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RESEARCH ARTICLE

Occurrence and behaviour of pharmaceutical compounds in a Portuguese wastewater treatment plant: Removal efficiency through conventional treatment processes

Vanessa de Jesus Gaffney[1] · Vitor Vale Cardoso[2] · Eugénia Cardoso[3] · Ana Paula Teixeira[3] · José Martins[3] · Maria João Benoliel[2] · Cristina Maria Martins Almeida[1,4]

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Abstract Wastewater treatments can eliminate or remove a substantial amount of pharmaceutical active compounds (PhACs), but there may still be significant concentrations of them in effluents discharged into surface water bodies. Beirolas wastewater treatment plant (WWTP) is located in the Lisbon area and makes its effluent discharges into Tagus estuary (Portugal). The main objective of this study is to quantify a group of 32 PhACs in the different treatments used in this WWTP. Twelve sampling campaigns of wastewater belonging to the different treatments were made in 2013–2014 in order to study their removal efficiency. The wastewaters were analysed by solid phase extraction (SPE) and ultra-performance liquid chromatography coupled with tandem mass detection (UPLC–MS/MS). The anti-diabetics were the most frequently found in wastewater influent (WWI) and wastewater effluent (WWE) (208 and 1.7 μg/L, respectively), followed by analgesics/antipyretics (135 μg/L and < LOQ, respectively), psychostimulants (113 and 0.49 μg/L, respectively), non-steroidal anti-inflammatory drugs (33 and 2.6 μg/L, respectively), antibiotics (5.2 and 1.8 μg/L, respectively), antilipidemics (1.6 and 0.24 μg/L, respectively), anti-convulsants (1.5 and 0.63 μg/L, respectively) and beta blockers (1.3 and 0.51 μg/L, respectively). A snapshot of the ability of each treatment step to remove these target PhACs is provided, and it was found that global efficiency is strongly dependent on the efficiency of secondary treatment. Seasonal occurrence and removal efficiency was also monitored, and they did not show a significant seasonal trend.

Keywords Wastewater · Influent, effluent · Pharmaceuticals · WWTP · UPLC–MS/MS

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✉ Cristina Maria Martins Almeida
calmeida@ff.ulisboa.pt

[1] iMed.UL (Institute for Medicines and Pharmaceutical Sciences, Portugal), Faculdade de Farmácia da Universidade de Lisboa (FFUL), Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal

[2] Empresa Portuguesa das Águas Livres, S.A.—EPAL, Direção de Controlo da Qualidade da Água, Av. de Berlim, 15 –, 1800-031 Lisbon, Portugal

[3] Empresa Portuguesa das Águas Livres, S.A.—EPAL, Direção de Operações de Saneamento, ETAR de Beirolas, Rua Chen He, 1990-513 Sacavém, Portugal

[4] FFUL, Laboratório de Bromatologia e Qualidade da Água, Avenida Prof. Gama Pinto, 1649-003 Lisbon, Portugal

Introduction

Nowadays, more than half the world's population lives in cities, and it is estimated that this number will reach 70% in 2050. Therefore, the supply of water in both quantity and quality for human consumption and the treatment of urban wastewater are two of the biggest challenges of the twenty-first century (Bertrand-Krajewski et al. 2000).

The management of the urban water cycle should ensure the health requirements of a growing population, providing both water quality and in quantity to ensure the sustainability of water resources. The urban water cycle covers not only the water supply but also the wastewater sanitation. It integrates all activities of collection, treatment and distribution of water supply, but also the collection, treatment and discharges of wastewater in the receptor media. From an environmental

perspective, the key steps in the lifecycle of pharmaceutical compounds are manufacturing, consumption and waste management. Throughout its lifecycle, the contamination pathways of pharmaceutical compounds depend on the lifecycle stage where emissions occur.

The occurrence of pharmaceutical active compounds (PhACs) and their metabolites in the water cycle are widespread documented across the globe and although their presence in drinking water is sporadic and in trace concentrations, all these issues have increased the attention of researchers, media and public (Gaffney et al. 2014).

Emerging pollutants are products or chemicals without regulatory status and whose effects on environment and human health are unknown (Deblonde et al. 2011). However, PhACs are recognized as 'emerging' contaminants due to their bioactivity, wide usage and potential health and ecological risks (WHO 2011).

Unlike other environmental contaminants, PhACs are well characterized due to a tight regulatory process and rigorous pre-clinical and clinical studies to assess their efficacy and safety before its commercialization approval (Salgado et al. 2013). Therefore, tests to assess the environmental risk of PhACs are required only for new formulated products. On the other hand, the effect of traditional water treatments on these contaminants is not well characterized. PhACs and their metabolites can be degraded, partially degraded or can resist the treatment processes remaining unchanged (Bila and Dezotti 2003; Gaffney et al. 2016). PhACs can be degraded through biotic (biological treatment or other) and abiotic processes (oxidation, hydrolysis or photolysis). The degradation products can also be cause for concern as they may have an equal or higher toxicity than the original PhACs (Andreozzi et al. 2003; Escher and Fenner 2011). Moreover, the efficiency removal might vary depending on the chemical properties and technologies implemented as well as the initial concentrations in the influents (Fatta-Kassinos et al. 2011; Igos et al. 2012; Kohler et al. 2012).

In terms of waste and surface waters, the most studied and detected PhAC belong to the following therapeutic classes: antibiotics, anti-inflammatory, analgesics/antipyretics, lipid regulators, beta blockers, radiocontrast agents, hormones, psychotropic drugs and anticonvulsants. However, higher concentrations of PhAC in wastewater treatment plants (WWTPs) have been measured in special circumstances (Chonova et al. 2016), such as in WWTP effluent from pharmaceutical industries (Hughes et al. 2013; Phillips et al. 2010) or hospital wastewater (Santos et al. 2013).

The motivation behind this study was the need to investigate the occurrence of target PhAC in effluents of WWTP and their removal efficiency along the several steps of Beirolas' WWTP. This study is designed to supplement the initial study on the occurrence of these compounds in raw water and water for human consumption (Gaffney et al. 2014, 2015). Moreover, national data on the profiles of these compounds in water treatment plant influents and effluents are scarce (Pereira et al. 2015; Salgado et al. 2010, 2012; Santos et al. 2013).

The 32 PhACs selected for this study are widely used pharmaceutical compounds that belonged to different therapeutic classes and were selected based on Portuguese consumption data provided by Infarmed (Portuguese Authority of Medicines and Health Products) (Infarmed 2010, 2011, 2014), environmental occurrence (Verlicchi and Zambello 2015), toxicity and persistence in the environment (Brozinski et al. 2013; Lahti and Oikari 2011; Memmert et al. 2013) and compounds proposed for inclusion in the Water Framework Directive (Directive 2013). According to a report from the Inspection General of the Environment and Spatial Planning on the environmental performance of WWTPs in Portugal, 80% of treated effluents are discharged into environmental waters, including surface waters (IGAOT 2004); thus, it is likely that PhACs or their metabolites that are resistant to treatment processes used in the WWTP may be detected in surface waters (Gaffney et al. 2014; Pena et al. 2007). The discharge of waste effluents in surface water is attenuated by the effect of dilution factor responsible for low detected concentrations of these compounds in this type of matrix (Miao and Metcalfe 2003). Other potential factors of reduction of these emerging compounds in surface waters include the adsorption of PhACs on suspended solids, colloids or dissolved organic matter and its subsequent deposition (Osenbrück et al. 2007), photodegradation due to sunlight exposure and also their bioaccumulation and biodegradation (Howard and Muir 2011).

The purpose of the current research was to determine the efficiency of Beirolas WWTP (Lisbon, Portugal) on the removal of target PhACs. Beirolas belongs to Lisboa and Vale do Tejo Company, which is actually managed by EPAL—Empresa Portuguesa das Águas Livres, S.A. This company has two objectives: (1) the collection, treatment and supply of water for public consumption and (2) the collection, treatment and disposal of effluents. The evaluation of the occurrence and removal of PhACs in the different treatments of this WWTP is important to define the best strategies to improve their removal to minimize their impact in the aquatic environmental system and to support future prioritization measures, namely prevent further deterioration and to protect and enhance the water quality and quantity of aquatic ecosystems and surface water.

Materials and methods

Reagents

All standards of PhACs are of analytical grade (highest purity available, ≥ 95%), suitable for analysis by chromatography,

and some of them meet the specifications of the pharmacopoeias (US Pharmacopoeia or USP, British Pharmacopoeia or BP), although belonging to different brands. The standards of 25 PhACs (acetaminophen, clofibric acid, acetilsalicylic acid, atenolol, bezafibrate, carbamazepine, ketoprofen, cortisone, diclofenac, erythromycin, fluoxetine, gemfibrozil, hydrocortisone, ibuprofen, indomethacin, metformine, naproxen, nimesulide, prednisolone, prednisone, propranolol, sulfadiazine, sulfamerazin, sulfamethazine, sulfapyridine) were provided by Sigma-Aldrich; 5 PhACs (caffeine, ciprofloxacin, sulfamethoxazole, sulfathiazole, testosterone) were provided by Fluka and 2 PhACs (metoprolol, oxazepam,) were provided by LGC; all three brands are from Spain.

Individual stock solutions of PhACs were prepared in methanol at 200 mg/L and stored at 5 ± 3 °C in the dark. Two intermediate solutions were prepared in methanol at two concentration ranges: (i) 9–52 mg/L and (ii) 0.17–42 mg/L. Daily, three working solutions prepared in ultrapure water were used (calibration curve).

Oasis HLB (200 mg, 6 mL) cartridges from Waters Corporation (Milford, Massachusetts, USA) were used for solid phase extraction. The organic extracts of SPE were filtered through a cellulose nitrate membrane (0.20 μm, Millipore).

Beirolas WWTP and wastewater sampling

Beirolas WWTP, designed for 213,500 population equivalent (PE), is located in Lisbon by the Tagus River, receiving urban and industrial wastewater from a combined sewage network. In some subcatchments, the flow rates are often increased by a factor of 20 or even higher due to some rainfall events compared with the average dry-weather flow; however, along the sewage network, there are some valves and other flow control devices to prevent damage in the pumping stations and therefore at the WWTP process. The peak plant capacity is 4600 m^3/h with an average daily flow rate of 54,500 m^3/day. The combined sewage is affected by infiltration below 10%, and there are one or two points that could be affected by higher tides but no more than 1.5%.

The wastewater treatment follows the traditional steps (Fig. 1): (i) pre-treatment with mechanical removal of coarse matter (screen, grit chamber and oil/water separators); (ii) primary treatment to remove suspended solids; overflow from the primary clarifiers goes to an equalization tank that prevents the occurrence of significant variations in influent flow rate to the biological treatment and (iii) secondary treatment (biological treatment) by activated sludge process which performs carbon, nitrogen and phosphorus removal.

The biological treatment is performed in a reactor of dispersed biomass by *Bardenpho* technology developed in three stages: (i) anaerobic zone where the highest removal of soluble chemical oxygen demand (COD) is and acts as microorganism selector, (ii) anoxic zone where removal of nitrates occurs (removal by denitrification) and (iii) aerobic zone where the oxidation of remaining COD and ammonia occurs.

After leaving the *Bardenpho* reactor, the effluent flows to secondary clarifiers where the sedimentation of biological sludge occurs and the sludge recirculation rate is 100%. The bioreactor aeration is a fine bubble aeration system to achieve large interfacial area between air and water and therefore sufficient oxygen mass transfer, with a sludge age of 12 days (solids retention time or SRT) in the winter (assuming a minimum temperature of 15 °C for wastewater) and 8 days in the summer (23 °C temperature) and with a concentration of liquid mixed suspended solids (MLSS) of 2–3 g/L. The hydraulic retention time (TRH) of the bioreactor is 10.3 h.

Effluent flows through sand filters, where the remaining fine solids are removed, and is then disinfected using ultraviolet light to be reused inside the plant and in other urban uses such as washing streets and watering trees, among others. The remaining effluent (more than 95% in volume) is discharged into the Tagus river estuary.

In Beirolas WWTP, the effluent of the screening chamber and influent before the disinfection unit were considered as the influent (WWI) and effluent (WWE), respectively. Therefore, sampling points were chosen in order to verify the efficiency of the wastewater treatment and to identify the potential influence of treated wastewater effluent on the quality of receiving waters.

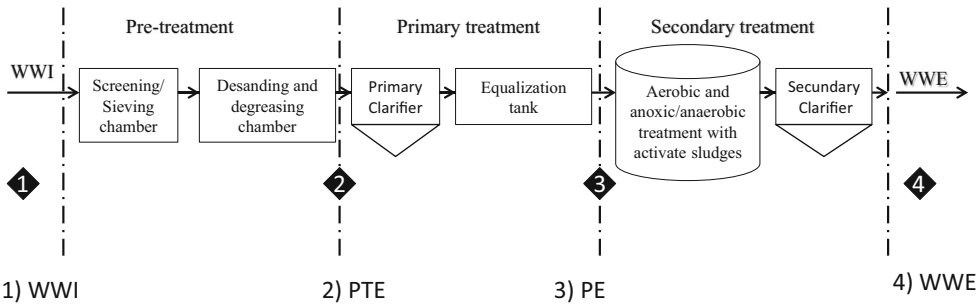

Fig. 1 Schematic diagram of the treatment processes in Beirolas WWTP and sampling points and its identification (*black diamond*)

Twelve sampling campaigns were performed between autumn 2013 and spring 2014: five sampling periods in autumn (15 and 29 October, 11 and 26 November, 11 December), four sampling periods in winter (7 and 21 January, 18 February, 11 March) and three sampling periods in spring (25 March and 15 and 29 April). Summer sampling was not performed due to logistic problems, such as personnel availability and production planning during the summer holiday period. Daily composite samples were obtained by mixing equal sample volumes (400 mL) collected every 1 h during 24 h (total volume of 9.6 L).

Four sampling points in each sampling period were controlled: WWI, PTE (pre-treatment effluent, grit and fat removal), PE (effluent of primary treatment) and WWE. The characterization of these samples during the 12 sampling periods is shown in Table S1 (Supporting information).

A total of 48 samples were analysed by SPE-ESI-UPLC-MS/MS at acid and basic conditions.

There are no specific sampling methods for PhAC. However, there are specific sampling methods for other organic contaminants also present in trace concentrations such as pesticides. This method was selected because it applies to organic compounds of a wide chemical nature (Clesceri et al. 2005). The sample is collected in accordance with their principles, using a glass amber bottle equipped with a screw cap having a TFE-fluorocarbon liner. The sample containers were not overfilled. Replicate samples were collected for replicate analysis. Upon reception, samples were acidified with hydrochloric acid (37%) to pH 2, vacuum filtered through a 1.0-μm glass-fibre filter Type 2 (Millipore, Sigma-Aldrich) followed by a 0.45-μm cellulose nitrate membrane (Millex 0.45 μm, Millipore) and stored at 5 ± 3 °C until analysis, which occurred within 7 days. Therefore, the PhAC concentration corresponds to their dissolved fraction.

SPE–LC–MS/MS analysis

One hundred microlitres of each sample was extracted by solid-phase extraction (SPE) with Oasis HLB cartridges using an automated AutoTrace[a] SPE workstation (Thermo Scientific), after addition of 400 μL of a 5-mg/L EDTA solution. The water was passed through the wet cartridges at a flow rate of 10 mL/min, the cartridges rinsed with 3 ml water (5 mL/min) and dried for 15 min using nitrogen. Analytes were then eluted with 8 mL of methanol in two 4 mL elution steps. This extract was filtered through a cellulose nitrate membrane of 0.20 μm, evaporated to dryness under a gentle stream of nitrogen (5 psi/35 °C) and reconstituted with 500 μL of ultra-pure water (Gaffney et al. 2014, 2015).

The LC–MS/MS analysis of PhAC was performed using a Waters UPLC Acquity system from Waters equipped with a binary pump, an automatic injector and a thermostated column compartment coupled to a mass spectrometer Quattro micro-API triple quadrupole and Aquity TDQ equipped with a Z-spray electrospray interface (Micromass, UK). Chromatographic separation was achieved with an Acquity BEH C18 column (2.1 × 50 mm, 1.7 μm) from Waters. The tandem mass spectrometer was operated with electrospray ionization (ESI) in positive and negative ionization modes using multiple reaction monitoring mode. The optimized and previously validated UPLC–MS/MS methods were adapted (Gaffney et al. 2014, 2015). The main changes were related to mobile phases, based on their pH, in order to achieve an adequate chromatographic profile and the best sensitivity possible for each PhAC.

A mixture of water-formic acid (acid method) and water-ammonium (basic method) was used for mobile phase A, and methanol was used for mobile phase B, both in different proportions (Gaffney et al. 2014, 2015).

During the recovery studies a strong matrix effects due to the use of electrospray as ionization technique was detected. The use of internal standards, the common practice used to compensate for matrix effects known to occur in ESI, could not be used in this study due to the number of pharmaceuticals analysed and to the fact that the percentage of matrix effects observed was not the same for all the compounds, even for pharmaceuticals belonging to the same therapeutical class. As so, the standard addition method was used to compensate for the ESI effects, where for each sample a matrix-matched calibration curve was used. The calibration curves were prepared by extracting 100 mL of the respective sample, evaporating the extract to dryness and re-dissolving it in 500 μL of ultra-pure water. Matrix-matched calibration curves with a minimum of 4 calibration points were constructed by adding 75 μL of the sample to 75 μL of each of the standard solutions prepared in ultra-pure water or 75 μL of ultra-pure water, in case of the non-spiked calibration point.

In order to use the standard addition method, the range of the calibration curve was within the linearity interval and the maximum spiking concentration used was two to five times the concentration of the pharmaceutical in the sample. The concentration (Cs) of the PhAC was estimated by extrapolation by applying Eq. (1):

$$C_s = \frac{a - y_b}{b \times CF \times Rec} \quad (1)$$

where a is the y-axis intercept of the calibration function, y_b is the signal of the blank control, b is the slope of the calibration unction, CF is the concentration factor (200 times) and Rec is the mean recovery of the pharmaceutical in the different wastewater matrices.

All instrumental and method validation parameters such as linearity and range, precision, accuracy and detection and quantification limits were determined. A detailed discussion of the methods and their validation is presented elsewhere (Gaffney et al. 2014, 2015). For quality and assurance purposes, at least one blank control (BC), two standard control

(SC), one duplicate (DD) and one recovery assay (REC) were performed for each batch of samples (daily sampling and chromatographic run) and they fulfilled the acceptance criteria defined in the laboratory for the analysis of trace organic compounds in water matrices (BC ≤ LOQ, standard error of SC ≤ 15%, DD ≤ 15%, and Rec = 100 ± 25%) (EC 1998). The determination coefficients (r^2) of calibration curves were over 0.995, and the coefficients of variation of the method (CV_m) were lower than 5%.

In this study, all PhAC concentrations lower than its limit of detection were represented by not detected (n.d.).

Calculation of removal efficiency

The removal efficiency of PhAC from WWTP was estimated by Eq. (2), assuming a constant WWTP influent and effluent flow rate, equal to the average daily flow rate and influent and effluent concentrations corresponding to their average daily values (based on 24-h composite water samples):

$$\text{Efficiency}\ (\%) = \frac{C_{inf} - C_{eff}}{C_{inf}} \times 100 \quad (2)$$

The C_{inf} is the average PhAC concentration measured in the influent, and C_{eff} is the average PhAC concentration measured in the effluent (the influent and effluent depend of each treatment step).

Statistical analysis

The statistical analysis was applied using Statistical Analysis Software IBM SPSS 22. Because data from PhAC concentration in each wastewater and in each season (autumn, winter and spring) were non-normally distributed and had non-homogeneous variances, we performed non-parametric statistical analysis by Kruskal–Wallis ANOVA to evaluate the differences of PhAC concentration or pharmaceutical class between seasons. To evaluate the differences between the removal efficiencies of PhAC, we performed a general linear model (GLM) using two fixed factors (season and step treatment) and the PhAC or pharmaceutical class as dependent variables. Both tests were performed with a confidence level of 95%.

Results and discussion

All figures and tables show the PhACs grouped in alphabetical order within their therapeutical classes. All data results are shown in Table 1. First, the discussion is focused on concentrations and detection frequencies of PhAC in the influent and effluent of Beirolas WWTP. Then, the removal efficiency of the different treatments is discussed in a separate section.

Occurrence and frequency

During a period of a 7-month monitoring programme, acetaminophen, diclofenac, ibuprofen, atenolol, metformin, carbamazepine and caffeine were found in all WWTP's influents analysed. However, only acetaminophen, metformin and caffeine were detected at high levels, with concentrations between 55 and 623 μg/L, between 70 and 325 μg/L and between 49 and 273 μg/L, respectively. Eight PhACs were not found in WWI (nimesulide, sulfadiazine, sulfamerazine, sulfamethazine, sulfathiazole, clofibric acid, prednisone and prednisolone). The remaining PhACs were quantified in collected samples with a frequency between 8 and 92%. The lowest frequencies (<50%) belonged to testosterone (8%), fluoxetine (17%), oxazepam (42%) and hydrocortisone (33%).

In WWE, other four PhACs were not detected (acetylsalicylic acid, ibuprofen, testosterone, hydrocortisone) because they were fully removed during the treatment.

The concentration range found both in WWI and WWE samples demonstrated that they are similar to those from other studies (Santos et al. 2013; Sousa et al. 2011; Verlicchi et al. 2012; Verlicchi and Zambello 2015). Also, the analgesic/antipyretic and non-steroidal anti-inflammatory drug (NSAID) therapeutic classes were the ones with the highest WWI concentration levels, as in other studies published (Kasprzyk-Hordern et al. 2009; Nebot et al. 2015; Salgado et al. 2010; Santos et al. 2013; Sim et al. 2010; Sousa et al. 2011; Verlicchi et al. 2012; Verlicchi and Zambello 2015).

Analgesic/antipyretic and NSAIDs

The variability of analgesics/antipyretics and NSAIDs in WWI were found to range between 0.46 and 623 μg/L; however, some PhACs of these classes were not detected in some samples (concentration lower than its detection limit). Relating to the most commonly investigated compounds (acetaminophen, diclofenac, ketoprofen, ibuprofen and naproxen), the major compounds are the same with those in other studies (acetaminophen and ibuprofen) but the order of prevalence of these major compounds (acetaminophen higher than ibuprofen) in WWI is consistent with some studies (Kasprzyk-Hordern et al. 2009; Nebot et al. 2015; Pereira et al. 2015; Sim et al. 2010), but differ from other studies (Gros et al. 2010; Santos et al. 2013; Sousa et al. 2011; Verlicchi et al. 2012).Usually, diclofenac is the third most abundant compound of this group but in this occurrence study, this place was occupied by naproxen.

Acetaminophen was the compound with the highest registered median influent concentration (118 μg/L), followed by ibuprofen (22 μg/L), naproxen (7.9 μg/L) and diclofenac (2.5 μg/L), with detection frequencies higher than 90%. These concentration values were higher than other Portuguese reported values for acetaminophen (ranging from

Table 1 Concentrations of PhAC in wastewaters of Beirolas WWTP calculated for samples collected over the period of the 7-month monitoring programme (October 2013–April 2014)

PhAC	Concentration (μg/L)							
	WWI		PTE		PE		WWE	
	Min–max (Med)	%	Min–max (Med)	%	Min–max (Med)	%	Min–max (Med)	%
Analgesics/Antipyretic								
Acetaminophen	55–623 (118)	100	50–517 (81)	100	0.02–302 (74)	100	n.d.–0.05 (0.01)	17
Acetilsalycilic acid	n.d.–63(17)	75	n.d.–63 (7.1)	42	n.d.–19 (1.8)	17	n.d.	0
Non-steroidal anti-inflammatory drugs (NSAID)								
Diclofenac	0.46–6.5 (2.5)	100	0.42–5.1 (2.3)	100	0.54–5.4 (1.9)	100	0.05–4.2 (1.5)	100
Ketoprofen	n.d.–1.7 (0.10)	67	n.d.–1.5 (0.05)	67	n.d.–1.4 (0.04)	67	n.d.–0.72 (0.01)	50
Ibuprofen	8–53(22)	100	6.8–42 (18)	100	6.2–39 (16)	100	n.d.	0
Indomethacine	n.d.–0.36 (0.15)	83	n.d.–0.32 (0.14)	83	n.d.–0.27 (0.12)	83	n.d.–0.2 (0.12)	83
Naproxen	n.d.–38 (7.9)	92	n.d.–34 (7.3)	92	n.d.–27 (8.2)	92	n.d.–3.3 (0.95)	92
Nimesulide	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Beta blocker								
Atenolol	0.57–2.9 (1.1)	100	0.25–2.0 (0.81)	100	0.26–1.2 (0.78)	100	0.22–0.69 (0.28)	100
Metoprolol	n.d.–1.1 (0.06)	83	n.d.–1.1 (0.06)	83	n.d.–1.1 (0.04)	83	n.d.–0.09 (0.05)	83
Propanolol	n.d.–0.49 (0.21)	92	n.d.–0.45 (0.22)	92	n.d.–0.39 (0.19)	92	n.d.–0.42 (0.17)	92
Antidiabetic								
Metformin	70–325 (208)	100	70–339 (203)	100	66–324 (192)	100	0.05–58 (1.7)	100
Anticonvulsionants								
Carbamazepine	0.82–6.5 (1.5)	100	0.58–6.0 (1.3)	100	0.46–2.2 (0.88)	100	0.32–1.6 (0.63)	100
Antidepressants								
Fluoxetine	n.d.–0.05 (0.003)	17	n.d.–0.05 (0.01)	17	n.d.–0.05 (0.01)	17	n.d.–0.03 (0.01)	17
Oxazepan	n.d.–1.7 (0.3)	42	n.d.–1.5 (0.3)	42	n.d.–1.9 (0.3)	42	n.d.–2.1 (0.3)	42
Antibiotics								
Ciprofloxacin	n.d.–4.2 (2.0)	75	n.d.–3.9 (1.9)	75	n.d.–3.6 (1.6)	75	n.d.–1.4 (0.35)	75
Erythromycin	n.d.–2.3 (0.5)	67	n.d.–2.8 (0.68)	75	n.d.–2.9 (0.62)	75	n.d.–2.78 (0.51)	75
Sulfadiazine	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Sulfamerazine	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Sulfamethazine	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Sulfamethoxazole	n.d.–5.3 (2.2)	92	n.d.–5.0 (1.3)	83	n.d.–4.9 (1.2)	83	n.d.–2.0 (0.69)	92
Sulfapyridine	n.d.–2.3 (0.5)	67	n.d.–1.6 (0.40)	67	n.d.–1.4 (0.43)	67	n.d.–1.5 (0.28)	67
Sulfathiazole	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Lipid regulators								
Clofibric acid	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Bezafibrate	n.d.–4.7 (1.1)	92	n.d.–4.7 (0.76)	92	n.d.–2.0 (0.50)	92	n.d.–0.52 (0.15)	92
Gemfibrozil	n.d.–1.3 (0.5)	83	n.d.–0.98 (0.52)	83	n.d.–0.97 (0.39)	83	n.d.–0.64 (0.09)	83
Sexual Hormones								
Testosterone	n.d.–0.19 (0.016)	8	n.d.–0.15 (0.01)	8	n.d.	0	n.d.	0
Corticosteroids								
Cortisone	n.d.–0.31 (0.035)	58	n.d.–0.31 (0.025)	50	n.d.–0.13 (0.025)	42	n.d.–0.09 (0.01)	8
Hidrocortisone	n.d.–0.33 (0.063)	33	n.d.–0.25 (0.05)	33	n.d.–0.22 (0.04)	33	n.d.	0
Prednisolone	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Prednisone	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Psychostimulants								
Caffeine	49–273 (117)	100	43–236 (96)	100	1.6–229 (83)	100	n.d. - 2.9 (0.49)	92

Min the lowest recorded concentration, *Max* the highest recorded concentration, *Med* median concentrations, % frequency of detection (percentage of samples with concentration higher or equal of LOD), *n.d.* not detected

0.08 to 9.3 μg/L), ibuprofen (ranging from not detected to 4.9 μg/L), naproxen (ranging from 0.09 to 1.6 μg/L) and diclofenac (ranging from not detected to 0.27 μg/L) (Santos et al. 2013); nonetheless, similar values were reported for WWE in other reports (Loos et al. 2013; Sousa et al. 2011; Verlicchi et al. 2012).

Our data may be correlated with the quantity of these target PhACs sold in Lisbon during 2013. In 2013, the sales reported by Infarmed in the district of Lisbon were 40,952 kg (acetaminophen), 18,710 kg (ibuprofen), 2894 kg (naproxen), 1198 kg (diclofenac), 916 kg (nimesulide), 73 kg (indomethacine) and 55 kg (ketoprofen) (Infarmed 2013). These data justify the high levels of acetaminophen found in the WWI. Additionally, this PhAC is sold without prescription and no data exists concerning non-prescription consumption (Infarmed 2010, 2011, 2013).

The other analgesic/antipyretic and NSAIDS such as acetylsalicylic acid, diclofenac, ketoprofen, ibuprofen,

indomethacine, naproxen and nimesulide were quantified at lower levels due to their lower usage in the community. However, although NSAIDS were in lower concentrations, their frequency in the WWE is high due to insufficient wastewater treatment.

Beta blocker and anti-diabetics

All three beta-blockers were found to be ubiquitous as they were quantified in samples at a higher representation than 80% (Table 1). Their median concentrations and frequencies in WWI decrease in the following order: atenolol (1.1 μg/L, 100%), propranolol (0.21 μg/L, 92%) and metoprolol (0.06 μg/L, 83%), and they are strongly correlated with the amount of PhACs dispensed in community (Infarmed 2013) and also due to their pharmaceutical nature. In 2013, the amount sold in Lisbon district was 351, 317 and 173 kg of atenolol, propranolol and metoprolol, respectively (Infarmed 2013). The median concentrations and frequencies in WWE obey the same order: atenolol (0.28 μg/L, 100%), propranolol (0.17 μg/L, 92%) and metoprolol (0.05 μg/L, 83%), but their values were lower than in WWI, especially for atenolol. These values were similar to those obtained by Verlicchi et al. (2012) but higher than the values obtained in the WWI analysed by Santos et al. (range values for atenolol, propranolol and metoprolol of 0.36–0.75, 0.003–0.024 and n.d.–0.015 μg/L, respectively) (Santos et al. 2013). Atenolol was also reported in another Portuguese study with concentrations of 0.065–0.48 and 0.119–1.3 μg/L in WWI and WWE, respectively (Salgado et al. 2010).

The anti-diabetic, metformin, belongs to the group of the 100 PhACs most sold in ambulatory sales on the Portuguese market (Infarmed 2013), with a total of 37,473 kg sold in Lisbon. After acetaminophen, it corresponds, in mass, to the second PhAC most sold in the district of Lisbon (Gaffney et al. 2014; Infarmed 2013). Therefore, it is natural that its concentration in the influent was high (70–325 μg/L) with a frequency of 100% both in WWI and WWE. Moreover, this PhAC represents the second highest absolute concentration in WWI with a concentration of 325 μg/L. However, there was a strong decrease of its range concentration in WWE (0.05–58 μg/L). This behaviour was similar to that obtained by Santos et al. (2013).

Anticonvulsants, antidepressants and psychostimulants

Carbamazepine has been found in WWTP effluents around the world. Its concentration in effluents are usually around hundreds of nanograms per litre, but can sometimes occur in micrograms per litre, with different values in several countries and even in different regions of the same country due to their different PhAC consumption (Ternes 1998; Zhang et al. 2008). The amount sold in Lisbon district was 1507 kg/year (Infarmed 2013). Carbamazepine was found in all WWI and WWE (100% of positive samples) with range concentrations of 0.82–6.5 and 0.32–1.6 μg/L, respectively. The highest concentration in WWE (1.6 μg/L) is lower than the highest concentration reported by Verlicchi et al. (20 μg/L) (Verlicchi et al. 2012) and other European studies (up to 4.3 μg/L) (Loos et al. 2013) but higher than the results retrieved by other Portuguese studies, where lowest concentration values were found in WWE (up to 0.5 μg/L) (Salgado et al. 2010; Santos et al. 2013). There are also reports of a slight increase when comparing carbamazepine concentration levels of WWTP influents and effluents, as a result of cleavage of the glucuronide conjugate (Vieno et al. 2006).

Benzodiazepines are effective anxiolytic and hypnotic drugs but they are also indicated as adjuvants to anaesthesia, muscle relaxation and anticonvulsive. These drugs, including diazepam, bromazepam, alprazolam, lorazepam and oxazepam, are widely investigated as potential environmental contaminants due to their high consumption globally (Koplin et al. 2002).

Benzodiazepines have generally a low rate of excretion of the unchanged compound, wherein one of the main metabolites of these drugs is oxazepam (Ruhoy and Daughton 2008). Therefore, this compound is probably one of the main compounds of this group in wastewaters and it was monitored as the main metabolite for several benzodiazepines and also as parent compound. Antidepressants are also prescription drugs (Khetan and Collins 2007). Regarding the risk for the aquatic environment, fluoxetine was classified as having a medium risk to the environment and it was also monitored (Daughton 2008; Kosma et al. 2010; Verlicchi et al. 2012).

The highest contamination level for these two antidepressants in WWI reached up to 1.7 μg/L (oxazepam) and 0.05 μg/L (fluoxetine), with median concentrations of 0.3 and 0.003 μg/L, respectively. The maximum concentration of oxazepam is in agreement with the values reported by Loos et al. (maximum of 1.8 μg/L) (Loos et al. 2013). The maximum concentration of fluoxetine in WWI is similar to values obtained by Santos et al. (2013) in WWTP influent (0.029 μg/L) of Coimbra city (centre of Portugal) but lower to the values registered by Verlicchi et al. (2012).

Caffeine was one of the PhACs most found in WWI with a maximum concentration of 273 μg/L and a median concentration of 117 μg/L. Its frequency in WWI was 100% and it was similar in the WWE (92%). However, the concentration in WWE reached up to 2.9 μg/L. These concentrations are higher than those reported by European studies (up to 36 μg/L) (Loos et al. 2013; Salgado et al. 2010), probably due to the high consumption of drinks containing caffeine in this region.

Antibiotics

The antibiotics showed widespread frequencies in WWI, with sulfamethoxazole having the highest frequency (92%),

followed by ciprofloxacin (75%), erythromycin (67%) and sulfapyridine (67%). The average contamination level for these PhACs reached up to 5.3 μg/L (sulfamethoxazole), 4.2 μg/L (ciprofloxacin), and 2.3 μg/L (erythromycin and sulfapyridine), with median concentrations of 2.2, 2.0, 0.5 and 0.5 μg/L, respectively. The other antibiotics analysed were below the detection limit. The frequency data in WWE were quite similar to those obtained in WWI, but the highest concentration and the median concentrations were lower, with exception of erythromycin. The antibiotics with the highest median concentration (sulfamethoxazol and ciprofloxacin) were those most frequently detected in WWI but not in WWE. The second major median concentration belongs to erythromycin.

The order of abundance of sulfamethoxazol, ciprofloxacin and erythromycin in WWI corresponds very well to other reports (Santos et al. 2013; Verlicchi et al. 2012), but lower range concentrations of these three PhACs were reported by these authors, namely 0.53–1.6, 0.10–0.30 and 0.009–0.22 μg/L for sulfamethoxazol, ciprofloxacin and erythromycin, respectively (Santos et al. 2013).

Lipid regulators

Concerning lipid regulators, clofibrid acid was not detected in any of the samples (WWI and WWE), and the frequencies of the other two compounds were 92 and 83% for bezafibrate and gemfibrozil in both samples (WWI and WWE), respectively. The maximum concentration of bezafibrate and gemfibrozil in WWI and WWE were 4.7 and 1.3 μg/L and 0.52 and 0.64 μg/L, respectively. Bezafibrate, as reported by other authors, was the most significant compound of this therapeutic class in WWI. In Lisbon, the consumption of both PhACs was similar, 291 kg of bezafibrate and 336 kg of gemfibrozil (Infarmed 2013). Its maximum concentration in WWI was lower than the value reported by Gros et al. in Spain (40 μg/L) (Gros et al. 2010) and the ones reported in other European countries (up to 9 μg/L) (Verlicchi et al. 2012), but higher or in agreement with those reported in Portuguese studies, up to 1.3 μg/L and up to 5.2 μg/L, respectively (Pereira et al. 2015; Santos et al. 2013; Sousa et al. 2011).

Sexual hormones

Androgenic steroids, such as testosterone, are used due to their high androgenic activity and indicated as replacement therapy for male hypogonadism and the main androgenic hormone used in this pathology, as well as in the delayed puberty of boys, osteoporosis and in some post-menopausal breast carcinomas (Infarmed 2010). Therefore, it is not a PhAC with high prescription (6 kg per year in 2013) (Infarmed 2013). Its presence in wastewater treatment plant is mainly due to its urinary elimination by man, and so, it should be a substance present in urban sewage and consequently in the influent of any WWTP. However, testosterone is usually not monitored in influents and effluents of WWTP. The results of this study, contrary to our expectations showed low frequency detection (8% in the influent), and the maximum concentration found in affluent was 0.19 μg/L, although the median was 0.016 μg/L. Moreover, testosterone was not detected in the effluent. It is assumed that this compound is strongly absorbed by organic matter or biodegraded by microorganisms present in wastewater (Yang et al. 2010).

Corticosteroids

Corticosteroids are usually not included in the list of PhACs to be monitored, whereby occurrence data relating to these compounds is scarce. Cortisone, prednisone, prednisolone and hydrocortisone were included in the list of PhACs to be monitored in this study, to evaluate their occurrence and fate in the WWTP. Only cortisone and hydrocortisone were found in WWI but with frequencies of 58 and 33%, respectively. Its median concentrations were 0.035 and 0.063 μg/L, respectively. Their presence in the WWE is negligible, either in concentration (up to 0.09 μg/L of cortisone) or in frequency detection (8% for cortisone).

Seasonal variation

The distribution profile for the majority of the therapeutic classes in these three seasons (Fig. 2) was similar in influent and effluents of WWTP during the first and second treatment stages (pre-treatment and primary treatment). However, it had a consistent variation in the final effluent of the WWTP (after secondary treatment), either on the total concentration of PhAC in each therapeutic class or in the most significant therapeutic class on each season. These changes are due to an increase in removal efficiency in the secondary treatment and also due to removal variability, inherent to each therapeutic class.

However, the differences between seasons are not significant and as the study did not include samples from the summer season, due to some logistic problems, the results reported here were different from the ones obtained by other authors (Golovko et al. 2014a, b; Sun et al. 2014).

In the influent, the mean concentration of analgesics/antipyretics is greater in winter (157 ± 211 μg/L), followed by concentrations obtained in autumn (81 ± 100 μg/L) and in spring (79 ± 65 μg/L). This distribution is characteristic of therapeutic classes whose consumption rate varies with weather conditions; in this case, increases with decrease in temperature probably due to an increase of flu, colds and febrile seizures.

Antibiotics, NSAIDs, anti-diabetics, beta-blockers and psychostimulants have higher concentrations in influent

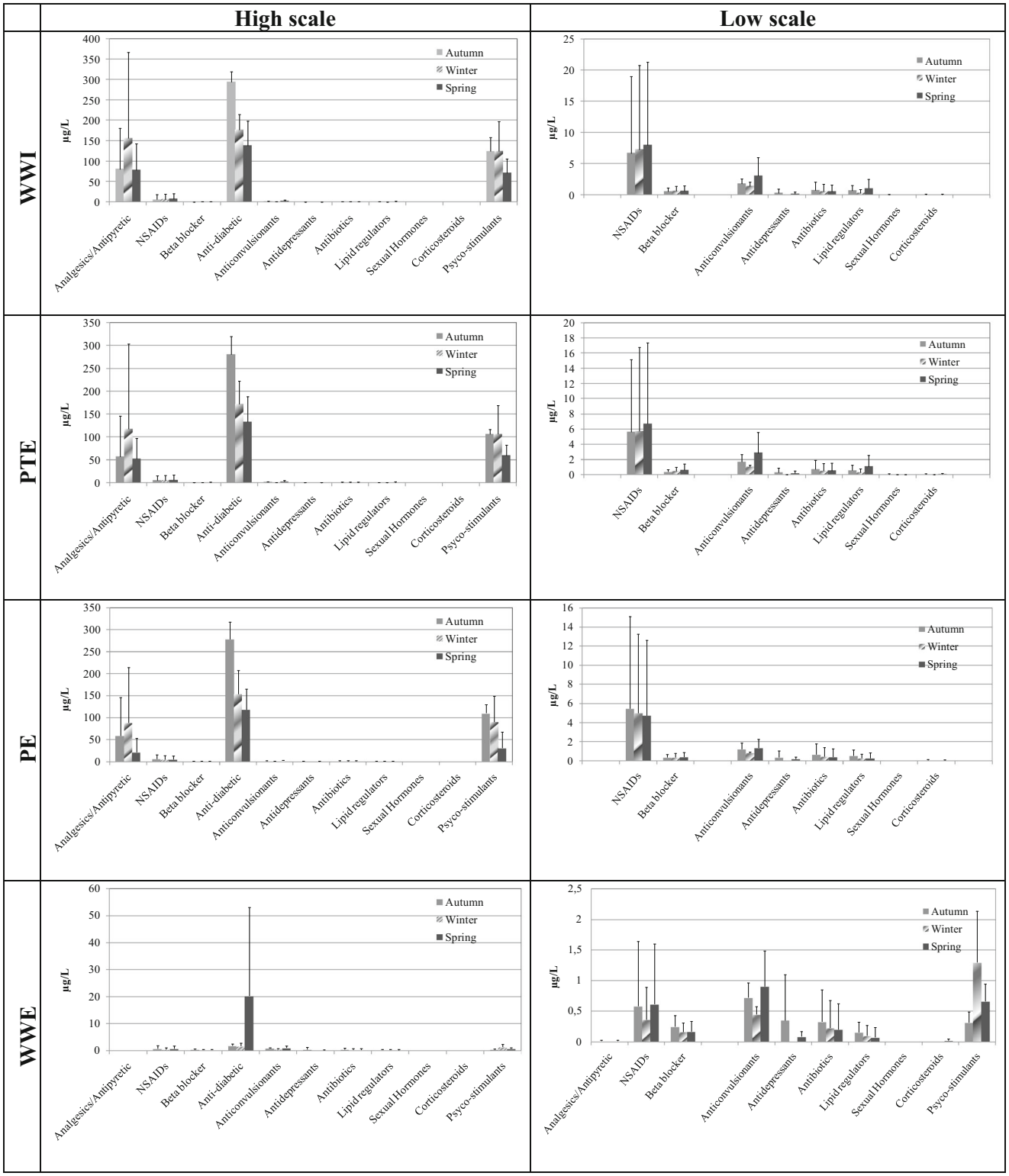

Fig. 2 Variability on mean concentrations of several pharmaceutical classes of PhAC in wastewaters of Beirolas WWTP collected in all sampling points during three seasons: autumn (n = 5), winter (n = 4) and spring (n = 3). The results were represented in two scales: high scale and low scale

during autumn, followed by winter and spring. These differences were more marked in antibiotics and anti-diabetics (represented exclusively by metformin). The concentration of metformin is 295 ± 26, 178 ± 38 and 139 ± 60 µg/L in autumn, winter and spring, respectively. The concentration of antibiotics is 0.80 ± 1.3, 0.59 ± 1.2 and 0.57 ± 1.1 µg/L in autumn, winter and spring, respectively.

In spring, the anticonvulsants had a higher concentration in the influent, although the concentrations obtained in the autumn and winter were similar, 3.1 ± 3.0, 1.8 ± 0.8 and 1.4 ± 0.6 μg/L, respectively.

In winter, the lipid regulators had a lower concentration in the influent, followed by those obtained in autumn and spring: 0.81 ± 0.5, 1.0 ± 0.8 and 1.6 ± 1.5 μg/L, respectively.

Figure 3 shows the concentration of PhACs in Beirolas influent in samples collected in the three seasons. The highest concentrations for the analgesic/antipyretic drugs obtained in winter were due to the concentrations obtained for acetaminophen because the concentration of acetylsalicylic acid was quite similar in all seasons. There was an increase of concentration of this PhAC during the fifth to seventh sampling period (11 December, 7 January and 21 January).

The seasonal variations of PhAC in NSAIDs in the influents were not similar (Fig. 3). Diclofenac and indomethacine did not show seasonal variations, but the concentration of ketoprofen and naproxen in the influent samples was higher in the fifth to sixth sampling periods (11 November and 7 January) compared to the samples collected in the other sampling periods.

The influent concentration of metformin was high during all seasons, and these data are in agreement with the prescription and use patterns of anti-diabetics because they are used for therapeutic reasons all over the year. However, metformin shows a slight decrease in spring. This fact, as observed for other PhACs, can be explained by the relative high water consumption in the warm season which may dilute the PhAC concentration in the urine and, consequently, to a reduction in the influent of WWTP.

The measured concentrations of all antibiotics in the influent and effluent showed a noticeable seasonal pattern (Fig. 3), with concentrations being lowest during spring (not detected or 0.21 μg/L for erythromycin) and peaking in winter (5.3 μg/L for sulfamethoxazole).

Concurrently, elevated concentrations of sulfamethoxazol and ciprofloxacin were found in the influent samples during the winter season. Erythromycin was found almost exclusively in winter season samples with concentrations up to 2.3 μg/L. Sulfapyridine was found almost exclusively in autumn season samples with concentrations up to 1.8 μg/L.

The influent concentration of caffeine was approximately the same during all seasons.

The highest concentrations for the beta blocker drugs obtained in winter were due to the concentrations obtained for atenolol and metoprolol because the concentration of propranolol was quite similar in all seasons. The maximum concentration of atenolol and metoprolol were found in 6 January, with a concentration of 2.9 and 1.1 μg/L, respectively.

The maximum concentrations of lipid regulators bezafibrate and gemfibrozil were higher in autumn and spring (up to 4.7 and 1.3 μg/L, respectively) than in winter (up to 1.7 and 1.3 μg/L, respectively).

The concentration of corticosteroids in influent was much lower than of the other therapeutic class' drugs, and they were not found in winter. Hydrocortisone was only detected in samples taken in autumn, and cortisone showed maximum concentrations in the last three spring samplings: 0.22, 0.23 and 0.31 μg/L, respectively. In spring, due to pollination, the cases of allergic rhinitis increase and this may justify the increased consumption of corticosteroids, and thus the increase of concentration of these compounds in the influent.

Figure 4 shows the concentration of PhACs in Beirolas effluent during the three seasons under study. PhACs in effluent were one to three orders of magnitude lower than those from influent. These results are a function of removal efficiency for each PhAC, which does not seem to have a uniform behaviour for PhACs within the same therapeutic class. The profile of PhAC in effluent will be discussed in parallel with the removal efficiency.

A snapshot of the seasonal variation of PhAC in Beirolas WWTP indicates that only some compounds belonging to some pharmaceutical classes, such as NSAIDs and antibiotics, showed a seasonal profile; their concentration in influent were higher during autumn and winter than during spring, which was attributed to increased human consumption of these PhACs during this period. The remaining PhACs did not show a dominant seasonal profile.

Removal efficiencies

Figure 5 shows the fate of the target pharmaceuticals grouped by their therapeutic classes along the different treatment processes in Beirolas WWTP (pre-treatment, primary treatment and secondary treatment). The total removal efficiency (or overall treatment efficiency) was also estimated for PhACs (individual or grouped by therapeutical classes) using the concentrations of target compounds in WWI (C_{inf}) and WWE (C_{eff}) of WWTP.

Significant differences between treatment steps in WWTP were observed, although no significant differences were observed between pre-treatment and primary treatment. Therefore, biological treatment by activated sludge played an important role in the elimination of PhACs because it was the most efficient treatment on the removal of the majority of PhACs.

These behaviours in pre-treatment and primary treatments were also observed in other studies (Verlicchi et al. 2012; Zorita et al. 2009), and in some cases, parent compounds may even be released during the process, probably caused by the simultaneous presence of deconjugable substances, that is, human metabolites, of these compounds in the raw influent (Carballa et al. 2004, 2005).

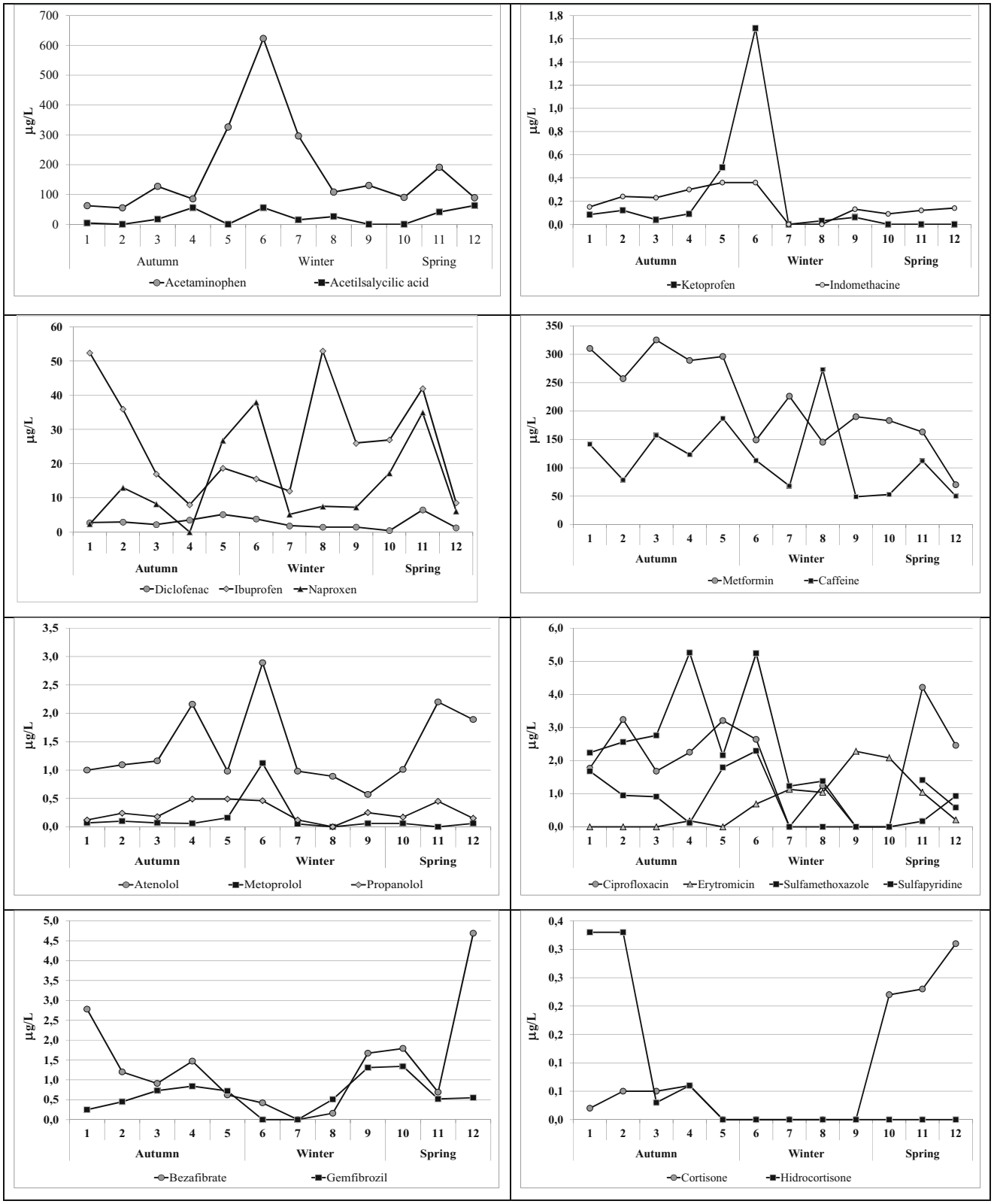

Fig. 3 Variability on concentrations of target PhAC in influent of Beirolas WWTP in 12 campaigns during three seasons: autumn, winter and spring

Except for testosterone (the single sexual hormone studied) with a removal efficiency (RE) of 100%, the removal efficiencies after pre-treatment ranged from 2.2% (anti-diabetics/metformin) to 84.2% (corticosteroids), with a removal efficiency of 16.3% for all target compounds belonging to these 11 therapeutic classes (16.3 and 30.6% for median and mean,

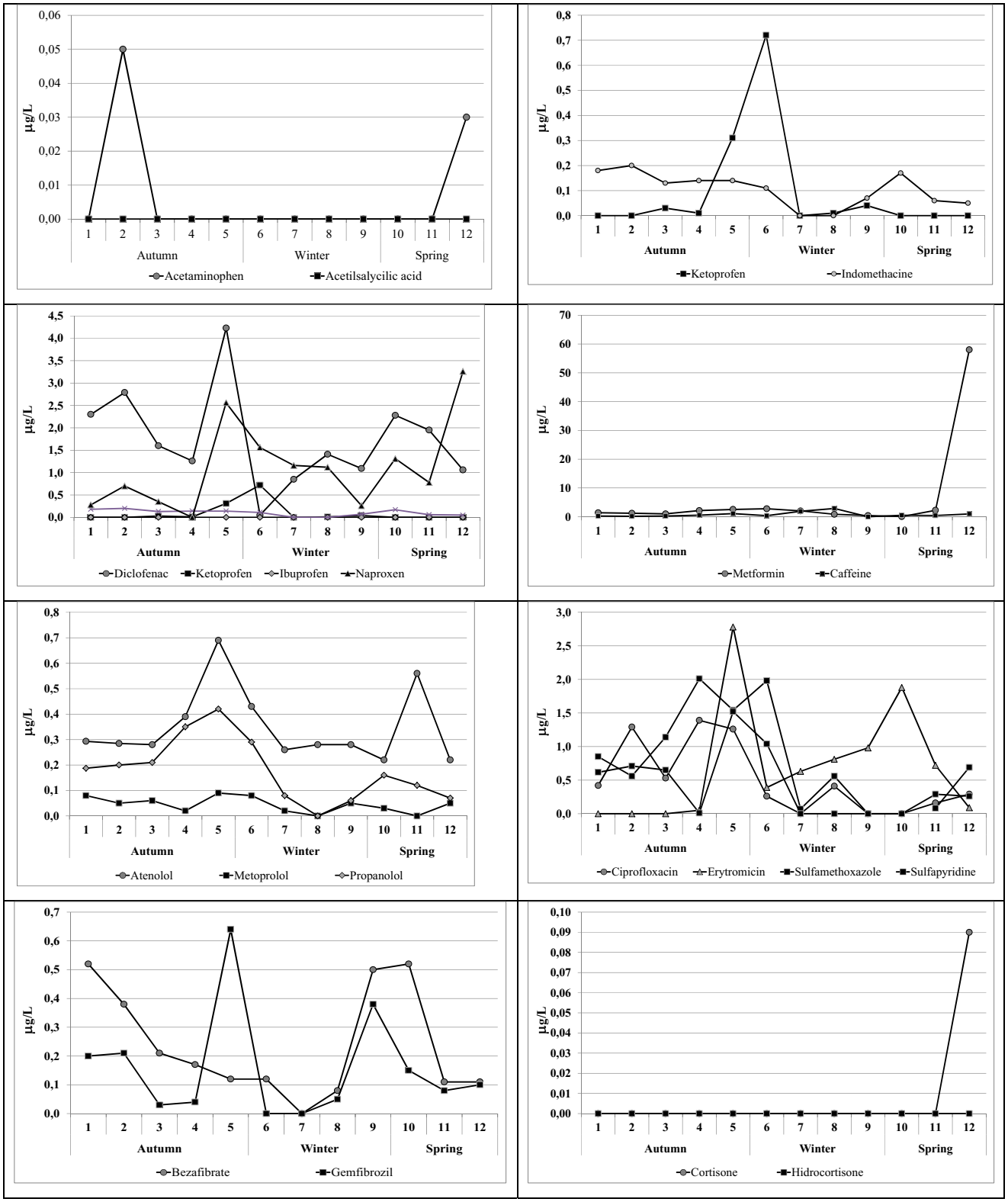

Fig. 4 Variability on concentrations of target PhAC in effluent of Beirolas WWTP in 12 campaigns during three seasons: autumn, winter and spring

respectively). Testosterone was completely removed in this phase, although its initial concentration was much lower (0.02 µg/L) than the remaining PhACs. The majority of the therapeutic classes had removal efficiencies lower than 30% (Fig. 5) after pre-treatment (NSAIDs, beta blockers, anti-diabetics, anticonvulsants, antidepressants, antibiotics, lipid

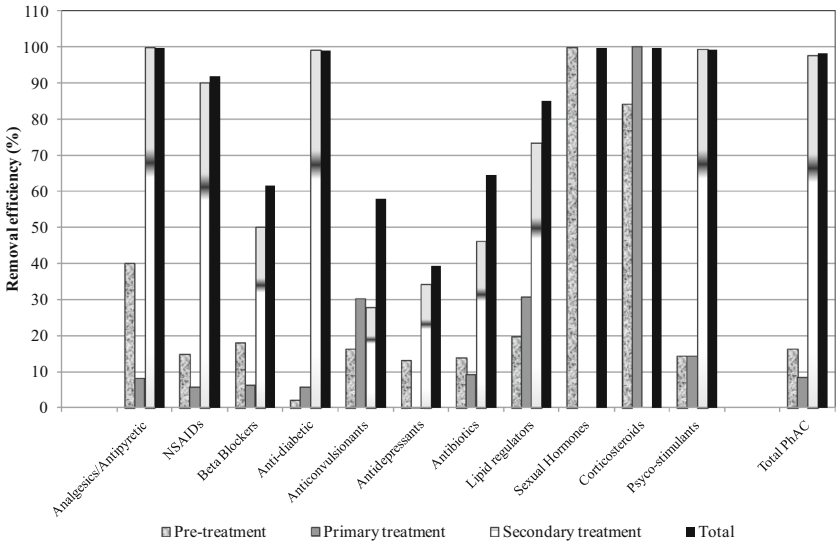

Fig. 5 Removal efficiency of specific therapeutic classes by several treatment phases of Beirolas WWTP and its global efficiency

regulators and psychostimulants). Analgesics/antipyretics and corticosteroids were the therapeutic classes most efficiently removed after pre-treatment with removal efficiencies of 40.1 and 84.2%, respectively.

The pattern of removal in the different therapeutic classes after the primary treatment was quite similar to the pre-treatment, but the median and mean of removal efficiency was 8.6 and 20.4%, respectively. These values were about 40 % lower than those obtained after pre-treatment.

The removal efficiency increased significantly with the secondary treatment with removal efficiencies between 28% (anticonvulsants/carbamazepine) and 100% (analgesics/antipyretics). Three therapeutic classes had removal efficiencies higher than 90%, such as NSAIDs, anti-diabetics and psychostimulants with removal efficiency of 90.2, 99.1 and 99.4%, respectively. Except for lipid regulators (73.4%), the efficiency removal of the remaining therapeutic classes was lower or equal to 50%.

Globally, the removal efficiency of all PhACs in this step was 97.8% with median and mean values of 73.4 and 69%, respectively. Therefore, the activated sludge treatment process (secondary treatment) had a positive influence on the ability of WWTP to remove PhACs. This fact may be explained by the diversity of microbial culture, which can degrade or transform some PhACs and also to break down the complex organic compounds, which might be useful in removing more strong and hydrophobic PhACs. This decreasing pattern and also this large variation between therapeutic classes and PhACs were also observed in other studies (He et al. 2013; Salgado et al. 2010; Sim et al. 2010).

Figure 6 shows the removal efficiency of each PhAC within the respective classes during the treatment at the WWTP. A rapid glance at these removal efficiencies shows that the different PhACs have different trends even when belonging to the same therapeutic class. However, the range of variability of removal efficiencies of secondary treatment is narrower and higher than in the two first treatments (pre-treatment and primary treatment) for the majority of PhACs. Nevertheless, some PhACs have very low global removal efficiencies, such as lower than 20% (indomethacine, metoprolol and propranolol) and between 21 and 50% (ketoprofen, diclofenac, oxazepan and sulfapyridine) and between 51 and 70% (carbamazepine, fluoxetine and sulfametoxazol).

Some of these low removal efficiencies can be explained by molecular structure and physicochemical properties of the PhACs. For example, carbamazepine is resistant to biodegradation at low concentrations (Zhang et al. 2008) and it is not sorbed to an appreciable degree (Ternes et al. 2004). Therefore, carbamazepine should pass through the WWTPs with the water phase in significant amounts. The low removal efficiency during the primary treatment indicates no significant adsorption of target compounds to the particles removed in this stage.

The compounds with higher values of pKa such as testosterone (pKa = 19.9) and corticosteroids (pKa = 12.58) have more affinity to the sludge than to the aqueous phase; therefore, their removal efficiencies in primary treatment were also higher than for the remaining PhACs. Most of the PhACs have log K_{ow} values of less than 3.0, so they are not expected to adsorb significantly to the particles. Some of the PhACs under study have higher K_{ow} values (ketoprofen, diclofenac, indomethacine, ibuprofen, gemfibrozil and bezafibrate), and they also have much lower pKa values than the pH of wastewater. Therefore, they are dissociated and expected to be >98% in the aqueous phase and not bound to the particles (Sui et al. 2010, Thomas and Foster 2005).

Some PhACs showed removal efficiencies higher than 50% in pre-treatment, namely acetylsalicylic acid (100%), fluoxetine (66.7%), testosterone (100%), cortisone (57.1%) and hydrocortisone (100%). Conversely, other PhACs such

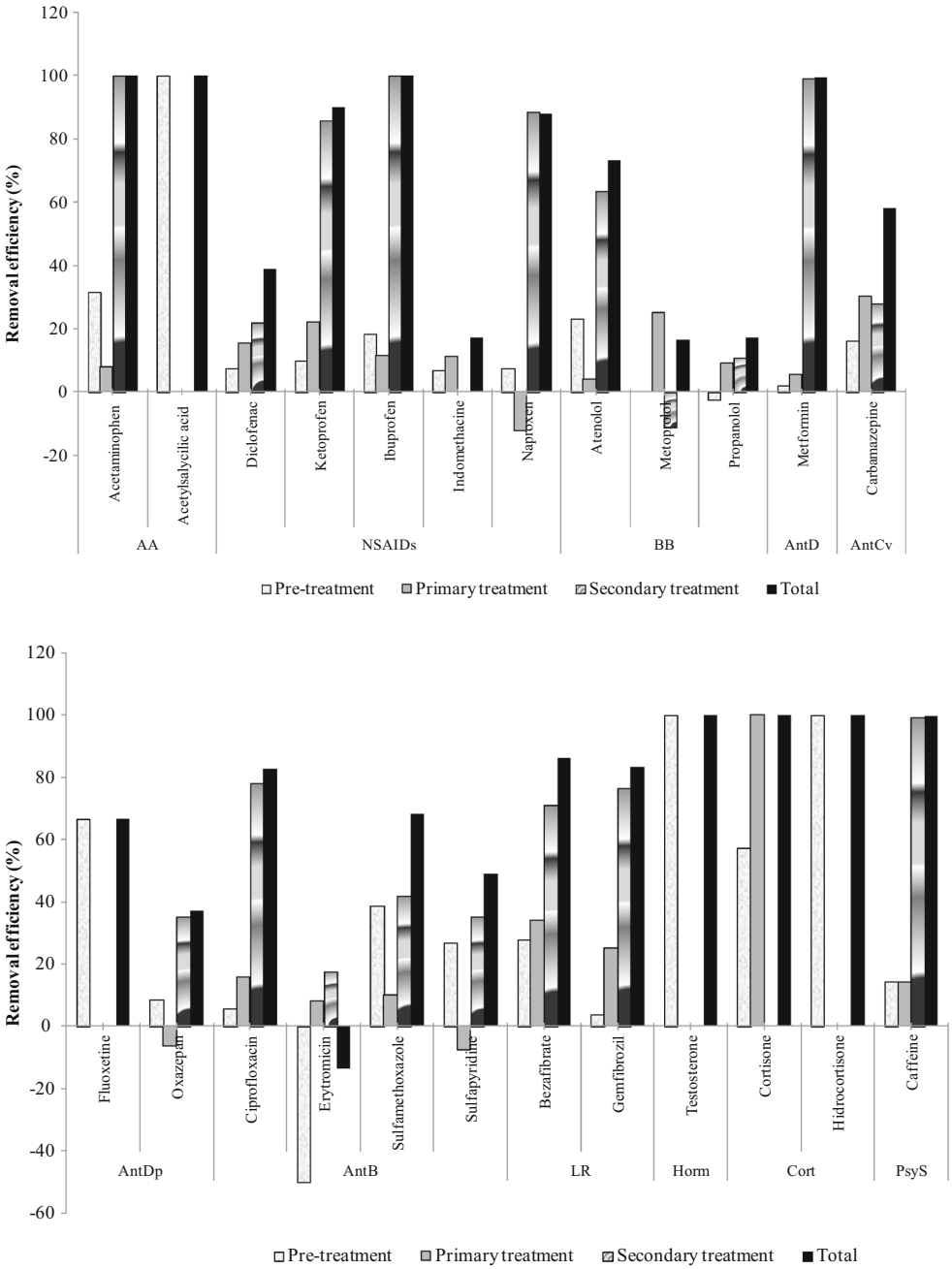

Fig. 6 Removal efficiency of PhAC (grouped by its therapeutic class) by several treatment phases of Beirolas WWTP

as propranolol and erythromycin had negative removal efficiencies: −2.4 and −50%, respectively. This negative removal efficiencies was also observed for other PhACs after primary treatment, namely naproxen (−12.1%), oxazepan (−6.3%) and sulfapyridine (−7.6%). These negative values can be explained by deconjugation of glucuronidated or sulphated PhAC and desorption from particles or hydrolysis of some compounds (for example, hydrolysis of acetylsalicylic acid to salicylic acid). These effects have been observed in several PhACs of different therapeutic classes, in particular diclofenac, ibuprofen, carbamazepine, estrone and iopromide (Kasprzyk-Hordern et al. 2009; Verlicchi et al. 2012; Zorita et al. 2009).

In the group of NSAIDs, diclofenac and indomethacine showed the lowest global removal efficiency, 38.9 and 17.2%, respectively. In contrast, the remaining NSAIDs under study have removal efficiencies higher than 85%. These values were similar to those obtained in other studies using activated sludge treatment (Kasprzyk-Hordern et al. 2009; Verlicchi and Zambello 2015).

Figure 7 shows the seasonal variation of removal efficiencies for the different therapeutic classes under study.

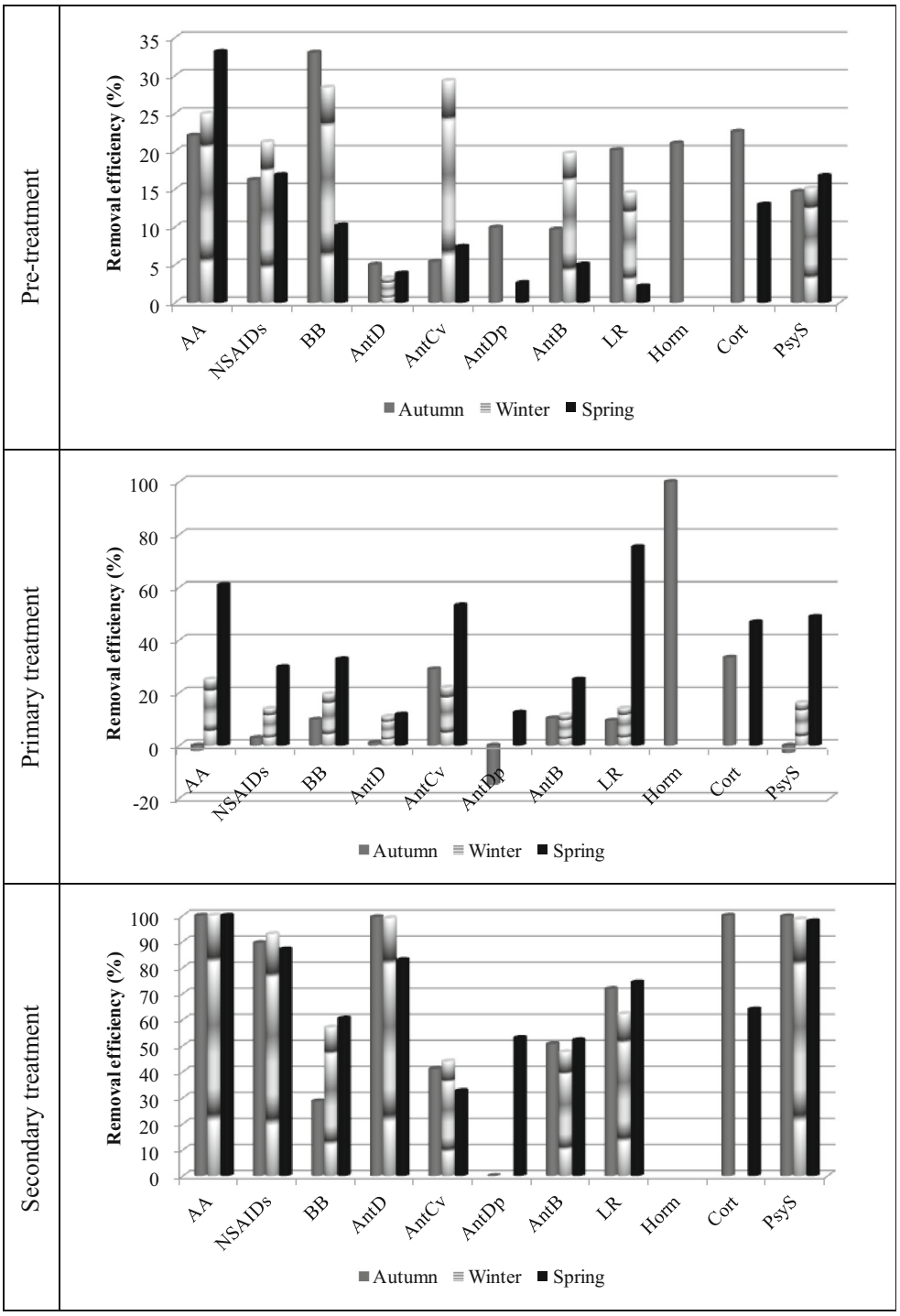

Fig. 7 Removal efficiencies of the different therapeutic groups and treatment procedures by three seasons (autumn, winter and spring)

No statistically significant differences were observed between the treatments carried out in the three seasons. However, the therapeutic classes' removal efficiencies for primary treatment were higher in spring than in autumn and winter. These results are consistent with those obtained by other authors where the treatment throughout the WWTPs was more efficient in warmer temperatures (Fernández et al. 2014; Sui et al. 2010).

It is always difficult to correlate the physical–chemical properties of pharmaceuticals (individual or therapeutical class) to their corresponding removal efficiency achieved in each treatment step or in overall treatment because many other factors contribute to it, in particular operating parameters such as biomass concentration, solid retention time, hydraulic retention time, pH, temperature, design and type of plant. Environmental factors such as the

weather also have influence. Although important, these factors were not the subject of this study. The objective was to evaluate the Beirolas WWTP under normal operating conditions by determining the type and concentration of PhACs in the affluent and what their behaviour in the various treatments is. Therefore, it was evaluated, the type and concentration of PhACs usually discharged into receiving environment (Tagus river).

After this first approach, this study should be supplemented with a detailed evaluation of the operating conditions of WWTP and to assess the factors that may increase the efficiency of this WWTP.

Conclusions

From the 32 PhACs considered in this study, acetaminophen (55–623 μg/L), metformin (70–325 μg/L) and caffeine (47–273 μg/L) were the compounds detected at higher levels in the influent of Beirolas' WWTP. Eight PhACs were not detected (nimesulide, sulfadiazine, sulfamerazine, sulfamethazine, sulfathiazole, clofibric acid, prednisone and prednisolone), and four PhACs were completely removed in the WWTP (acetylsalicylic acid, ibuprofen, testosterone, hydrocortisone).

Several PhACs (e.g. acetaminophen, acetylsalicylic acid, metformin, gemfibrozil, bezafibrate and caffeine) showed significant concentration decrease rates in the WWTP, mainly in the biological treatment.

The overall removal efficiencies varied strongly between individual PhAC. Therefore, it was difficult to establish a general trend for each therapeutic class but, in most cases, the results indicated that the elimination of the PhAC was incomplete. However, Beirolas WWTP seems to operate well in removing most of the investigated PhACs, even if some improvements are required to completely remove these target molecules from the final effluents, and thus minimize their impact in receiving waters (mainly surface waters).

Lack of a clear seasonal tendency in the occurrence and removal of these target compounds were observed. However, further research in these issues is warranted and needed.

These results reinforce the importance of monitoring studies, as defined by Directive 2013/39/EU, in order to minimize their environmental impact and support future decisions on environmental policy.

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