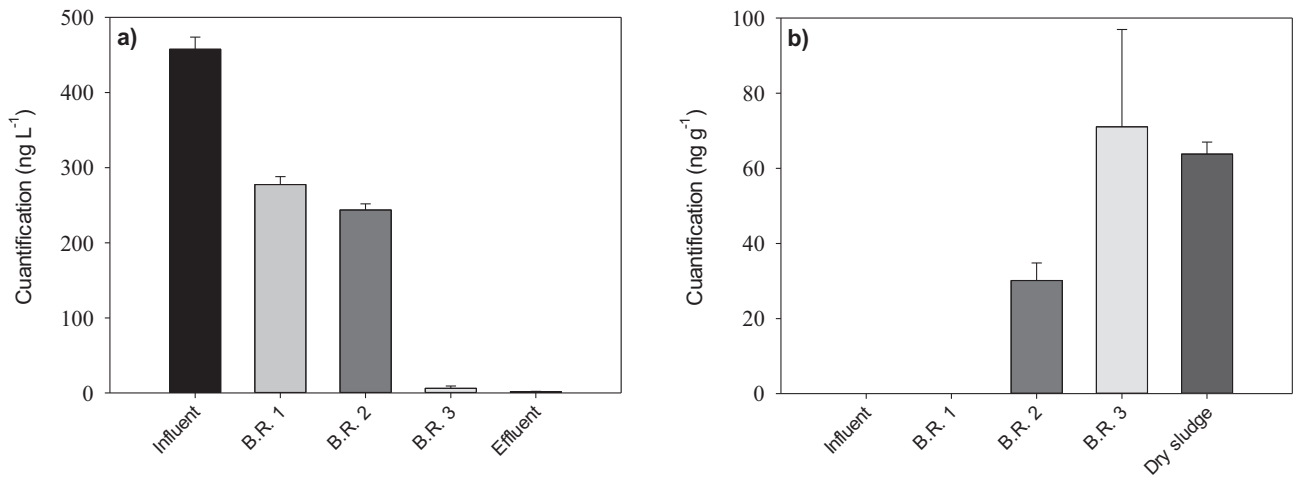
Fig. 3. Ibuprofen concentration in a) water and b) sludge samples obtained from different parts of the WWTPs. Medium ± error standard (n = 3).
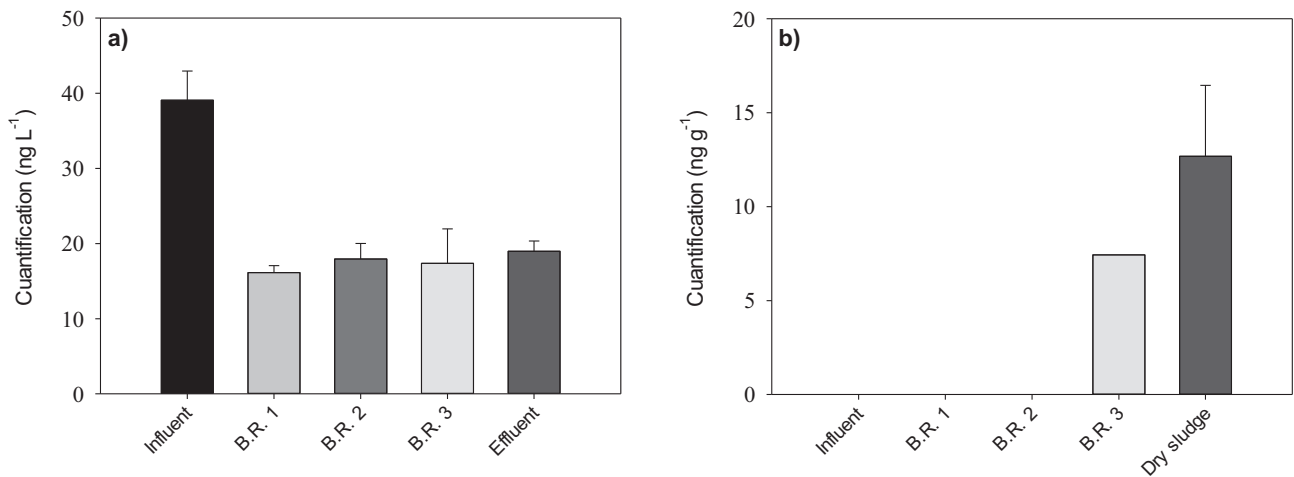

Fig. 4. Ketoprofen concentration in a) water and b) sludge samples obtained from different parts of the WWTPs. Medium ± error standard (n = 3).
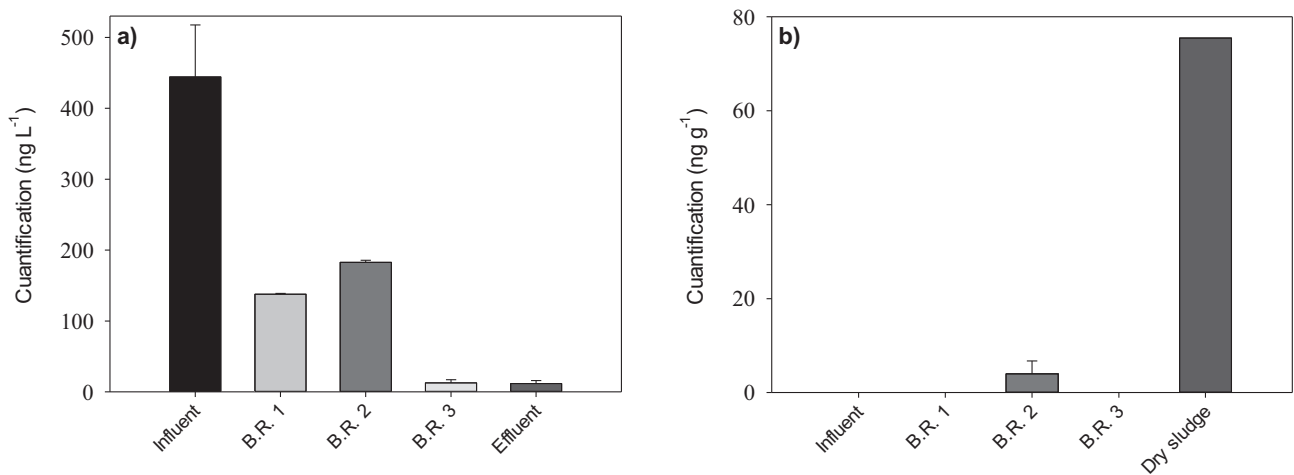

Fig. 5. Naproxen concentration in a) water and b) sludge samples obtained from different parts of the WWTPs. Medium ± error standard (n = 3).

[25] for different species. Each of the reported PNECs is 1000 times lower than the toxicity concentration value found for the most sensitive species assayed, so apparently, the effluent concentration detected are harmless for the biota of the ecosystems. Some similar

happen with the possible sludge application like amendment in agricultural culture. The current assessments for human health due to the consumption of edible tissues of plants that have been grown in soil amended with biosolids is very low if we consider

the lowest therapeutic dose of every one of the pharmaceutical compounds.

3.2. Calculations of biological degradation and sorption into sludge of PhACs calculations

The intrinsic biodegradation rates (K_{biol}) were calculated as they represent a better understanding of the soluble degradation of PhACs within an activated sludge wastewater treatment process with a known MLSS. These values were calculated for the target pharmaceutical compounds analysed in the WWTP. At the sampling time, the MLSS was 4.09 g L^{-1} and the results obtained from the calculations are shown in the Table 1.

According to the classification scheme for pharmaceutical biodegradation establish by Joss [18] in a bath experiment with activated sludge with nutrient- removed WWTPs, the removal status of CBZ and NPX are classified as of "no removal" (<0.1 g$_{ss}^{-1}$ h^{-1}) since are removed by biodegradation below the 20%; while DCF, IBP and KTF have a "partial biodegradation" (<0.1 < K_{biol} < 10 L g$_{ss}^{-1}$ h^{-1}) with an expected biological transformation between 20 and 90%. These results are similar to the obtained by Urase [32] in a study with various full-scale municipal WWTPs, with K_{biol} values for DCF, IBP, KTF and NPX of 2.02->10, 0.05–1.06, 0.34->10 and 0.46–1.92 L g$_{ss}^{-1}$ h^{-1}, respectively. Nevertheless, in Joss [17] study, the K_{biol} results of DCF, IBP and NPX were <0.1, 21–35, and 1.0–1.9 L g$_{ss}^{-1}$ h^{-1}, respectively. These differences between the studies can be explained by substantially different experimental concentration of pharmaceuticals concentration, sludge origin (sludge age, wastewater composition, and flow scheme) or sludge handling prior to batch experiments (e.g. artificial substrate dosing, sludge storage).

With respect to the sorption of pharmaceuticals onto the sludge. It mainly occurs by (a) absorption, in which hydrophobic interactions occur between the aliphatic and aromatic groups of a compound and the lipophilic cell membrane of microorganisms as well as the fat fractions of sludge, and (b) adsorption, involving the electrostatic interactions of the positively charged groups with the negatively charged surfaces of the microorganisms and sludge [33]. This process depends on many factors, including pH, redox potential, stereochemical structure and chemical nature of both the sorbent and the sorbed molecule [34].

The sorption coefficients for the pharmaceutical compounds are shown in Table 1. All results are below from the value 0.50 L g$_{ss}^{-1}$ required for significant sorption onto sludge [33]. The values obtained for DCF and CBZ were considerably higher than those for the other compounds. In this work CBZ is quite sorbed onto the sludge (0.47 L g$_{ss}^{-1}$) being this result very high in contrast to Ternes [33] who described a hardly attached onto sludge where the distribution coefficient between water and secondary sludge (K_d) was 0.001 L g$_{ss}^{-1}$. The sorption behaviour of DCF onto sludge was in the same order than CBZ, and also higher than the found by Ternes [33] where the water–sludge distribution coefficient was 0.02 L g$_{ss}^{-1}$. Nevertheless, for Joss [17] the value obtained for DCF was 0.016 L g$_{ss}^{-1}$, more similar to the results obtained from this work.

Table 1
Constant biodegradation rates (K_{biol}) and sorption equilibrium in the sludge (K_d) for the different studied compounds.

Compound	K_{biol} (L g$_{ss}^{-1}$ h^{-1})	K_d (L g$_{ss}^{-1}$ h^{-1})
Carbamazepine	−0.87	0.47
Diclofenac	1.31	0.11
Ibuprofen	1.22	0.03
Ketoprofen	0.81	0.04
Naproxen	−1.91	0.08

The constant biodegradation rates (K_{biol}) and sorption equilibrium in the sludge (K_d) were calculated following the Eqs. (1) and (2), respectively.

In the IBP, KTP and NPX cases the Kd values were lower than those obtained for CBZ and DCF (Table 1), but consistent with other results [35]. Although for authors like Joss [17], the sorption coefficient for the activated sludge values for IBP and NPX were 0.007 and 0.013 L g^{-1}ss, respectively, which are lower than the obtained in the present experiment. According to Ternes [33], this can be due to their acidic structures, which could explain their presence mainly in the aqueous phase. At neutral pH, like in this WWTP case (7.5), the target pharmaceutical substances show little tendency absorb into sludge. For that reason, it can be said that the acidic operational conditions seem to be preferable for the removal of acidic pharmaceutical substances because the limiting step for the removal was not biodegradation but the transfer of substances from the water phase to the sludge phase [32].

3.3. Mass balance and removal percentages of the pharmaceutical compounds in the WWTP

The mass balance was estimated in order to evaluate the pharmaceutical degradation inside the WWTP. The results obtained (Table 2) are similar to the obtained in the water and sludge concentrations. Again, CBZ is present only at the aerobic phase and effluent of the WWTP, while the other compounds suffer a considerably degradation process through the different treatments steps. For the sludge, it can be observed a great sorption of pharmaceutical compounds that finally are partly recirculated and partly removed from the WWTP.

With the results from the mass balance, a determination of the pharmaceutical degradation by the microorganism was made using Eq. (5). Later, an estimation of the components elimination due to microorganism degradation and to sorption, was made (Table 3).

For CBZ, it seems that the major part of the elimination of this medicine is through the sorption into the sludge (Table 3), but it is important to remark that only the 28% of the CBZ is excreted in faeces, while the 72% is excreted in urine [2].However, as seen before, the concentration in sludge was higher than in water (Table 2), maybe the reason for that behaviour is that in the 72% of the CBZ excreted in the urine only the 1% correspond to the parent compound [2] that is the analysed in this work and the other correspond to its metabolites (mainly CBZ-diol, 3-OH-CBZ, 2-OH-CBZ, CBZ-acridan and CBZ-epoxide) that are not analysed. Probably this is the main reason of their higher degradation into the sludge of the parent compound CBZ.

Something similar to CBZ happen with DCF. The elimination of DCF seems to be mainly due to the retention onto the sludge. Since only the 35% of the DCF is excreted in faeces and the 65% is excreted in urine, but only the 6% in the parental compound and the other correspond to their metabolites (mainly 4′-OH-DCF, 4′-5-diOH-DCF, 5-OH-DCF and 3′-OH-DCF) [2].

In general, for PhACs, even if the compounds fall into the same therapeutically group, their biodegradability and sorption can show great variability such as it has been found in this study. For example, Salgado [12] reported that, between NSAIDs, DCF presented a low biodegradation rate (<25%), while IBP and KTF were biodegraded in a much higher extent (>75%). Also, it is important to consider that the compounds that tend to be sorbed onto sludge are expected to be better eliminated by activated sludge treatment, like those used in this study, rather than by other low-cost secondary treatments such as trickling filter beds, anaerobic lagoons and constructed wetlands [36].

If we consider only concentrations of pharmaceutical compounds found in waters samples, from the influent and the effluent and study their eliminations, we observed a great elimination of all compounds (>80%) with the exception of CBZ and KTP (Fig. 6). The low removal efficiency of carbamazepine can be explained by its

Table 2
Mass balance of the pharmaceutical compounds thought the different treatment steps of the WWTP (mg d^{-1}).

Compound	Influent	B.R. 1	B.R. 2	B.R. 3	Effluent	Recirculated sludge	Removed sludge
Carbamazepine	5.11	N.A.	1.53	11.2	11.3	N.A.	4181
Diclofenac	98.7	64.8	44.2	20.2	7.43	N.A.	5841
Ibuprofen	585	363	318	6.74	2.02	26486	12364
Ketoprofen	567	180	239	14.0	12.9	3486	N.A.
Naproxen	49.9	21.1	23.5	19.3	21.2	N.A.	1293

N.A. Not available. The mass balance was calculated following the Eqs. (3) and (4), respectively.

Table 3
Mass loss of the pharmaceutical compounds (m$_{sor}$) and (m$_{bio}$) and percent of elimination due to the biodegradation (R$_{bio}$) and sorption (R$_{sor}$).

Compound	m$_{sor}$ (mg d^{-1})	m$_{bio}$ (mg d^{-1})	R$_{bio}$ (%)	R$_{sor}$ (%)
Carbamazepine	4181	0.00	0.00	100
Diclofenac	5841	0.00	0.00	100
Ibuprofen	12364	14704	54.3	45.7
Naproxen	1293	0.00	0.00	100
Ketoprofen	N.D.	4040	99.7	0.32

The percent of elimination has been calculated following the Eqs. (5), (6) and (7).
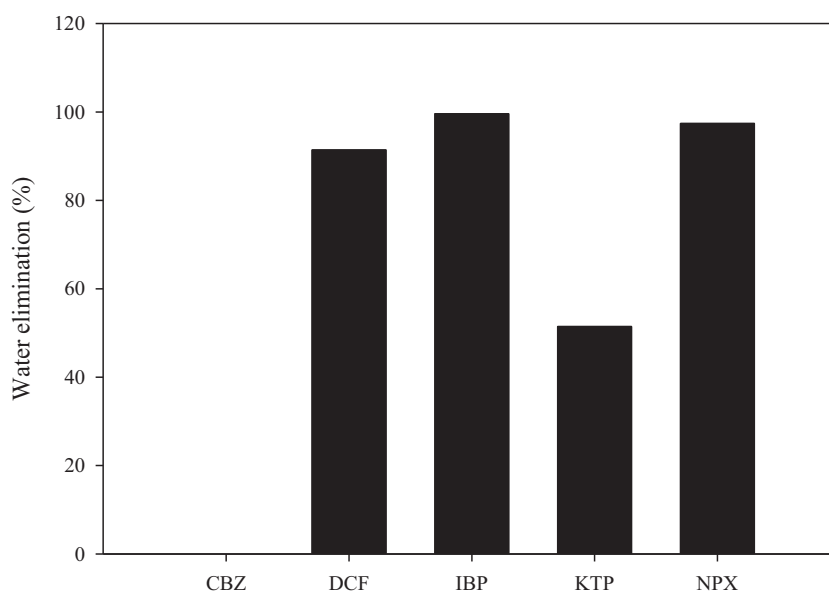
Fig. 6. Removal efficiency of pharmaceutical compounds in the water.

properties, since it is resistant to biodegradation at low concentrations [2], and also two other theories said before: pharmaceutical compounds can be enclosed in faecal particles and is to be released from these particles when the microbes broken down these faeces [24]; and/or the undetected metabolites of the pharmaceutical compounds are being retransformed into the parent compounds by microorganism action [12,25]. The negative removal rate has also been ascribed to the daily concentration fluctuations during the sampling period, the analytical uncertainty, or desorption of molecules from sludge and suspended particulate matter [37,38]. In the DCF case, the removal efficiency was around 80% (Fig. 6). In other works, diclofenac was also significantly removed (81.4%) in a Korean WWTP [23] whereas it was showed insignificant reduction (5%) in a Spanish WWTP [39]. In general, these results are in agreement with others obtained by [19] for CBZ, IBP and NPX removal efficiency and with other data collected by Verlicchi [25] in WWTPs with activated sludge treatment.

The elimination of KTP in effluent waters were 51.4%, results lower than the expected by the authors of this work, nevertheless, several authors [25] showed eliminations from 30 to 92% depending on the operating sludge retention time, obtaining a major elimination with higher retention time (92 days).

It has been observed that, in water samples, degradation of pharmaceuticals depends on its own physical properties, but also to many other factors such the operation parameters inside the WWTP like biomass concentration, sludge retention time, hydraulic retention time, pH, temperature, configuration and type of plant between others [25].

4. Conclusions

According to K$_{biol}$, NPX and CBZ are not degraded to a significant degree (<20% removal), while DCF, IBP and KTF showed partial biodegradation (between 20% and 90%). KTP and IBP underwent almost quantitative elimination due to the microbial activity. Considering the sorption coefficient onto sludge (K$_d$) we observed that the higher values were obtained for CBZ, DCF and NPX, indicating that these compounds are greatly absorbed onto sludge. These

results are consistent with the percentage eliminations due to the sorption that were about 100% for these compounds.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2017.01.048.

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