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Pharmaceuticals in a Mediterranean Basin: The influence of temporal and hydrological patterns in environmental risk assessment

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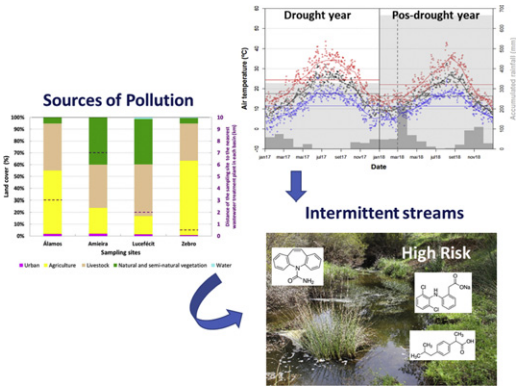
HIGHLIGHTS

- Streams of Guadiana Basin contaminated with pharmaceuticals;
- Intermittent regimes more sensitive to the pharmaceutical's contamination;
- Use of optimised risk quotient for obtaining a more real scenario of risk;
- Drought intensifies the risk of pharmaceuticals;
- Diclofenac, ibuprofen and carbamazepine with high risk, at intermittent streams;

GRAPHICAL ABSTRACT



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ABSTRACT

Occurrence of pharmaceuticals in the aquatic environment is nowadays a well-established issue that has become a matter of both scientific and public concern. Tons of different classes of pharmaceuticals find their way to the environment at variable degrees, after their use and excretion through wastewater and sewage treatment systems.
The main goal of this study was to correlate the dynamics and the environmental risk of pharmaceuticals with different temporal and hydrological patterns, at the Guadiana Basin (South of Portugal). Water samples were collected bimonthly during 2017 (classified as a drought year) and 2018 (post-drought year) in: Zebro, Álamos and Amieira (intermittent hydrological streams), and Lucefécit (perennial hydrological stream). The pharmaceuticals quantified in higher concentrations, out of 27 investigated, were diclofenac (up to 4806 ng L^{-1}), ibuprofen (3161 ng L^{-1}), hydrochlorothiazide (2726 ng L^{-1}) and carbamazepine (3223 ng L^{-1}). Zebro and Álamos presented the highest contamination by this group of environmental hazardous substances, which may be correlated with the presence of wastewater treatment plants upstream the sampling point of each stream. Furthermore, the highest concentrations occurred mainly during the dry period (2017), when the flow was nearly inexistent in Zebro, and in Álamos after the first heavy rainfalls.

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In specific periods, the high concentrations of pharmaceuticals detected may induce risk for the organisms of lowest trophic levels, damaging the balance of the ecosystems at these streams. The risk quotient optimised approach (RQf) integrating exposure, toxicity and persistence factors, ranks the pharmaceuticals investigated in terms of risk for the aquatic ecosystems as follows: diclofenac, ibuprofen and carbamazepine (high risk), clarithromycin (moderate risk), acetaminophen, ofloxacin and bezafibrate (endurable risk), and hydrochlorothiazide (negligible risk).

1. Introduction

Aquatic emerging pollutants present a new global water quality challenge, with potential serious threats to human population, ecosystems balance and services (Ivanová et al., 2018). Among emerging pollutants, pharmaceuticals are the unique group structurally designed to maximize their intrinsic biological activity at low concentrations and developed to induce a continued action at biological systems. These properties highlight the risk associated with the unintentional presence of pharmaceuticals in the aquatic environments (Ebele et al., 2017; Ginebreda et al., 2010). Further, around 5000 different pharmaceutical forms are used in human and veterinary medicine in the European Union, covering a broad range of chemical structures and physic-chemical proprieties (Hughes et al., 2013). Complex mixtures of these compounds along with their metabolites are continuously introduced into the environment via distinct pathways, such as the disposal of unused, expired medicines or by untreated discharges and/or wastewater treatment plant (WWTP) effluents (e.g. from hospitals, veterinary practices and agriculture activities, namely, livestock) (Mandaric et al., 2019; Zhou et al., 2019). In water bodies, pharmaceuticals may be removed from the dissolved phase through adsorption to suspended particles and may accumulate in sediments, from which they can be remobilized by resuspension. The abiotic cycle and fate of these pollutants in the environment depend, not only on their physical-chemical proprieties, but also on other variables like seasonal changes as temperature variability, drought periods, and water hydrology (Nunes et al., 2019).

In the aquatic environment, pharmaceuticals may induce a variety of physiological changes, reversible or not, in non-target aquatic organisms (Godoy and Kummrow, 2017), such as macroalgae (Rosa et al., 2019), molluscs (Gonzalez-Rey and Bebianno, 2012; Joyce and Vogeler, 2018), crustaceans (Hossain et al., 2019; Oropesa et al., 2017, 2016) and fish (Mathias et al., 2018; Perussolo et al., 2019). Furthermore, chronic exposure to trace levels of pharmaceutical mixtures can also induce antibiotic resistances (Sabri et al., 2018) and endocrine disruption effects (Xu et al., 2019). Nevertheless, only recently, pharmaceuticals have emerged as one of the major concerns in Europe and worldwide (Ebele et al., 2017). Example of this is the fact that any of the 45 priority substances identified under the Directive 2013/39/EU (ECC, 2013) are pharmaceuticals, and only in 2007, diclofenac and carbamazepine were identified as future emerging priority candidates (Ebele et al., 2017). Following this, in 2015, six pharmaceutical compounds (17-α-ethynilestradiol, 17-β-estradiol, estrone, diclofenac, erythromycin, clarithromycin, and azithromycin) were included in the first European watch list of substances for Union-wide monitoring in the field of water policy (ECC, 2015). More recently, in 2018 (ECC, 2018), this list has been modified to include the antibiotics amoxicillin and ciprofloxacin and exclude diclofenac after the information gathered on it on the previous years. Thus, further studies are needed to identify, within this group of emerging substances, those that present a greater potential risk to the aquatic environment and introduce them in lists of priority substances for specific assessment and management at European member state's water bodies (Ribeiro et al., 2015). In this context, in order to increment the effectiveness of the rank systems, making them more realistic and useful at regional scale, it is essential: (i) to have high-quality methodologies with enough sensitivity to detect very low levels of these substances, allowing the chemical fingerprint characterization of the abiotic matrices (Brack et al., 2018; Sousa et al., 2019); (ii) to refine environmental risk assessment processes, integrating occurrence patterns, frequency of detection, chemical properties of pharmaceuticals, bioaccumulation, bioamplification and aquatic toxicity effects (Brack et al., 2018; Zhou et al., 2019); (iii) to develop risk assessment processes taking into consideration different temporal and spatial scenarios, mainly in regions where the impact of climate changes may be more stressed (Mandaric et al., 2019; Pereira et al., 2017); and (iv) to establish correlations among pharmaceuticals risk assessment and ecological status of water bodies (Brack et al., 2015).

Nowadays, occurrence, frequency of detection, and risk assessment studies have mostly been dealt with at a global scale, which is evident from the large number of research articles published (Fekadu et al., 2019; Gogoi et al., 2018; Li et al., 2019; Reis et al., 2019). In the Mediterranean region, one of the most affected by climate changes, some studies have already been developed at a local scale, assessing different temporal and spatial scenarios of pharmaceuticals risk for the aquatic environment (Barbosa et al., 2018; Desbiolles et al., 2018; Mandaric et al., 2019; Osorio et al., 2016; Sousa et al., 2019). All these studies focused on the importance of the work at local scale, namely at the level of basin, river and stream, to get answers about the influence of hydrologic patterns, attenuation processes, variability of temporal factors, and pollution pathways correlated with land cover and land uses, in the dynamics of emerging pollutants at the water systems. In fact, Mediterranean streams/rivers are characterized by inter-annual hydrological fluctuations encircling floods in spring and autumn and droughts in summer (Palma et al., 2018), which can cause streams intermittent regimes, processes that are becoming more frequent with the intensification of drought periods. Consequently, intermittent streams and rivers in the Mediterranean area, under climate-change conditions, are among the most frequent and dynamic freshwater systems (Acuña et al., 2017, 2014). Hence, the local studies are the midpoint to obtain specific results of different patterns allowing the outline of regional strategies that may be overlapped for global goals.

The main goals of this study were: (i) to analyze the presence and respective concentrations of pharmaceuticals belonging to different therapeutic classes in Mediterranean streams, with different flow regimes and affected by several land uses; (ii) to assess the joint effects of hydrological (river flow) and chemical stressors on the occurrence and distribution of pharmaceuticals, during drought and post-drought periods; and (iii) to estimate and analyze the environmental risk of pharmaceuticals, using an improved method. The results will add knowledge on the behavior of pharmaceuticals under a drought climate scenario, followed by a normal climate year, at streams with different flow regimes, contribution extremely significant for climate studies at the Mediterranean region. The results, integrated in European databases of pharmaceuticals risk assessment, will provide additional information on pharmaceuticals at regional and global scales and help identify those that deserve priority consideration in the European water policies.

2. Materials and methods

2.1. Site description and sampling procedures

The Guadiana basin integrates the international Hydrographic Region of Guadiana which has a total area of 66,800 km^2, of which 55,220 km^2 (83%) belong to Spain and 11,611 km^2 (17%; most of it at the Alentejo region) belong to Portugal. It is the fourth largest watershed in the Iberian Peninsula, after the Douro, Ebro and Tagus basins

(APA, 2016). The drainage basin of the Guadiana river section in Portugal and the corresponding land cover map are shown in Fig. 1. Land cover has been obtained from the 2018 CORINE Land Cover (CLC) inventory, produced with satellite imagery (Sentinel-2 and Landsat-8 for gap filling), available from Copernicus Land Monitoring Service (2018) (https://land.copernicus.eu/).

The Hydrographic Region of Guadiana basin is limited to the north by the Tagus river basin to the east by the Júcar region and to the south by the Guadalquivir region and the Tinto rivers, Odiel and Piedras (APA, 2016). The Guadiana River rises in the lagoons of Ruidera in Spain, at an altitude of 868 m, developing over 800 km to the mouth of the Atlantic Ocean, next to Vila Real de Santo António (Portugal). In Portugal,

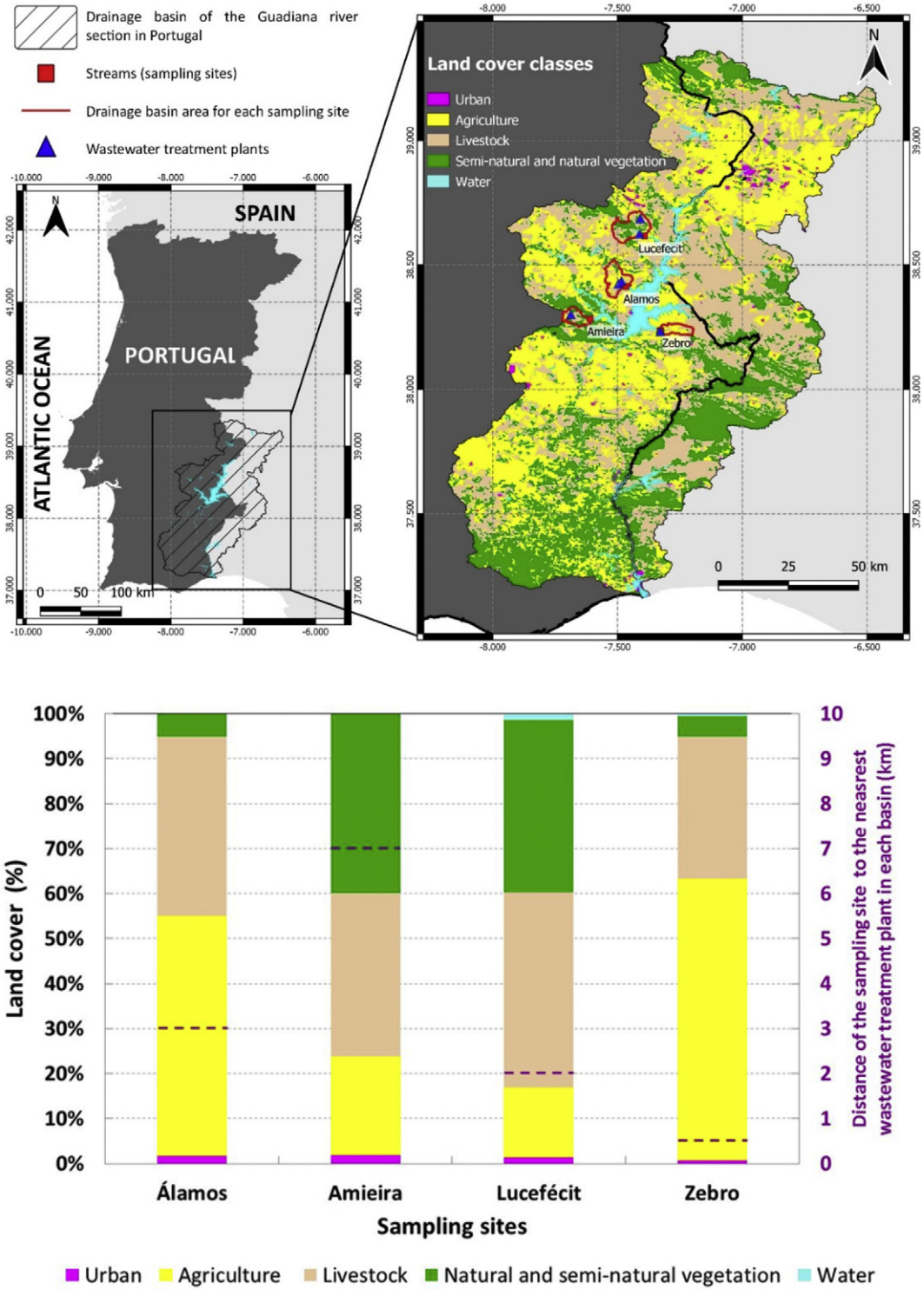

Fig. 1. a) Map of the study area and corresponding land cover map. The line pattern fill (left panel) delineates the drainage basin of the Guadiana river section in Portugal and the red lines (right panel) represent the drainage basin areas for each sampling site. The red squares represent the stream sampling sites (from North to South: Lucefécit - Lf; Álamos - Al; Amieira - Am; and Zebro – Zb) and the blue triangles indicate the location of the wastewater plants. b) The bars show the percentages of the land cover classes in the drainage basins of the stream sampling sites (left axis) and the dashed lines indicate the distance to the nearest wastewater treatment plant (right axis).

the river has a total length of 260 km, presenting nine sub-basins that integrate the main tributary waterlines to the rivers of Guadiana, Alcarrache, Ardila, Caia, Chança and Xévora (APA, 2016) (Fig. 1).

The soils are mostly heavy, essentially derived from schists and granite. This region is characterized by a very rich natural heritage, with a high conservative value, in terms of habitats and species of flora and fauna, such as Mediterranean oaks, cork oak (*Quercus suber* L.) and holm oak (*Quercus rotundifolia* Lam.) woodlands interspersed within the agricultural landscape. Few patches of forest plantations (eucalyptus and pines) are also found (APA, 2016). Fig. 1 also shows the distribution of land cover types, with agriculture and livestock activities widely disseminated in the Guadiana basin, and natural and semi-natural vegetation (including Montado landscape) more often found in the south-eastern regions. The lower panel of Fig. 1 presents the percentages of the land cover classes in each drainage basin, as well as the distances to the nearest wastewater treatment plant, as a way of identifying the main sources of pollution in the areas. Table 1 also presents information on the sources of pollution and wastewater treatment types and flow rates.

The climate at Guadiana basin is quite homogeneous, with dry Mediterranean characteristics, as hot summers, high levels of insolation and evapotranspiration and severe winters. The average annual temperature is in almost the whole basin near 16 °C, with values of 24 to 28 °C in the warmer months (July/August), and with average air temperature around 9 °C in the coldest month (January). The weighted average annual rainfall is 550 mm (561 mm in Portugal and 540 mm in Spain). The annual distribution of precipitation is extremely irregular, falling to 386 and 422 mm in dry years and rising to 722 and 766 mm in wet years, being the majority (±80%) concentrated in the period of October–April (APA, 2016).

From a climatological point of view, the years of 2017 and 2018 in Portugal were quite different, with 2017 classified as extremely warm and dry in comparison with the climatological normal of 1971–2000, whereas 2018 was considered normal with respect to the same reference (www.ipma.pt; last accessed on 25/09/2019). The year of 2017 was characterized by extremely hot weather especially in April, June and October, with temperature values much higher than normal from April to October, in particular the maximum temperature. This, combined with the anomalously low precipitation values, caused high evapotranspiration values and significant deficits of soil moisture, which also contributed to a very severe forest fire season in the country. From April 2017 on, great part of the country was gradually affected by severe and extreme drought conditions, according to the Portuguese Institute for the Sea and Atmosphere (IPMA), including the Guadiana basin. In 2017 this area presented a mean annual temperature that was 1.5 °C higher than the normal, accompanied by a decrease of precipitation of roughly 55% of the normal, taking as reference the 1971–2000 climatological normal for the region. Fig. 2 presents the temperature and precipitation data measured in 2017 and 2018 at a meteorological station installed in the lower part of Guadiana basin at Alqueva reservoir (Potes et al., 2017). The differences between both years can be clearly distinguished with the annual values of average temperatures (minimum, mean and maximum represented by the blue, black and red horizontal lines, respectively) showing higher values in 2017 than in 2018. In contrast, the annual accumulated precipitation (represented by the light grey bars) in 2017 is roughly half of that in 2018. The monthly temperature lines for 2017 show that high temperatures were registered for a longer period than in 2018 and the dark grey bars confirm that after March 2017 precipitation was scarce, which caused the severe drought conditions. The precipitation that occurred in March 2018 triggered a relief of the drought conditions, which is represented by the dashed grey vertical line in Fig. 2. The year of 2018 does not present significant deviations from the climatological normal, with the mean annual temperature showing a slight deviation of +0.2 °C and the precipitation presenting a slightly higher annual value (112% of the climatological normal).

Four sampling sites were selected in streams located at the lower part of the Guadiana basin and draining to the Alqueva reservoir: Zebro (Zb; 38°14′15.54″N, 7°19′40.32″W); Álamos (Al; 38°24′50.46″N, 7°28′2.16″W); Amieira (Am; 38°16′58.80″N, 7°36′35.20″W) and Lucefécit (Lf; 38°36′59.19″N, 7°23′31.65″W), represented in Fig. 1, along with the land cover classes. The selection of these streams took into consideration: (i) the differences in hydrological regimes: Amieira and Álamos are intermittent streams, which dry to a series of disconnected pools part of the year, during the study this happened in July and September; Zebro with an intermittent regime presents water all year long but without flow during some periods; Lucefécit presents a perennial regime, with flow during the whole year; (ii) the type of pollution correlated with the land cover patterns; and (iii) the lack of knowledge about their chemical status. The characterization of each stream is displayed in Table 1.

Table 1
General characteristics, chemical and potential ecological status classification, and principal sources of pollution identified during the cycle of assessment of 2009–2015 (APA, 2016), including wastewater treatment types and flow conditions (ARHAlentejo, 2011). The flow was characterized qualitatively during the present study (Jn: January; Mr.: March; M: May; Jl: July; Sp: September; Nv: November; 17:2017; 18:2018).

Site	Typology	Basin	Sub-basin	Length (km)	Flow conditions	Sediments Texture (%)	Ecological potential	Chemical status	Principal sources of pollution	Wastewater treatment plant: type and flow rate (m^3/day)
Álamos	Small rivers of Southern Portugal (S1 ≤ 100 km^2)	Guadiana	Guadiana	17.0731	Low flow (M17, Sp17, Nv17, Jn18, M18, Sp18) Very fast (Mr17, Mr18 Nv18) No flow (Jl17, Jl18)	Sand: 65 Silt: 25 Clay: 9	Less than good	Unknown	Agriculture Livestock Urban	Reguengos de Monsaraz: Activated Sludge with Aeration (927) S. Pedro do Corval: Activated Sludge with aeration (157)
Amieira	Small rivers of Southern Portugal (S1 ≤ 100 km^2)	Guadiana	Degebe	17.6246	Low flow (Nv17, Jn18, M18) Moderate (M17, Mr18), Very fast (Nv18) No flow (Jl17, Sp17, Jl18, Sp18)	Sand: 90 Silt: 3 Clay: 8	Unknown	Unknown	Livestock Agriculture Urban	Portel: Stabilization Ponds (Natural Lagooning) (239)
Zebro	Small rivers of Southern Portugal (S1 ≤ 100 km^2)	Guadiana	Guadiana	13.295	Low flow (Jn18, Mr18, Nv18) No flow (Mr17, M17, Jl17, Sp17, Nv17, M18, Jl18, Sp18)	Sand:89 Silt:4 Clay:9	Less than good	Unknown	Agriculture Urban Livestock	Póvoa de S. Miguel: Percolator Bed (96)
Lucefécit	Small rivers of Southern Portugal (S1 ≤ 100 km^2)	Guadiana	Guadiana	44.101	Low flow (M17, Jl17, Sp17, Nv17, Jn18, M18, Jl18, Sp18, Nv18) Very fast (Mr18)	Sand: 59 Silt: 31 Clay: 10	Less than good	Unknown	Livestock Agriculture Urban	Terena: septic tank and macrophyte trench (150) Alandroal: Activated Sludge with Nitrogen and Phosphorus Removal (483)

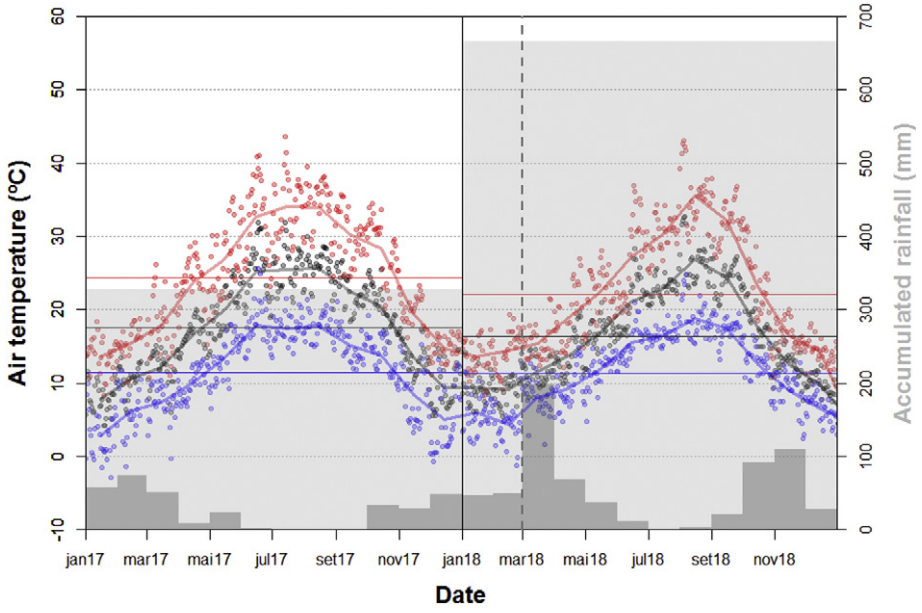

Fig. 2. Daily and monthly mean (black dots and line), maximum (red dots and line) and minimum (blue dots and line) air temperature and monthly rainfall (dark grey bars) from 2017 to 2018 in the Alqueva region (Guadiana Basin). The light grey bars represent the annual rainfall in this period and the black, red and blue horizontal lines, constant each year, denote the mean, maximum and minimum annual temperatures. The dashed grey vertical line indicates the end of the drought period.

Pharmaceuticals were evaluated during 12 sampling campaigns from January 2017 to November 2018 (in the months of January (Jn), March (Mr), May (M), July (Jl), September (Sp), November (Nv), for both years). The wet period included the months of November, January and March, and the dry period included the months of May, July and September, these periods were defined by APA, for the Alentejo region (ARHAlentejo, 2011). The water collection in the months of July and September for the intermittent streams was done at the existing pools. Due to technical problems with some laboratory facilities, it was not possible to collect the samples of January and March 2017 in Álamos and Lucefécit. During the study period, a total of 44 water samples (1 L) were collected at a depth of 30 cm and stored in amber PET bottles in the dark at −18 °C until analysis.

In situ measurements at each sampling site using a multiparametric YSI 6820 MPS probe® allowed to determine the water temperature (T; °C), pH, electrical conductivity (EC; μS cm^{-1}) and dissolved oxygen (DO; %).

The flow conditions of Guadiana river (sub-basin of the streams analysed) were obtained from the database of National Water Resources Information System (2019; https://snirh.apambiente.pt/). The existent data (until 2010) showed significant variations of river flow rates, between 0.8 m^3 s^{-1} (30/7/2009) and 552.0 m^3 s^{-1} (5/11/2006). This high flow variability, also observed in other Iberian rivers (Pereira et al., 2017), is justified by the climate-driven oscillations, which increment the water scarcity. Since no information about the specific hydrology of the studied streams was available at the national database, the flow conditions were qualitatively assessed, using the following scale: no flow, slow flow, flow, and very fast flow (results displayed in Table 1).

2.2. Chemicals

A total of 27 target pharmaceuticals representative of several therapeutic classes, and some of them included in the European Watch Lists, were selected for analysis (ECC, 2000, 2018). The list of analytical reference standards comprised the analgesics (ANALG) and no steroids anti-inflammatories drugs (NSAIDs) acetaminophen (ACET), diclofenac (DIC), ibuprofen (IBUP), indomethacin (IND), ketoprofen (KET), naproxen (NAP), and propyphenazone (PROPy), the antibiotics ofloxacin (OFLOX), sulfadiazine (SULFD), sulfamethazine (SULFM), trimetroprim (TRIM), sulfamethoxazole (SULFAMT), and clarithromycin (CLAR), the psychotropic drugs diazepam (DIAZ), lorazepam (LORAZ), paroxetine (PAR), and carbamazepine (CARB), the lipid regulators bezafibrate (BEZ), gemfibrozil (GEM), and fenofibrate (FEN), the β-blockers atenolol (ATEN), metoprolol (MET), propranolol (PROP), and sotalol (SOT), the diuretics furosemide (FUR) and hydrochlorothiazide (HYDR), and the opioid codeine (COD). All compounds were high purity (mostly 90%) and were bought from Sigma Aldrich (St. Luis, MO, U.S) or Cerilliant (Texas, USA). The CAS numbers, molecular formulas, molecular weights, and other relevant properties of all target compounds are reported in Electronic Supplementary Material (ESM, Table S1).

Isotope-labelled compounds (IS) acetaminophen-d_4, bezafibrate-d_4, carbamazepine-d_{10}, codeine-d_3, diazepam-d_5, diclofenac-d_4, furosemide-d_5, fenofibrate-d_6, gemfibrozil-d_6, ketoprofen-d_3, indomethacin-d_4, hydrochlorothiazide-d_2, lorazepam-d_4, metoprolol-d_7, naproxen-d_3, paroxetine-d_4, propranolol-d_7, sotalol-d_6, sulfamethoxazole-d_4, sulfadiazine-d_4, and sulfamethazine-d_4 were purchased from Sigma Aldrich (St. Luis, MO, U.S), Cerilliant (Texas, USA), Alsachim (Illkirch-Graffenstaden, France) or Santa Cruz Biotechnology (Dallas, TX, USA) (ESM, Table S2).

LC-MS grade acetonitrile (ACN) (≥99.9%), methanol (MeOH) (≥99.9%), ethyl acetate (EtAc) (≥99.9%), dimethyl sulfoxide (DMSO) (≥99.9%), and HPLC water were purchased from Merck (Darmstadt, Germany). Formic acid (≥96%, ACS reagent) and ammonium acetate were supplied by Sigma-Aldrich.

Individual stock standard solutions (concentration of 1000 μg mL^{-1}) were prepared in either 100% methanol or 100% DMSO, depending on the solubility of each compound. Working solutions mixtures containing all the aforementioned analytes and the isotopically labelled compounds (1 μg mL^{-1}), for analysis and calibration purposes, were prepared by diluting adequate volumes of the individual stock solutions with MeOH. All solutions were stored at −20 °C.

2.3. Chemical analysis

Pharmaceuticals were analysed in water following a method based on solid phase extraction (SPE) and analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The details of the method can be found in the Electronic Supplementary Material (ESM). In brief,

water samples (200 mL), previously filtered and spiked with a mixture of isotopically labelled compounds, were extracted with Oasis HLB (60 mg, 3 mL) cartridges (Waters Corporation, Milford, MA, US), using 2 × 4 mL of MeOH for elution. The extracts were then evaporated, reconstituted into 1 mL of water/MeOH (90:10 v/v), and filtered (through 0.45 μm PTFE syringe filters). LC-MS/MS analysis of the extracts (5 μL injection volume) was performed using a SCIEX ExionLC™ AD system (Sciex, Redwood City, CA, U.S.) and a Hibar® HR Purospher® STAR RP-C18 column (100 mm × 2.1 mm i.d., 2 μm particle size, Merck, Darmstadt, Germany) for chromatographic separation. MS detection was carried out in both positive and negative electrospray ionization modes (in separate runs) using a SCIEX X500R QTOF system (Sciex, Redwood City, CA, U.S.) with a Turbo V™ source. High resolution data were acquired using multiple reaction monitoring (MRMHR) following a workflow consisting of a TOF-MS survey (100–850 Da for 80 ms of Accumulation time (AT)). The MRMHR scanning mode was used for accurate quantification of product ion transitions (see Tables S3 and S4 in ESM), optimised with the Guided MRMHR tool from SCIEX. All relevant selected detection conditions (transitions, ion spray voltage, source temperature, declustering potential, collision energies, etc.) can be found in the ESM.

Qualitative and quantitative data analyses were performed using SCIEX OS™ Software version 1.5 (Sciex, Redwood City, CA, U.S.). Two high resolution product ions were used for each compound, the most abundant for quantification and the second most abundant for confirmation. Linearity of the method was evaluated using the internal standard calibration approach with a calibration curve constructed between 0.1 and 300 μg L^{-1} (equivalent to 0.5 and 1500 ng L^{-1} in surface water, taking into consideration a method concentration factor of 200 in the aqueous extract). Calibration curves were constructed using linear weighted least-squares regression (1/x as weighting factor) by plotting the ratio of the analyte signal to that of its corresponding IS (added at a fix concentration of 50 ng L^{-1}) as a function of the analyte and surrogate standard concentration ratio. The corresponding coefficient of determination together with the Method Detection Limits (MDLs) and Method Quantification Limits (MQLs) obtained for each compound can be found in Tables S3 and S4 of ESM. MDLs and MQLs, experimentally calculated using a signal-to-noise ratio of 3.3 and 10, respectively, from the mass spectrometry signal observed in the real samples, were between 0.05 and 1.3 ng L^{-1}.

2.4. Environmental risk assessment

To determine the pharmaceuticals that promoted a higher negative impact (higher risk) in the streams, and rank them accordingly as potentially harmful compounds specific for the Guadiana basin (Mediterranean region), a risk quotient optimised approach (RQf) was developed on the basis of the respective EU guidelines (ECC, 2003) and further improved to include the frequency of MECs (measured environmental concentrations) exceeding PNECs (predict no-effect concentrations) (Zhou et al., 2019). This approach allows highlighting the pollutants that repeatedly appear at potentially harmful concentrations at a given site and pinpoint them in the high risk compounds prioritization process (Desbiolles et al., 2018). To this end, the authors applied as a starting point the methodology previously used by them for evaluation of the risk assessment of pollutants (Palma et al., 2014), based on the calculation of the ratio between the MEC and the PNEC (RQ = MEC/(PNEC/AF)) in different scenarios: general (using the average of the quantified individual concentrations for each compound at each site) and worst (using the maximum concentrations), in each of the years 2017 (drought) and 2018 (pos-drought conditions). For the calculation of the PNEC, NOEC values were used and, in their absence, EC_{50} values for three trophic levels (algae, crustacean, fish), adjusted by an appropriate assessment factor (AF; 10, 50, 100 or 1000, depending on the toxicity values used) to overcome the uncertainty of this conservative approach. Ecotoxicological data was obtained from the United States EPA ECOTOX database (2019) (https://cfpub.epa.gov/ecotox/), and from Zhou et al. (2019). The toxicicity and PNEC data, as well as some chemical properties influencing the environmental persistence and bioaccumulation of compounds, are displayed in Table 2.

The optimised risk quotient value (RQf) is determined by multiplying the mean of the various RQ values obtained for a compound and site by the frequency (F) with which its MEC exceeds the corresponding PNEC in the considered period, that is, by the percentage of cases where the RQ is equal or higher than 1, according to the following equation:

$$RQf = RQ_{mean} \times F$$

According to Zhou et al. (2019), the RQf was classified into 5 groups: high environmental risk: RQf > 1; moderate environmental risk: 0.1 ≤ RQf < 1; endurable environmental risk (small scale adverse effect): 0.01 ≤ RQf < 0.1; negligible environmental risk: 0 < RQf < 0.01; no environmental risk: RQf = 0 (safe).

2.5. Statistical analysis

Water data were assessed by descriptive statistics (mean, range, standard deviation and frequency of detection) and the seasonal and annual variations of the pharmaceuticals were analysed. Since most of the data were not normally distributed, the Kruskal–Wallis ANOVA by

Table 2
Logarithm of the octanol-water partition coefficient ($LogK_{ow}$), degradation time in water (DT50), toxic value for the most sensitive species (ng L^{-1}) and PNEC value of the pharmaceuticals analysed in the Guadiana Basin.

Compound	$LogK_{ow}$ (25 °C)	Water DT50 (days)	Toxic value (mg L^{-1})	PNEC value (ng L^{-1})
Analgesics/NSAIDs				
Acetaminophen	0.46	3.10[a]	0.005 ($NOEC_{algae}$)	500
Diclofenac	4.51	5[b]	0.00001 ($NOEC_{crustaceans}$)	1
Ibuprofen	3.97	20[c]	0.0001 ($NOEC_{fish}$)	10
Indomethacin	4.27	17–18[d]	2.9 ($NOEC_{algae}$)	2900
Ketoprofen	3.12	–	1.24 ($EC50_{crustaceans}$)	1240
Naproxen	3.18	14[c]	36.7($EC50_{fish}$)	36,700
Propyphenazone	1.94	–	0.571($EC50_{algae}$)	571
Antibiotics				
Ofloxacin	−2.00	10.6[b]	0.005 ($NOEC_{algae}$)	100
Sulfadiazine	−0.09	9.8[e]	1.88 ($EC50_{crustaceans}$)	1880
Sulfamethazine	0.89	72–180[f]	1 ($NOEC_{algae}$)	20,000
Sulfamethoxazole	0.89	5.2[e]	0.12 ($NOEC_{crustaceans}$)	2400
Trimethoprim	0.91	6[e]	0.157($NOEC_{fish}$)	15,700
Clarithromycin	3.16	–	0.002 ($NOEC_{algae}$)	20
Psychotropic drugs				
Diazepam	2.82	34[a]	0.983 ($EC50_{crustaceans}$)	983
Lorazepam	3.98	–	1. 683 ($EC50_{algae}$)	1680
Paroxetine	2.57	–	0.22 ($NOEC_{crustaceans}$)	2200
Carbamazepine	2.45	63[c], 100[b]	0.0001 ($NOEC_{crustaceans}$)	100
Lipid regulators				
Bezafibrate	4.25	–	0.023 ($NOEC_{crustaceans}$)	230
Gemfibrozil	4.77	–	0.078 ($NOEC_{crustaceans}$)	1560
Fenofibrate	5.19	–	0.703 ($EC50_{fish}$)	7030
β-Blockers				
Atenolol	0.16	–	5 ($NOEC_{fish}$)	100,000
Metoprolol	1.88	–	7.3 ($NOEC_{algae}$)	73,000
Propranolol	3.48	16.8[b]	0.004 ($NOEC_{fish}$)	400
Sotalol	0.24	–	26.39 ($EC50_{algae}$)	26,390
Diuretics				
Furosemide	2.03	22[g]	19.78 ($NOEC_{algae}$)	197,800
Hydrochlorothiazide	−0.07	–	56.18 ($EC50_{algae}$)	56,180
Opioids				
Codeine	1.19	–	0.976 ($EC50_{crustaceans}$)	976

$LogK_{ow}$ (Log Octanol-Water Partition Coefficient) values were obtained from CHEMSpider (http://www.chemspider.com/); toxic values and PNEC where obtained from United States EPA ECOTOX database (https://cfpub.epa.gov/ecotox/) and (Zhou et al., 2019); the values of DT50 in water where obtained from: [a]Loffler et al., 2005; [b]Andreozzi et al., 2003; [c]Tixier et al., 2003; [d]Yamamoto et al., 2009; [e]Nguyen Dang Giang et al., 2015; [f]TOXNETdatabase: https://toxnet.nlm.nih.gov/; [g]Liu et al., 2019.

Ranks test was performed to assess statistical differences among pharmaceuticals and sampling locations, period and flow.

A correlation test was performed to evaluate possible associations among pharmaceuticals, hydrological and temporal patterns. Hence, considering the number of samples (<50), and that most data failed the Shapiro-Wilk normality test, correlations between parameters were assessed using the Spearman's rank coefficients, as a non-parametric measure computed over ranked data. The Spearman coefficient (R) was used to evaluate the statistical significance of the correlations, for a confidence level of $p < 0.05$.

All statistical analyses were performed with the STATISTICA 7.0 (Software™ Inc., PA, USA, 2004).

3. Results and discussion

3.1. In-situ physical-chemical parameters, meteorology and land cover

The results of the in-situ parameters are displayed in Fig. 3. In general, pH showed neutral to slightly alkaline values in all streams, with values between 7.01 (July 2017; Lucefécit) and 9.05 (July 2017, Zebro). The water temperature during 2017 ranged between 6.5 °C (January; Amieira) and 29 °C (July; Zebro) while in 2018 it varied between 10.5 °C (January, Lucefécit) and 34 °C (September; Amieira in the intermittent phase). These values are closely connected to air temperatures that in 2017 presented the highest monthly maximum in July (34 °C), with abnormally high values already from April, whereas in 2018 the highest monthly maxima were observed later, in August and September (36 °C and 32 °C, respectively) (Fig. 2). The higher water temperatures with long daylight periods may be important factors for the increment of biodegradation rates and phototransformation mechanisms of some pharmaceuticals (Barbosa et al., 2018). Furthermore, temperature and pH may influence the dynamics of pharmaceuticals among the water/sediment abiotic compartments, due to some variability of the logarithm of octanol-water partition coefficient ($LogK_{ow}$) (Mandaric et al., 2019; Nunes et al., 2019). Thus, the high solubility in water that occurs for compounds with a $LogK_{ow} < 2.5$ (25 °C), such as sulfamethoxazole, propyphenazone and trimethoprim (see Table 2), may increment the degradation processes and contribute to the low concentrations quantified in the streams. Zebro and Álamos were the streams that presented higher values of electrical conductivity, positively correlated with the urban ($R = 0.41$; $p < 0.05$) and livestock land cover classes ($R = 0.59$; $p < 0.05$), which was already reported by other authors (Bonansea et al., 2016; Rodrigues et al., 2018). To refer also that Álamos and Zebro drainage basins present very low percentages of natural and semi-natural vegetation areas (about 5%), in comparison with Amieira and Lucefécit basins that present almost 40% of this land cover type (Fig. 1b).

3.2. Pharmaceuticals occurrence

Relatively to pharmaceuticals, the study analysed 27 compounds, belonging to 7 different therapeutic classes, in 44 samples from four

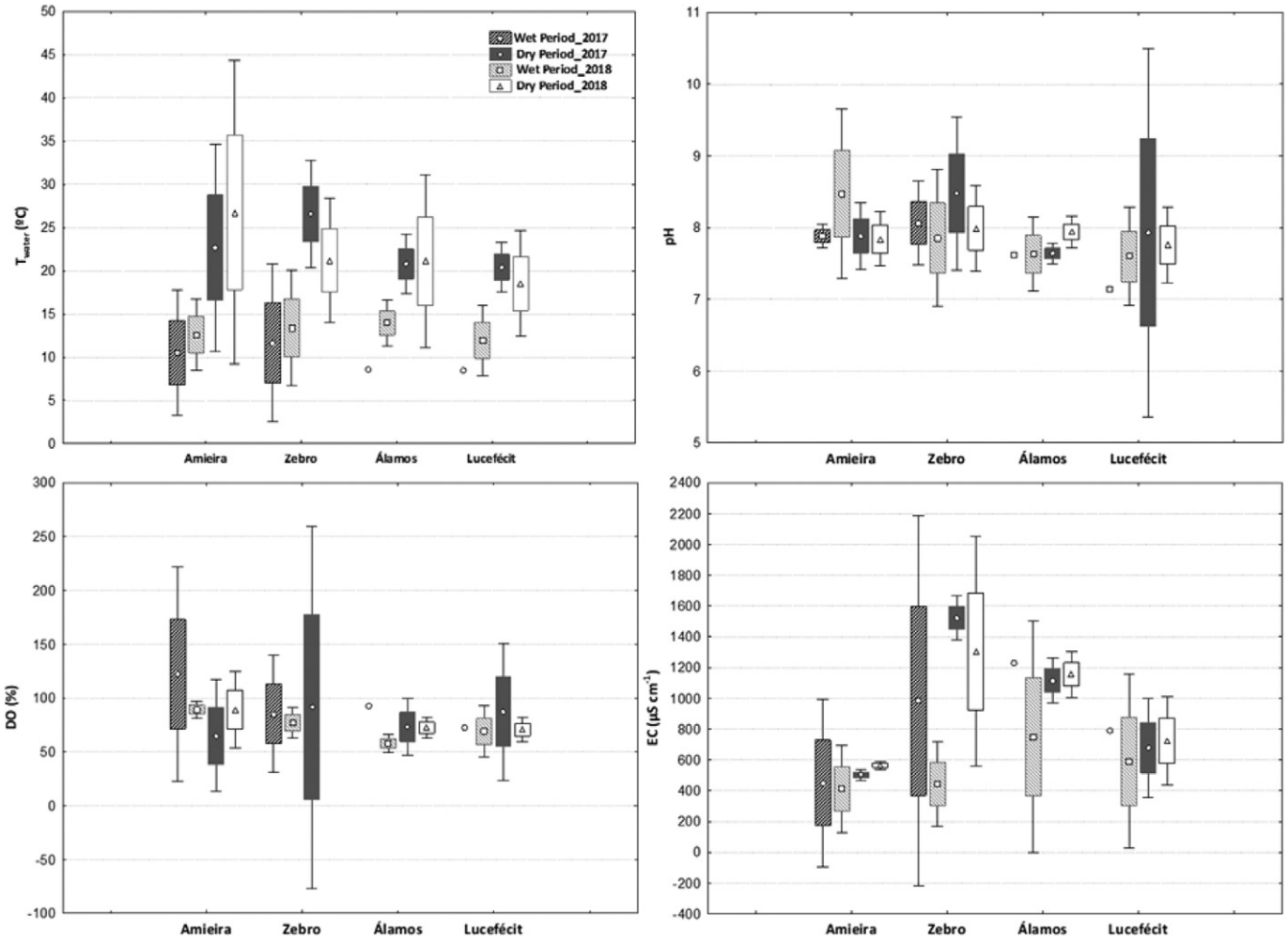

Fig. 3. Spatial and temporal (seasonal and yearly) variations of the in situ parameters measured in each stream. The mark across the box represents the mean, and the top and bottom of the box show the standard error, and the whiskers represent the standard deviation.

streams, distributed by two seasonal periods in two years, with distinct climate characteristics (2017 a drought year; 2018 a pos-drought year). The results of the principal therapeutic classes, detection frequencies, means, maximum concentrations and dynamics of the most representative pharmaceuticals, are displayed in Table 3, Figs. 4a, b and 5.

3.2.1. Pharmaceutical therapeutic groups

In general, the group of ANALG/NSAIDs was the most frequently detected (25–71%), followed by the class of diuretics (2–40%), and psychotropic drugs (1–38%), for both years (see Fig. 4a). Similar results were reported for other Mediterranean surface waters, such as waters from the Evotras River, at the Balkan Peninsula (Mandaric et al., 2019), Mondego and Tâmega rivers, in Portugal (Barbosa et al., 2018), and the natural Park of Pego-Oliva Marshlands in Valencia (Vazquez-Roig et al., 2012). In fact, Fekadu et al. (2019), in a study about pharmaceuticals in European and African freshwaters, reported that ANALG/NSAIDs were the most used and quantified drugs in European countries. An analysis of the consumption data in Portugal, obtained through the World Health Organization (WHO) Collaborating Centre for Drug Statistics Methodology (2019), shows that the averages of the defined daily doses (DDD) during the years of study (2017–2018) for NSAIDs, diuretics and psychotropic drugs were 70.8–71.7, 51–52 and 20–21 DDD per 1000 inhabitants per day, respectively (https://www.oecd.org/els/health-systems/pharmaceuticals.htm).

Among the analysed compounds of the ANALG/NSAIDs group, the most represented were diclofenac (total sum of concentrations 21,569 ng L^{-1}), ibuprofen (14,908 ng L^{-1}) and naproxen (12,709 ng L^{-1}). Hydrochlorothiazide (21,816 ng L^{-1}) was the most quantified drug within diuretics, while carbamazepine (7978 ng L^{-1}) predominated within the class of psychotropic drugs. The importance of these drugs (relatively to the detection frequency and concentrations), in each therapeutic class, has already been reported in other Mediterranean surface waters (Mandaric et al., 2019; Paíga et al., 2016; Pereira et al., 2017).

Fig. 4b shows that the year of 2017 (drought year) presented the highest concentrations of pharmaceuticals highlighting the correlation of extreme climate conditions with the increased presence of this group of emerging compounds in the waters ($R_{ibuprofen} = -0.47$, $p < 0.05$; $R_{ofloxacin} = -0.69$, $p < 0.05$; $R_{metoprolol} = -0.36$, $p < 0.05$).

3.2.2. Detection frequencies and concentrations of pharmaceuticals

All the 27 pharmaceuticals assessed were found in some or most of the samples analysed. In general, all the samples presented >15 compounds at levels higher than the corresponding MDLs. No pharmaceutical was detected in 100% of the samples. Propyphenazone and paroxetine were the least recurrent compounds, quantified occasionally, at Álamos (568 ng L^{-1}; January 2018) and Zebro (111 ng L^{-1}; Maio 2017), respectively (Table 3). Despite that, the concentrations detected were higher than in other Portuguese rivers, such as at Lis River (north Portugal), where paroxetine was detected at maximum concentrations of 25 ng L^{-1} (Paíga et al., 2016). Propranolol was only quantified at Álamos, with a FD of 70% and a mean concentration of

Table 3
Frequency of detection (FD) (%), and mean (ng L^{-1}) and maximum concentrations (Max) (ng L^{-1}) of individual pharmaceuticals in surface water samples, from 4 sampling stations in the Guadiana Basin, during the period of study (January 2017–November 2018).

	Amieira			Zebro			Álamos			Lucefécit		
	FD (%)	Mean (ng L^{-1})	Max. (ng L^{-1})	FD (%)	Mean (ng L^{-1})	Max. (ng L^{-1})	FD (%)	Mean (ng L^{-1})	Max. (ng L^{-1})	FD (%)	Mean (ng L^{-1})	Max. (ng L^{-1})
Analgesics/NSAIDs												
Acetominophen	45	173.91	699.60	42	77.41	176.48	30	30.00	62.50	44	5.23	7.87
Diclofenac	82	23.72	72.45	100	808.33	4208.00	100	1152.57	4806.00	89	18.09	80.25
Ibuprofen	91	72.14	255.80	83	1020.46	3082.00	100	364.02	3161.00	44	85.40	258.00
Indomethacin	27	5.38	11.18	83	32.15	121.44	100	42.32	124.00	22	43.21	80.25
Ketoprofen	45	27.47	112.64	92	68.92	321.40	100	14.72	38.49	56	12.88	43.91
Naproxen	55	50.61	90.05	83	978.77	2868.00	90	298.79	2293.50	44	9.09	18.49
Propyphenazone	nd			nd			10	568.00	568.00	nd		
Antibiotics												
Ofloxacin	45	31.70	75.72	75	31.28	106.00	60	27.87	115.18	33	31.38	80.94
Sulfadiazine	nd			8	75.24	75.24	nd			11	18.49	18.49
Sulfamethazine	9	24.94	24.94	8	3.61	3.61	10	1.62	1.62	33	59.58	156.20
Sulfamethoxazole	27	12.93	19.29	17	69.88	110.55	40	105.65	204.30	11	1.70	1.70
Trimethoprim	36	5.33	9.00	17	21.89	38.42	50	6.36	11.36	11	3.09	3.09
Clarithromycin	55	6.02	9.93	75	50.64	243.40	90	57.62	306.30	33	4.05	4.49
Psychotropic drugs												
Diazepam	9	1.13	1.13	25	4.70	9.32	70	4.50	7.12	11	0.78	0.78
Lorazepam	45	35.72	96.47	67	57.43	112.82	70	76.78	140.68	78	27.08	73.64
Paroxetine	nd			17	60.65	110.98	nd			nd		
Carbamazepine	100	49.37	91.32	67	63.61	425.20	100	689.65	3235.00	89	6.90	26.14
Lipid regulators												
Bezafibrate	nd			100	328.02	1672.40	70	20.81	120.30	11	0.48	0.48
Gemfibrozil	9	4.59	4.59	75	22.95	66.96	90	69.74	428.65	nd		
Fenofibrate	18	2.89	3.22	8	1.91	1.91	10	6.75	6.75	22	16.21	30.51
β-Blockers												
Atenolol	36	16.55	23.66	83	44.13	130.08	70	90.50	307.40	nd		
Metoprolol	27	12.13	22.46	58	115.08	333.20	80	48.64	147.80	33	1.65	2.06
Propranolol	nd			nd			70	46.71	188.00	nd		
Sotalol	9	3.36	3.36	25	10.84	12.57	90	240.03	656.80	nd		
Diuretics												
Furosemide	18	5.49	9.90	92	886.79	6894.00	100	288.87	1073.40	33	16.46	43.91
Hydrochlorothiazide	73	57.48	239.44	100	877.94	2678.00	100	1057.53	2726.00	100	49.96	156.20
Opioids												
Codeine	9	5.99	5.99	58	56.12	131.96	40	30.16	73.60	nd		

nd – not detected.

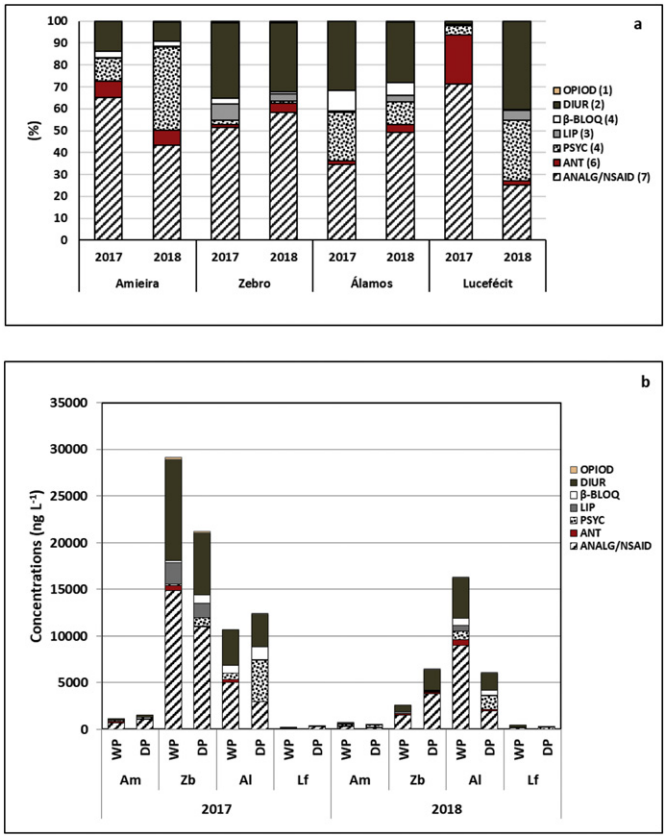

Fig. 4. Spatial and temporal variability of the therapeutic groups analysed: (a) Percentages of therapeutic groups in each stream, in both years. The total number of pharmaceuticals analysed per group are given in brackets. (b) Sum concentration of the therapeutic groups (ng L^{-1}) in each period (dry period: DP; wet period: WP) at the four streams (Amieira: Am; Zebro: Zb; Álamos: Al; Lucefécit: Lf). OPIOD: opioids; DIUR: diuretics; β-BLOQ: β-blockers; LIP: lipid regulators; PSYC: psychiatric drugs; ANT: antibiotics; ANALG/NSAID: analgesics and nonsteroidal anti-inflammatory drugs.

46.7 ng L^{-1} (Table 3), levels higher than those detected at the Douro river (North Portugal) (Madureira et al., 2010) and at freshwaters of the Pego-Oliva Marshlands (Valencia) (Vazquez-Roig et al., 2012). In general, the low levels detected, in spite this β-blocker be commonly used in Portugal (https://www.oecd.org/els/health-systems/pharmaceuticals.htm), are probably due to its low excretion rate in an unchanged form (<1%) (Ternes, 1998) and its high removal rates through WWTPs (96%) (Ternes, 1998). Accordingly, the concentrations detected may be correlated with low efficiency removal rates of the drug in the WWTPs located at Reguengos de Monsaraz and S. Pedro do Corval (both nearest at the sampling point; Fig. 1).

The most recurring and abundant pharmaceuticals were the anti-inflammatories diclofenac (FD: 82–100%; max. 4806 ng L^{-1} at Álamos-Nv2017) and ibuprofen (FD: 44–100%; max. 3161 ng L^{-1} at Álamos-Jn2018), the diuretic hydrochlorothiazide (FD:73–100%; max. 2726 ng L^{-1} at Álamos-Nv2017), and the antiepileptic drug carbamazepine (FD: 67–100%; max. 3235 ng L^{-1} at Álamos-Sp2017) (Table 3, Fig. 5). Most of the maximum individual concentrations occurred in 2017 (year of drought), in the temporary stream of Álamos, when the flows were very low or inexistent. Additionally, at Álamos the concentrations of diclofenac have always been above 9 ng L^{-1} (maximum acceptable detection limit proposed for this compound in the 2015 Watch List, which should be at least as low as the predicted no-effect concentration (PNEC)) (ECC, 2000). In fact, the concentrations found were much higher than those reported in other Portuguese rivers (Barbosa et al., 2018; Madureira et al., 2010; Paíga et al., 2016; Pereira et al., 2016) and also in other Mediterranean surface waters (Desbiolles et al., 2018; Mandaric et al., 2019; Stasinakis et al., 2012; Vazquez-Roig et al., 2012), indicating high contamination of the Guadiana basin streams by this drug. Up to now, only one study has assessed pharmaceuticals in the Guadiana River (near the estuarine area: Alcoutim; during the hydrological years of 2014/2015), at the time the researchers did not detect any of the 23 pharmaceuticals analysed (Pereira et al., 2017). Relatively to the anti-inflammatory drugs, the levels detected are probably more correlated with their high consumption rates, poor removal in the local WWTPs and hydrological regime of the streams (evident by the significant correlation among this drugs and flow: $R_{ibuprofen} = -0.47$, $p < 0.05$; $R_{diclofenac} = -0.32$, $p < 0.05$), than with their properties, provided that diclofenac and ibuprofen have $LogK_{ow} > 3.5$ (see Table 2), which would favor their adsorption to the sediments, and very short degradation times in water. In contrast, the concentrations of carbamazepine may be correlated with its properties ($LogK_{ow}$ of 2.45; large degradation time in water (up to 120 days)) and with the fact that it is one of the drugs least removed at WWTPs worldwide (<10%) (Paíga et al., 2016; Pereira et al., 2016; Ternes, 1998). These four pharmaceuticals presented significantly high variability among streams ($p < 0.005$; Kruskal-Wallis ANOVA by Ranks). Concerning the analgesic acetaminophen, the concentrations detected in Amieira (<MDL-670 ng L^{-1}) and Zebro (<MDL-177 ng L^{-1}) were similar or lower than those detected in the Lis River (up to 527 ng L^{-1}) and the Leça River (up to 925 ng L^{-1}) in the North of Portugal (Gonçalves et al., 2013; Paíga et al., 2016) and in other Mediterranean freshwaters (Vazquez-Roig et al., 2012). Detection frequency was always below 45% in all 4 streams, not supporting the pseudo-persistence of acetaminophen reported by other authors (Fairbairn et al., 2016; Paíga et al., 2016).

Regarding lipid regulators, bezafibrate occurred in all samples from Zebro with a mean concentration of 328 ng L^{-1} (Table 3). The concentrations at Zebro (0.3–1672 ng L^{-1} in Nv2017) and at Álamos (<MDL-120 ng L^{-1} in Jn2018) were higher than those reported at the Mondego (FD = 5%; 15.32 ng L^{-1}) and Tagus rivers (FD = 17%; 15.86 ng L^{-1}) (Pereira et al., 2016). Similar pattern was observed for gemfibrozil, with the highest FDs and concentrations observed at Álamos (90%; <MDL-429 ng L^{-1} in Jn2018) and Zebro (75%; <MDL-67 ng L^{-1} in Sp2017). These concentrations are higher than those detected at the Llobregat Basin (Spain) (Osorio et al., 2016). The present results may be justified by the inefficiency of the WWTPs located nearest the surface waters, since the removal rate for fenofibrate is 83% and for gemfibrozil is 69%, as reported by Ternes (1998).

The antibiotic group is one of the less detected at the streams. The less detected drugs belong to the classes of sulfonamides and penicillins. The macrolide clarithromycin was the most recurrent one, presenting FD > 55%, except at Lucefécit. The maximum concentrations detected at Álamos (306 ng L^{-1} in Jn2018) and Zebro (243 ng L^{-1} in Nv2017) were of the same range as those reported for the Ave river (North of Portugal) (Sousa et al., 2019). Clarithromycin surpassed the maximum acceptable method detection limit of 90 ng L^{-1} set in the 2015 Watch List (ECC, 2000) only occasionally, but with a considerably frequency (21%) in the case of the more strict limit of 19 ng L^{-1} established later in the 2018 Watch List (ECC, 2018). In spite that the presence of macrolide antibiotics may be related to the season, since these drugs are prescribed for infectious respiratory diseases more usually in winter and spring than in summer and fall, no significant statistical correlation was found between the drug and the period (wet or dry).

3.2.3. Spatial and temporal analysis

The spatial analysis showed that Zebro (with an intermittent regime having water all the year but without flow in some periods) was the most contaminated stream with a total pharmaceutical's concentration of 59,369 ng L^{-1} (Fig. 4b). The direct discharge of the domestic wastewaters from the wastewater treatment plant (WWTP) of Póvoa São Miguel (Municipality of Moura, Fig. 1) to the stream, located at a distance of only 500 m of the sampling point, may be one of the main reasons that justify these results, since this WWTP is undersized and

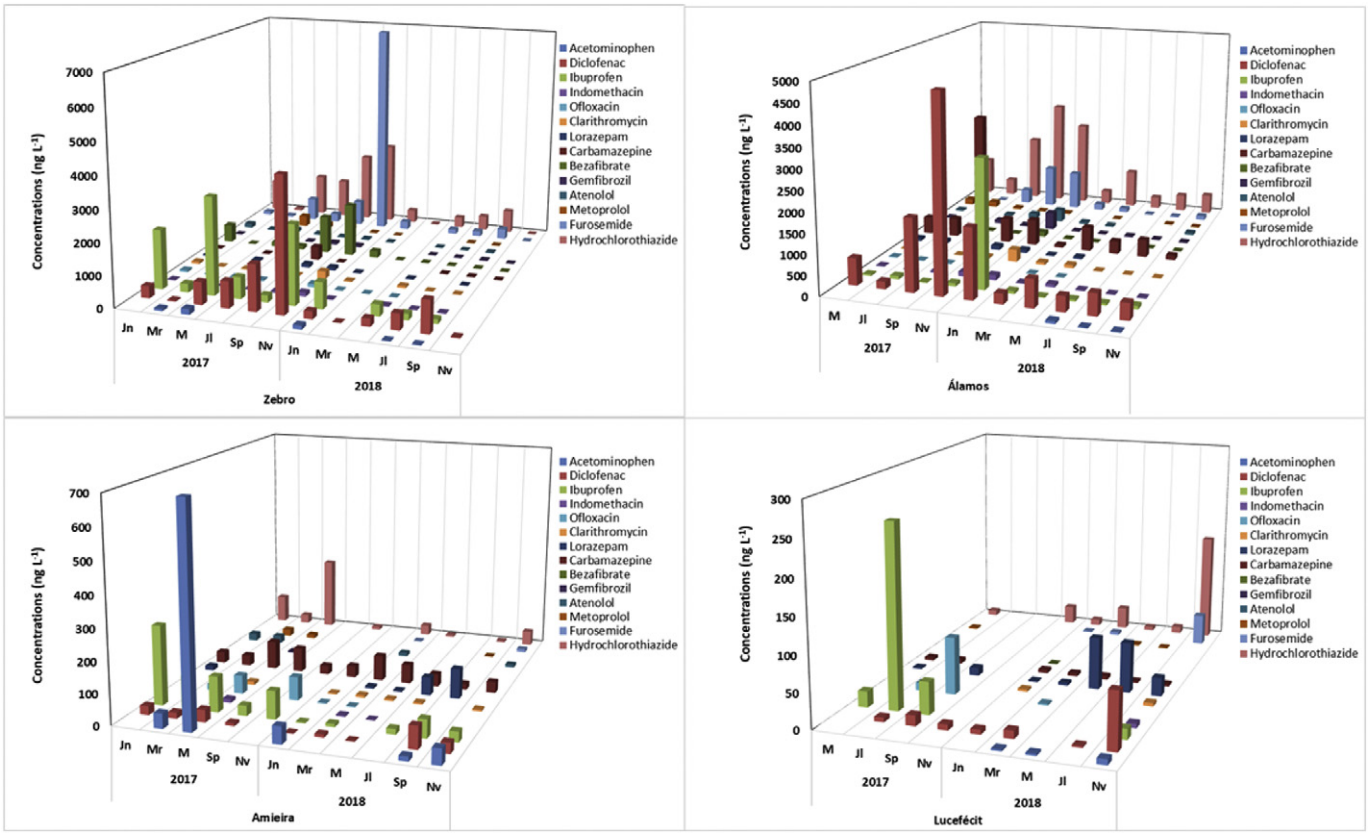

Fig. 5. Spatial (streams) and temporal (January: Jn; March: Mr; May: M; July: Jl; September: Sp; November: Nv) patterns observed for the pharmaceuticals quantified at higher concentrations (ng L^{-1}), during 2017 and 2018.

obsolete. Numerous authors have previously reported the importance of WWTPs in the pollution processes of pharmaceuticals (aus der Beek et al., 2016; Desbiolles et al., 2018; Vazquez-Roig et al., 2012), being this group of emerging contaminants considered markers of wastewater contamination (Čelić et al., 2019). The high concentrations observed could be also due to the absence of flow most of the time, which raises the accumulation processes, hypothesis supported by the significant negative correlations observed between flow and the concentrations of some therapeutics groups, such as ANALG/NSAIDs ($R = -0.42$; $p < 0.05$) and PSYC ($R = -0.31$; $p < 0.05$). Furthermore, in 2017, when Zebro did not present any flow (see Table 1) due to the drought conditions, the higher concentrations occurred during the wet period (31,738 ng L^{-1}; see Fig. 4b), when the temperatures and the photodegradation rates were lower. In contrast, during the post-drought year (2018), Zebro presented low flow in the wet months (Jn, Mr and Nv), and the higher concentrations of pharmaceuticals were observed in the dry period. Hence, the results highlighted that pharmaceuticals in surface waters were more dependent on flow than on temperature or daylight, as previously observed in other studies (Mandaric et al., 2019; Pereira et al., 2017). In fact, the highest individual concentrations at Zebro were achieved by furosemide (6894 ng L^{-1} at Nv2017), followed by diclofenac (4208 ng L^{-1} at Nv2017) and ibuprofen (3082 ng L^{-1} at M2017) (see Fig. 5). A different scenario occurred at Álamos, with an intermittent regime, having periods with the water concentrated only in isolated pools. In fact, in the drought year (2017), this stream presented a total concentration of pharmaceutics of 45,463 ng L^{-1}, mainly during the dry period, due to the accumulation at the pools (Fig. 4b). Results of the post-drought year showed higher levels of pharmaceuticals in the wet period, corresponding to the period when the stream presented moderate to very fast flow (due to heavy rainfall events). The high accumulation in the pools increments the adsorption to the sediment's particles for drugs with $LogK_{ow} > 2.5$, process more relevant in sediments that show higher percentages of fine particles, as occurs at Álamos (silt + clay = 33%). Consequently, when the heavy rainfall events take place (more frequently in the Mediterranean region; Reoyo-Prats et al., 2018), the intensification of flow (in the case of Álamos, in Mr and Nv 2018), and the increased resuspension of sediments, leads to a particularly high increase of concentration levels of pharmaceuticals. This process is more important in temporary rivers, where the accumulation rate is higher (Mandaric et al., 2019; Reoyo-Prats et al., 2018).

Amieira, also with an intermittent hydrologic regime, presented the highest concentrations of pharmaceuticals in 2017 (2697 ng L^{-1}) during the dry period, mostly corresponding to acetaminophen (670 ng L^{-1} in M2017) followed by hydrochlorothiazide (239 ng L^{-1}). This stream presented lower concentrations of pharmaceuticals than Álamos, which may be justified by textural characteristics of the sediments, constituted mostly by sand particles (90%), with low adsorption capacity. Additionally, Amieira is 40% covered by natural and semi-natural vegetation (Fig. 1b), which acts as a buffer to the input of contaminants (Shi et al., 2017). Further, these results also support those observed in Álamos, in the sense that in temporary rivers the low flow and the water scarcity are factors of upmost importance for the increase of pharmaceuticals` concentrations in surface waters.

Lucefécit was the less contaminated stream (1219 ng L^{-1}), probably due to its hydrology, perennial with a constant flow during the whole year, as well as to the characteristics of its sediments, constituted 41% by fine particles, which increment the dilution processes and the adsorption mechanisms, with the corresponding natural attenuation of the system (Mandaric et al., 2019). Although the livestock activity (one of the most important activities for pharmaceutical contamination; de Jesus Gaffney et al., 2015; Gogoi et al., 2018) represents >40% of the land cover, the area also presents a considerable percentage of natural and semi-natural vegetation (about 38%), which may act as a barrier

to the pollution inputs. At this stream, ibuprofen was the pharmaceutical that achieved the highest concentration (258 ng L^{-1} in Jl2017), followed by hydrochlorothiazide (156 ng L^{-1} in Nv2018).

3.3. Environmental risk assessment (ERA)

The prioritization lists of pharmaceuticals are based on the concept of environmental risk assessment (ERA), which conventionally takes into consideration the potential toxic effect of a specific pharmaceutical and its exposure level, determined by the risk quotient (RQ). Traditional pharmaceutical ranking usually assesses the risk of the substance under general (using the average of the quantified concentrations) and worst (maximum concentrations) scenarios. To refine the methodology, making it more realistic, we applied a risk quotient optimised approach (RQf), allowing highlighting the pharmaceuticals most frequently detected at concentrations eventually of concern. Fig. 6 shows the RQs corresponding to mean and maximum concentrations, and the RQf values corresponding to mean concentrations, of the compounds (8) showing risk in at least one of the sites investigated, for each stream and year. The pharmaceuticals hydrochlorothiazide, ofloxacin and acetaminophen showed moderate RQs and in general irrelevant RQf (Amieira: $RQf_{acetomiphen}$ – moderate risk, F = 20%; Zebro: $RQf_{ofloxacin}$ - endurable risk, F = 18%; Álamos: $RQf_{ofloxacin}$ – endurable risk, F = 17%) (Fig. 6). These results indicate that a potential moderate risk exists for these substances, but the possibility of the organisms to be exposed to unsafe levels of these compounds is lower than 20%. Pereira et al. (2017) also reported low risk of acetaminophen ($0.01 \leq RQ < 0.1$) in several Portuguese rivers (Tâmega, Mondego, Trancão, Tagus, Xarrama, Álamo). Vazquez-Roig et al. (2012) reported that acetominophen induced a moderate risk to crustaceans (RQ = 0.321) and ofloxacin a high risk to algae (RQ = 3.137) in surface waters of Pego-Oliva Marshlands (Valencia). Zhou et al. (2019) in a review about risk assessment of pharmaceuticals in European countries, using RQf, reported moderate risk for both substances ($RQf_{ofloxacin}$ = 0.74, F = 30%; $RQf_{acetaminophen}$ = 0.31, F = 30%). Bezofibrate showed RQf > 1 in Zebro, during the drought year (2017). Clarithromycin presented RQf > 1 in Zebro in 2017 (F = 50%) and Álamos in 2018 (F = 40%). In spite that high risk according to RQs was estimated in only a few occasions, the frequency of organisms' exposure to eventually dangerous levels is considerable. Furthermore, the high consumption of clarithromycin and its $LogK_{ow}$ of 3.16, contributing to its potential persistence and bioaccumulation (Palma et al., 2015), are important factors that justify the follow up of this substance. Clarithromycin presented moderate risk for algae ($0.1 < RQ < 0.1$) in the Portuguese rivers of Trancão, Tâmega and Xarrama (Pereira et al., 2017), and a moderate risk (RQf = 0.41) in the European context (Zhou et al., 2019). In fact, Zhou et al. (2019) has already proposed that substances with $LogK_{ow} > 3$ and those with high or moderate risk, should be both considered as priority, at the same risk level. Furthermore, clarithromycin was already included in the Watch List published in 2015 (ECC, 2015) and continues to be present in the one issued in 2018 after revision of the first one which means that it requires further monitoring for the purpose of supporting possible feature prioritization (ECC, 2018).

Carbamazepine presented RQf > 1 in Zebro 2017 (F = 50%), Álamos 2017 (F = 100%) and 2018 (F = 100%) and Amieira 2017 (F = 100%) and 2018 (F = 100%). These results highlight the danger that this drug represents to aquatic ecosystems. Studies in surface waters in the Mediterranean region reported lower risk values for this substance (Vazquez-Roig et al., 2012); however, the present results are in line with what was reported for Europe (RQf = 10.13) (Zhou et al., 2019).

Diclofenac, followed by ibuprofen, are the pharmaceuticals exhibiting the highest risk, with frequencies of organisms exposure to unsafe concentrations of these pollutants higher than 70% in Zebro, Álamos and Amieira. The fact that both compounds present $LogK_{ow} > 3$, and thus high capacity to bioaccumulate within the trophic chain, and high long-term toxicity, makes these substances a threat for the aquatic ecosystems. In the prioritization list of pharmaceuticals for European countries, diclofenac appeared with a RQf of 153.65 (F = 62%), and

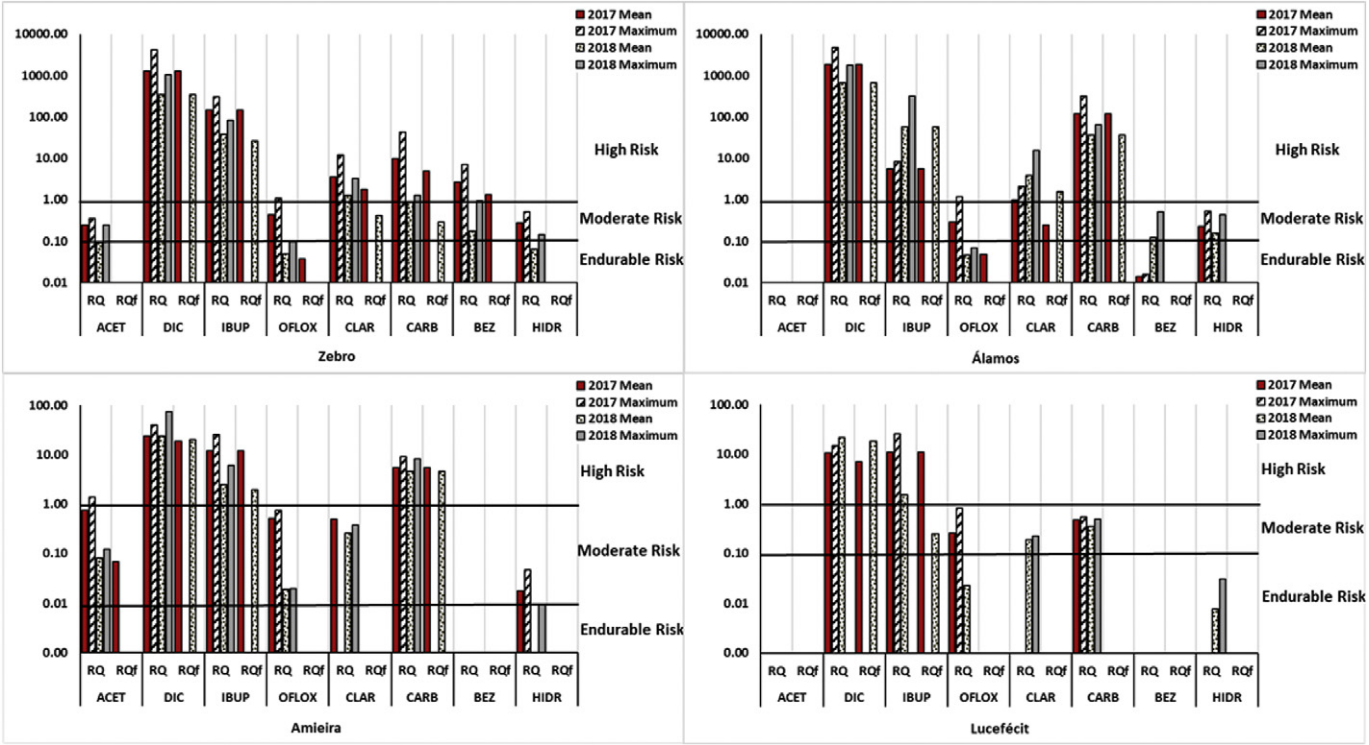

Fig. 6. The RQs calculated for mean and maximum concentrations, and the RQf values calculated for mean concentrations, retrieved from the analyses of the samples collected in 2017 and 2018, at each stream. ACET: acetaminophen; DIC: diclofenac; IBUP: ibuprofen; OFLOX: ofloxacin; CLAR: clarithromycin; CARB: carbamazepine; BEZ: bezafibrate; HIDR: hydrochlorothiazide.

ibuprofen with a RQf of 15.73 (47%) (Zhou et al., 2019). Moreover, in 2016 aus der Beek et al. (2016) reported that diclofenac is the most often detected pharmaceutical in the environment and that, due to its high ecotoxicological potential, it is becoming one of the most hazardous pharmaceuticals at global scale. Likewise, a European demonstration program for chemical identification and effect-based monitoring of organic pollutants in surface waters has recently included 8 pharmaceuticals in a list of 21 target compounds prioritized on the basis of their frequencies and extent of exceedance of PNECs (Tousova et al., 2017). Four of them are ranked in our work as having high or moderate risk (RQf), namely, diclofenac, ibuprofen, carbamazepine and clarithromycin. As clarithromycin, diclofenac was included in the first Watch List. However, unlike clarithromycin, diclofenac has been removed from the updated version of the Watch List published in 2018. The question now, after the most recently published studies and the present one, is whether diclofenac should be reconsidered as a potentially hazardous priority substance.

Finally, spatial and temporal analysis of the data showed that Zebro and Álamos were the streams under higher environmental risk by pharmaceuticals, mainly during dry periods. Therefore, the climatic conditions affected the hydrological regime, incrementing the stress factors to the aquatic ecosystems. Streams with temporary hydrological regimes (with no flow or without surface water in the whole channel during a period) became more vulnerable to the contamination by organic pollutants, in this case by pharmaceuticals, as highlighted by the results. In fact, Lucefécit, the only stream that always presented flow, showed lower risk scenarios. In this stream, diclofenac and ibuprofen were the compounds showing higher risk, mainly in the dry period (2017) with F higher than 70%.

4. Conclusions

This work put into evidence the presence and persistence of pharmaceuticals in streams of the Guadiana basin. Notwithstanding the high variability of concentrations observed among streams, periods and years, all 27 pharmaceuticals investigated were detected at quantifiable concentrations at least once. The most abundant pharmaceuticals were diclofenac, ibuprofen, carbamazepine and hydrochlorothiazide, all of them measured at levels that represent moderate to high risk for the aquatic ecosystems.

The streams investigated presented different hydrological regimes and sources of pollution. The presence of the pharmaceuticals found in the waters is attributed mainly to the continuous discharge of these substances from WWTPs, due to their incomplete removal at the facilities, as well as to intensive livestock.

The results obtained also showed the negative influence of drought periods, with the strengthening of no flow regimes, and the consequent increment of pharmaceuticals` concentrations. In the streams with temporary regimes, which are expected to increase in the Mediterranean region due to climate changes, the highest concentrations of pharmaceuticals were quantified in the dry period if the stream presented sediments with textures constituted mostly by sand as in the case of Amieira. On the contrary, in Álamos (with a higher percentage of fine particles), the highest concentrations of pharmaceuticals were detected after the rainfalls, due to the resuspension of the substances from the sediments.

The results showed that the use of the optimised risk assessment method, considering the frequency of concentrations above PNECs, is more realistic, and allows the prioritization of pharmaceuticals integrating the persistence as a key factor for the risk in the ecosystem. Consequently, the results support its application for screening-level risk assessment. The rank of the 8 pharmaceuticals that presented risk at the streams in the Guadiana basin is: (i) High Risk: diclofenac, ibuprofen, carbamazepine; (ii) Moderate Risk: clarithromycin; (iii) Endurable Risk: acetaminophen, ofloxacin, bezafibrate; (iv) Negligible risk: hydrochlorothiazide. This work displays that this group of 8 substances should be integrated in programs of water resource management, given priority to high-risk pollutants that are widely distributed and frequently detected.

Results highlighted that some surface waters from South Portugal are impacted with pharmaceuticals, particularly when looking up the increment of risk scenarios for the aquatic ecosystems in drought periods and after heavy rainfalls. Therefore, further research is needed to increment the knowledge about the influence of hydrological regimes, the effect of the intensification of dry periods, the response of different biological systems to the recovery, and the chronic toxic effects of the priority substances to improve water management programs in the Mediterranean region.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.136205.

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