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Patterns of pharmaceutical contamination in streams of European cities across urbanisation gradients: Potential impacts on One Health

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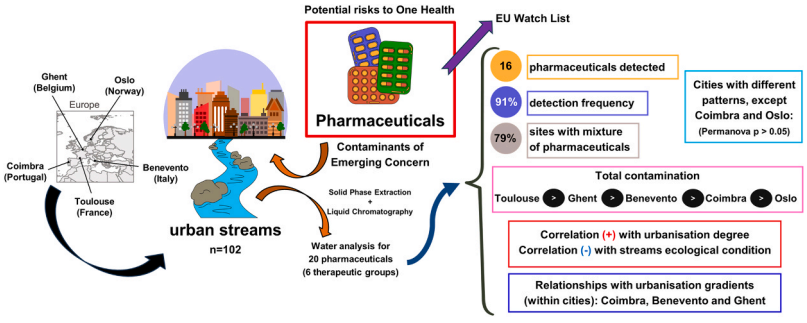
HIGHLIGHTS

- Sixteen pharmaceuticals were detected, mostly above concentrations ever reported.
- Pharmaceuticals and their mixtures were in 91 % and 79 % of sites, respectively.
- Pharmaceuticals from European Watch List 2025 were found in 4 cities.
- Urbanisation's influence was negative, while the ecological condition was positive.
- Three cities had relationships between pharmaceuticals and urbanisation gradients.

GRAPHICAL ABSTRACT



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ABSTRACT

Pharmaceuticals are Contaminants of Emerging Concern, with known effects on aquatic organisms such as increased mortality and reproduction inhibition. This study aimed to provide a comprehensive overview on occurrence of pharmaceuticals in European urban streams, and to assess the patterns and factors influencing their

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Urban areas
Water quality

presence in the water. Sixteen pharmaceuticals from 6 therapeutic groups were detected in 102 sites sampled in 2022–2023 within 5 European cities (Coimbra-Portugal, Toulouse-France, Benevento-Italy, Ghent-Belgium, and Oslo-Norway). Pharmaceuticals and their mixtures were observed in 91 % and 79 % of the sites, respectively. Irbesartan, bisoprolol and carbamazepine were found in more than 50 % of the sites. Acetaminophen had the highest median concentration (near 1 μg/L). Toulouse had the higher total pharmaceutical concentration, followed by Ghent, Benevento, Coimbra and Oslo. Contaminants from the European Union Watch List (ofloxacin, propranolol, fluoxetine) were detected. The pharmaceutical patterns differed among cities, except between Coimbra and Oslo. The contamination by pharmaceuticals, considering all sites, was predicted by population and tree cover densities, but also by morphological condition, riparian zone, water pollution and imperviousness within some of the cities. This study reveals a high contamination of urban streams by pharmaceuticals, which could lead to risks for the health of aquatic organisms and humans.

1. Introduction

The presence of pharmaceuticals in water bodies at trace concentrations (ng/L-μg/L) is considered an emerging issue, because they persist in freshwater ecosystems and may lead to multidrug-resistant microorganisms [1,2]. Therefore, pharmaceuticals are classified as Contaminants of Emerging Concern (CECs) that provide additional risks for the health of animals, plants and humans, *i.e.*, for One Health [3–5]. More urgently, the environmental persistence and accumulation of antibiotics contribute to the rise of antimicrobial resistance (AMR), one of the gravest public health challenges nowadays. From a human health perspective, the presence of pharmaceuticals in drinking water sources, even at trace levels, raises concerns about long-term exposure effects, particularly for vulnerable populations such as pregnant women and children.

The European Union has used the Watch List mechanism to prioritise the monitoring of emerging contaminants since the 2013 update of the Water Framework Directive (WFD). The goal of the sequential watch lists is to gather data to establish future priorities, focusing on contaminants for which the available information is still insufficient to assess their risks and draw conclusions about their effects [6]. The current Watch List was recently published in March 2025 [7] and includes 29 substances, including pharmaceuticals belonging to different therapeutic groups (*e.g.*, the antibiotics ofloxacin, oxytetracycline and tetracycline, the antihypertensive propranolol, as well as the antidepressant fluoxetine).

Pharmaceuticals can reach freshwater ecosystems through multiple pathways, including human and animal excretion, improper disposal of medications, pharmaceutical manufacturing waste, stormwater runoff, and particularly through wastewater discharges. Wastewater treatment processes are not yet effective enough to remove all pharmaceuticals, resulting in the release of improperly treated water, making Wastewater Treatment Plants (WWTPs) a major source of pharmaceuticals for contamination of connected water bodies. WWTPs usually discharge treated waters into larger rivers, although some streams also receive them [8]. Other infrastructures can also contribute to the leakage of pharmaceuticals, reaching rivers and streams, namely Combined Sewer Overflows (CSOs), Sanitary Sewer Overflows (SSOs), septic tanks, landfill sites, and industrial wastewater treatment plants [9,3,10], as well as the irregular discharges.

Due to the growth of the urban population, as well as the increased use of pharmaceuticals associated with longer life expectancy [11] and the number of new drugs being frequently approved, pharmaceutical contamination of freshwater ecosystems is expectedly more significant in urban areas. Among freshwater ecosystems, streams (*i.e.*, small water courses with *ca.* 1–5 m wide) are prone to higher concentrations of pharmaceuticals, which could lead to higher risks for One Health compared to larger rivers due to: (i) lower flow rates, making contaminants dispersal slower and poor dilution; (ii) proximity to the population, as they spread throughout the cities; and (iii) high levels of artificialisation, water retention and abstraction [12]. However, urban streams provide important ecosystem services that can be jeopardised by water contamination, and that are of great importance for biodiversity and human well-being [13–16].

Pharmaceutical contamination in streams impact the health of aquatic organisms by increased mortality, impaired growth, morphological alterations, endocrine disruption, reproduction inhibition, hatching disorder and bioaccumulation [17,18,5,19,20]. It has consequences at ecosystem level, such as decreased gross primary production and biomass [21]. Also, Environmental Risk Assessments (ERA) of urban streams concluded that pharmaceuticals such as paracetamol, caffeine and fluoxetine pose a high risk to aquatic invertebrates [5,8].

Despite the important health risk for both humans and aquatic biodiversity, studies focusing on pharmaceuticals in urban streams, compared to those in urban rivers, are still fewer worldwide (*e.g.*, [22–25]), as well as in Europe (*e.g.*, [26–29]). Moreover, differences in the analytical methods used and the pharmaceuticals analysed across studies make direct comparisons challenging, limiting a comprehensive understanding of the global occurrence and impact of pharmaceuticals in urban streams [12].

To address this gap, a standardised, multi-city assessment of 20 pharmaceuticals from different therapeutic groups was conducted across 102 urban streams in five European countries (Portugal, Italy, France, Belgium, and Norway). In addition, the present study aimed to assess the distribution patterns and the factors related to urbanisation and stream condition influencing the presence of these contaminants in freshwater ecosystems. For this purpose, streams were selected along urbanisation gradients within each city, and pharmaceutical occurrence was related to both urban land use and stream ecological conditions. This design allowed to evaluate both anthropogenic and environmental drivers of pharmaceutical contamination across diverse European settings, using a consistent analytical protocol. The tested hypotheses were: (i) the patterns of pharmaceuticals in urban streams differ among cities; (ii) the concentration and diversity of pharmaceuticals are influenced by urbanisation; and (iii) by ecological conditions of streams (*e.g.*, riparian vegetation, habitats, hydromorphology), independently of the city.

The findings of this study will contribute to the design of enhanced water management practices aligned to the One Health goals, by improving the ecological condition of urban streams. Better ecological conditions can promote the natural filtration of pharmaceuticals by sediments and plants [30,31], decreasing their availability in water and reducing health risks for human and animals, when promoting biodegradation.

2. Materials and methods

2.1. Study areas and sampling sites

This study was performed in five cities, located from the southwest to the north of Europe: Coimbra (Portugal), Benevento (Italy), Toulouse (France), Ghent (Belgium) and Oslo (Norway) (Fig. 1). According to Köppen-Geiger classification [32], the cities have temperate and cold climates, varying from a hot-summer mediterranean climate in Coimbra and Benevento to a warm-summer humid continental climate in Oslo (Table 1). The city with the largest number of inhabitants is Oslo, followed by Toulouse, Ghent, Coimbra and Benevento (Table 1). Regarding

nature conservation, the northern European countries have longer traditions in the conservation and restoration of rivers, with 32 projects started before 2000 against 21 from the central countries and 13 from the southern ones [33]. Nevertheless, these environmental practices are more disseminated throughout Europe nowadays, with a total of 84 registered projects in France, 40 in Italy, 34 in Norway, 13 in Belgium and 7 in Portugal [33].

Approximately 20 samples were collected in each city (including their outskirts and nearby urban areas), in a total of 102 sampling sites (Fig. 1; Supplementary Materials (SM) – Table S1), from different streams. The streams were previously selected based on a gradient of urbanisation considering constructed areas near the streams, but also the integrity of the riparian vegetation, banks, aquatic habitats, water pollution (mostly by nutrients), and longitudinal connectivity (presence

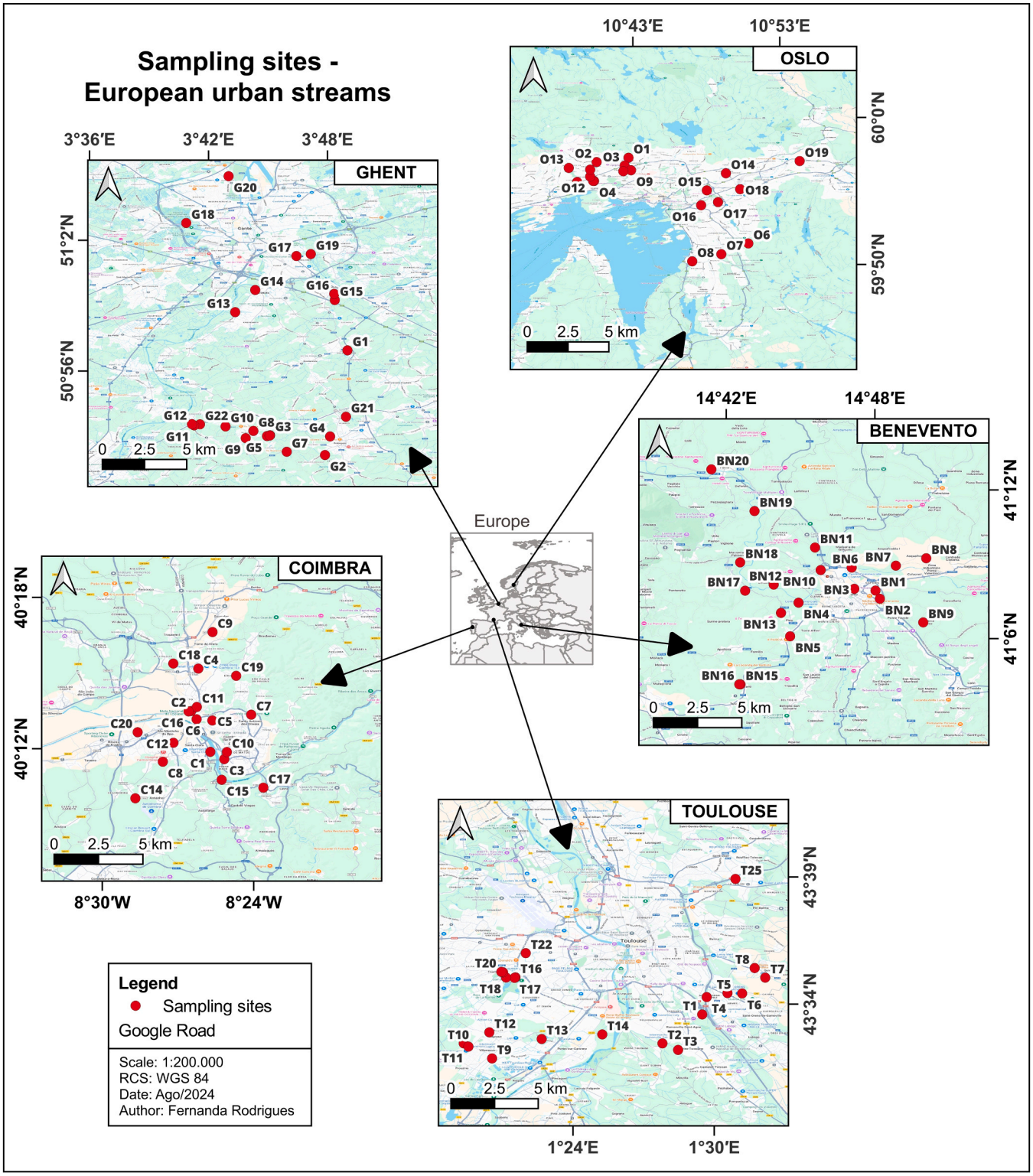

Fig. 1. Location of the sampling sites (n = 102) in the five European cities: Coimbra (Portugal; n = 20), Benevento (Italy; n = 19), Toulouse (France; n = 21), Ghent (Belgium; n = 22), and Oslo (Norway; n = 20).

of transversal barriers). Each sampling site included at least a 100 m stretch of open and accessible stream, with a width between 1.5 and 8 m, a depth between 0.25 and 0.7 m, and a flow velocity between 0 and 1.5 m/s during the sampling season, allowing for a standardised sampling.

The stream condition and level of urbanisation around the stream was assessed *in situ* using five categorical variables, in an approach similar to the one previously described [41]: urbanisation level visible from the stream (UL), stream connectivity (SC), riparian zone (RZ), morphological condition (MC), and water pollution (WP). At each stream site, each variable was assessed by attributing scores from 1 (near natural condition) to 5 (very different from the natural condition) (Table 2).

In addition, public databases were used to obtain information about population density (PopD), imperviousness density (IMD) and tree cover density (TCD) inside a buffer zone of 500 m ratio (geodesic distance) around each sampling site. All spatial data were projected using the ETRS 1989 LAEA (Lambert Azimuthal Equal-Area) projection to ensure accurate area and distance calculations. PopD (inhabitants/km^2) was extracted from a dataset of population statistics across Europe by local administrative units provided by Eurostat [42] and derived as a weighted mean within the 500 m buffer around each sampling site. The weighting was based on the proportion of each local administrative unit's area that intersected the buffer. IMD (%) and TCD (%) were calculated using raster data from Copernicus Land Monitoring Service at 10 m resolution ([43,44], respectively) as the weighted mean within each buffer, using the area of the raster cells as the weight. The calculations were carried out with the 'exactextractr' package (version 0.10.0) in R software.

2.2. Water sampling and pharmaceuticals analysis

One litre of water was collected from each urban stream between November 2022 and September 2023 under similar environmental conditions regarding temperature and hydrological patterns in each region, against the flow and below the surface, using a high-density polyethylene (HDPE) bottle pre-cleaned with 10 % nitric acid, then with ultrapure water, and rinsed with water *in situ* before sampling. The samples were transported in a cooler box at *ca.* 4 °C, acidified to pH = 3 with formic acid (98–100 % P. A., Merck), and stored at least at -20 °C until processing.

The pharmaceuticals set for analytic determination were selected *a priori* based on the following criteria, according to a previous study [45]: (i) being used for treatments of human diseases; (ii) high consumption rates; (iii) persistence in the environment; (iv) toxicity level; (v) frequency of detection; and (vi) technical capacity for detection. Consequently, the 20 pharmaceuticals analysed in this study belong to six therapeutic groups: antibiotics; anticonvulsants; antihypertensives; analgesics/antipyretics; lipid regulators; and psychopharmaceuticals.

Water samples were filtered (after the defrost) in three consecutive steps using a vacuum pump with a paper filter (no. 4, diameter of 9 cm, Whatman) and with nylon membrane filters (0.45 and 0.22 μm pore sizes, diameters of 4.7 cm, Filter-Lab). For the extraction procedure [45], 500 μL of an internal standard solution comprising 10 μg/mL of sulfameter (previously prepared using sulfameter with purity > 99 %, Sigma-Aldrich) were added to 500 mL of the filtered water sample. A solid phase extraction (SPE) cartridge (Oasis PRiME HLB 6 cc, 200 mg, 6 mL, Waters) was pre-conditioned with 2 mL of methanol (≥ 99.9 % for HPLC, Honeywell) and 2 mL of ultrapure water (Milli Q). The purification and concentration of the sample were carried out by loading the water sample in the cartridge and washing it with 5 mL of methanol: water solution (10:90), and then drying at low vacuum pressure. The elution was performed with 6 mL of methanol, and this extract was evaporated under N_2 flow (Air Liquid) at 40 °C. Then, the extract was redissolved with 500 μL of formic acid 0.1 % (v/v) in water (previously prepared using formic acid 99–100 % P.A., Merck) and filtered with a 0.45 μm filter. During all steps of the sample preparation, light exposure was avoided.

The detection and quantification were performed by injecting 10 μL of the final extract into the UHPLC-ToF-MS system (UHPLC coupled with a high-resolution mass spectrometer, a Time-of-Flight Triple TOFTM). The UHPLC equipment was composed of a vacuum degasser, an autosampler with a programmed temperature (10 °C), a binary pump, and a compartment for the chromatographic column at a controlled temperature (40 °C). The column used was a reverse-phase one. The mobile phases used were A—formic acid 0.1 % (v/v) in water, and B—acetonitrile Merck (Darmstadt, Germany), at a flow rate of 500 μL/min and following the gradient as described: 0–5 min – from 97 % to 40 % [A]; 5–9 min – from 40 % to 0 % [A]; 9–10 min – from 0 % back to 97 % [A]; and 10–12 min (end of chromatographic run) – maintained 97 % [A]. For the detection, the mass spectrometer ToF-MS operated with ionisation in positive mode and an electrospray ion source (ESI+) working in full-scan data acquisition mode. In terms of identification criteria, the parameters evaluated were the exact mass, with a maximum acceptable error of 5 ppm, relative retention time deviation below 1 % (Commission Implementing Regulation (EU) 2021/808), and isotope pattern similarity with a maximum of 10 % difference (between the obtained and the theoretical). This method of analysis was previously validated for the identification and quantification of the 20 chosen pharmaceuticals [45]. The limits of detection (LODs) are listed in this previous study and the concentrations bellow them were considered zero.

2.3. Data analyses

Descriptive statistics (sum, median, average, standard deviation, and detection frequency) were used to compare pharmaceuticals and therapeutic groups across cities and within cities. For multivariate analyses, pharmaceutical concentrations were *a priori* transformed by Log(x + 1), to reduce differences in scale. The Euclidean distance was the distance

Table 1
Location, climatic conditions and socio-economic characteristics of the five European cities studied. Lat. – latitude; Long. – longitude; climate type, precipitation and temperature according to Köppen-Geiger classification (historical period 1991–2020); GDP – Gross Domestic Product (million euro; reference year: 2023).

City	Country	Lat. Long.[1]	Climate type[2]	Precipitation[2]	Temperature[2]	Population (municipality)	GDP[8] (country)
Coimbra	Portugal	40.2056 -8.4196	Temperate	Dry summer	Hot summer	144,822[3]	267,384
Benevento	Italy	41.1333 14.7500	Temperate	Dry summer	Hot summer	56,048[4]	2128,001
Toulouse	France	43.5995 1.4332	Temperate	No dry season	Hot summer	504,078[5]	2822,455
Ghent	Belgium	51.0500 3.7167	Temperate	No dry season	Warm summer	263,703[6]	596,321
Oslo	Norway	59.9167 10.7500	Cold	No dry season	Warm summer	716,261[7]	448,717

[1] [34]; [2] [32]; [3] [35]; [4] [36]; [5] [37]; [6] [38]; [7] [39], [8] [40].

Table 2
Description of the scores of the categorical variables used to assess the urbanisation level and stream condition *in situ*.

Categorical variable	Score 1	Score 2	Score 3	Score 4	Score 5
Urbanisation level (UL)	No urbanisation or just 1 or 2 houses or non-impervious small roads, green areas/gardens surroundings	Urban areas with some constructions (houses, roads), but stream surroundings have small impervious areas	Urbanisation with housing/ residential areas and roads with soil sealing up to 50 % of the site length	Very dense urban areas, extended surroundings with soil sealing surface	Heavily urbanised around the site, mostly impervious surroundings along the stream section
Stream connectivity (SC)	No barriers or existence of a "bypass-type" device	Small barriers that allow the passage for most species all over the year	Some barriers that allow the passage of certain species	Barriers that can allow the occasional passage of a single species	Perfectly defined artificial barrier (dams/weirs) preventing completely the passage of water or fish
Riparian zone (RZ)	Continuous riparian corridor with native species	Semi-continuous riparian corridor with native species	Occasional clumps of vegetation in the margins including some non-native trees/plants	Regularly spaced or isolated trees in the margins, presence of many non-native species	Lack of riparian vegetation, completely removed or from most *ca.* 70 % the site length
Morphological condition (MC)	Natural (no obvious human intervention)	Some human interventions, but with natural material and multiple types of habitats available (*e.g.*, stones, sand, submerged roots, aquatic plants, riffles/rapids and pools)	Part of the stream is reinforced with artificial material, but the natural shape is still maintained and lack of some habitats	Most sections are reinforced with artificial material, most natural habitats are missing	Fully artificial open channel using concrete or similar material, highly linearised
Water pollution (WP)	Very clear running water, no turbidity	Water looks unpolluted but has few suspended dissolved solids, very low turbidity	Turbid water	Water very turbid or with bad smell	Water looks polluted, with foam, colours or bad smell, heavily turbid

measure used for ordination analyses. The multivariate analyses were performed in PRIMER 6 (version 6.1.16) & PERMANOVA+ (version 1.0.6) software (PRIMER-E Ltd, Plymouth UK).

To analyse the differences among cities, a Permutational Multivariate Analysis of Variance (PERMANOVA; unrestricted permutation of raw data as permutation method; permutations = 9999) was used based on pharmaceutical concentrations. In addition, the Similarity Percentages (SIMPER) analysis was performed to highlight the pharmaceuticals that had the highest contributions to the within city similarity (to a 90.00 % cut off of cumulative contribution), for the five cities. To understand the global patterns in terms of pharmaceutical contamination in all the studied urban streams, a non-metric Multidimensional Scaling (nMDS) was performed based on the Euclidian distance matrix. In addition, to understand which were the most influential pharmaceuticals in the distribution of all sites, vectors based on the Spearman correlation between each pharmaceutical and the ordination space were superimposed on the nMDS.

Finally, Distance-based Linear Models (DistLM) were used to identify the influence of urbanisation and stream condition on pharmaceutical concentrations across cities and within each city. The most parsimonious model was obtained through a stepwise forward selection procedure, based on the R^2, and the significance was assessed with 9999 permutations. Samples with missing values for those variables (n = 3) were removed from this analysis. The multi-collinearity among urbanisation and stream condition variables were previously tested though correlations and no variable was excluded (*i.e.*, all correlations < 0.75).

2.4. Comparison with literature data

The concentrations of the pharmaceuticals investigated in this study were compared to the occurrences reported in the literature for urban streams, based on the compiled information in Rodrigues *et al.* [12], and to the occurrences reported for urban rivers, based on the publications listed in the same reference. In addition to the references provided by Rodrigues *et al.* [12], the existence of more recent studies was verified. To compare the concentrations found in this study with the previously reported, the available average and maximum concentrations were extracted for the same pharmaceuticals obtained for urban streams and rivers.

3. Results

3.1. Pharmaceuticals in the European urban streams

Sixteen out of the 20 pharmaceuticals analysed were detected across all therapeutic groups and 91 % of the sites presented pharmaceuticals (Table 3; Supplementary Materials (SM) – Table S2). There was no ubiquitous pharmaceutical, but three were found in more than 50 % of the sites, namely the antihypertensives irbesartan and bisoprolol and the anticonvulsant carbamazepine. Irbesartan had the highest detection frequency (73 % of the sites) and mixtures of pharmaceuticals were observed in 79 % of the sites.

Concentrations substantially varied among and within therapeutic groups (Supplementary Materials (SM) – Fig. S1, Fig. S2). The highest concentration was detected for irbesartan at 31,659 ng/L (T13, Toulouse) and the lowest concentration obtained corresponds to bisoprolol at 0.06 ng/L (sampling site C11, Coimbra). The second and third highest concentrations were of acetaminophen (analgesic/antipyretic – 16,789 ng/L; G6, Ghent) and losartan (antihypertensive – 1676 ng/L; G1, Ghent), respectively. Seven out of 20 analysed pharmaceuticals had concentrations > 1 μg/L for at least one sample, encompassing almost all therapeutic groups: ofloxacin (antibiotic); carbamazepine (anticonvulsant); irbesartan and losartan (antihypertensives); acetaminophen (analgesic/antipyretic); and citalopram and venlafaxine (psychopharmaceuticals).

Three pharmaceuticals included in the current Watch List (2025) were analysed and detected: ofloxacin in Coimbra and Ghent, propranolol in Benevento, Toulouse and Ghent, and fluoxetine in Coimbra and Toulouse (Fig. 2). The detection frequencies were below 15 %, except for propranolol, which was higher than 35 % in Toulouse and Ghent. One sample had concentration > 1 μg/L for ofloxacin in Ghent (1625 ng/L; G21), which was considered an outlier. Furthermore, compared to trimethoprim, another antibiotic included in the previous Watch List (2022), ofloxacin was much less persistent in the cities.

3.2. Differences in pharmaceuticals among cities

Pharmaceuticals were detected in all sites from Benevento and Ghent. One of the sites of Coimbra (C10) had the highest number of pharmaceuticals detected (n = 14), followed by a site of Toulouse (T13; n = 12). The highest detection frequencies within cities were for irbesartan in Benevento (100 %) and Toulouse (86 %), for bisoprolol in

Table 3
Summary of the pharmaceuticals detected for each therapeutic group in the 102 stream sites: number of pharmaceuticals detected and analysed; % of sites where the pharmaceuticals were detected; and highest and median concentrations of the pharmaceuticals detected.

Therapeutic group	Pharmaceuticals detected/analysed	Detection frequency (%)	Highest concentration (ng/L)	Median concentration (ng/L)
Antibiotic	4/7	24	1625 (ofloxacin)	41
Anticonvulsant	1/1	58	1218 (carbamazepine)	98
Antihypertensive	6/6	84	31,659 (irbesartan)	158
Analgesic/antipyretic	1/1	31	16,789 (acetaminophen)	825
Lipid regulator	1/1	6	534 (bezafibrate)	108
Psychopharmaceutical	3/4	38	1599 (citalopram)	114

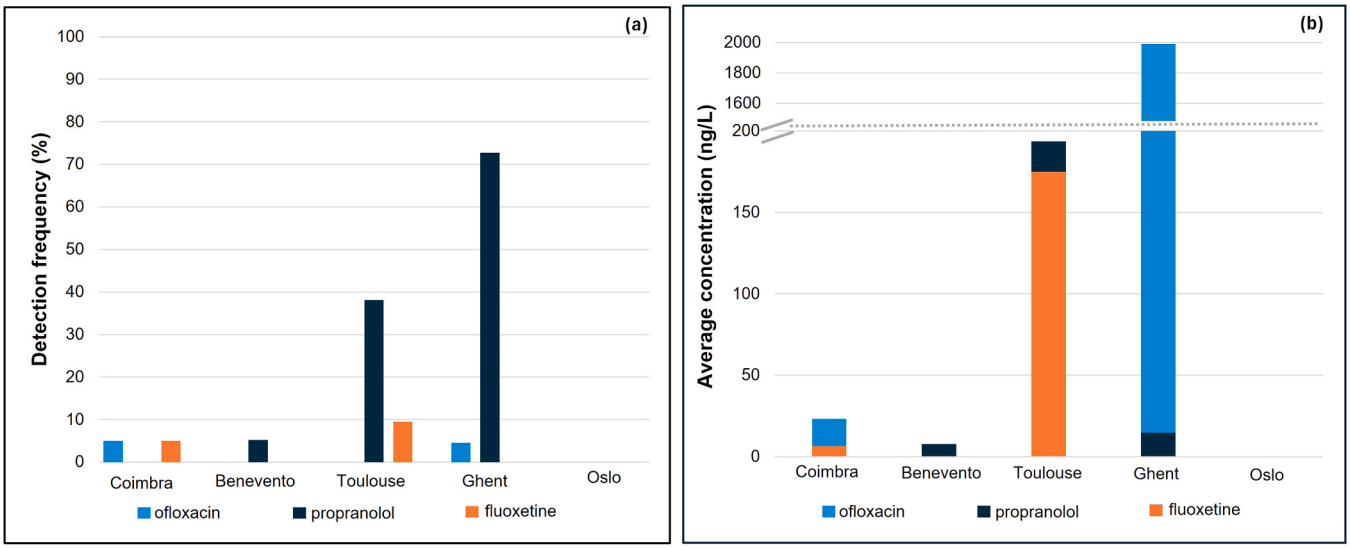

Fig. 2. The (a) detection frequencies and (b) average concentrations of pharmaceuticals from the Watch List (2025) in each city.

Ghent (100 %), and for carbamazepine in Coimbra (70 %).

The highest concentrations detected were for irbesartan in Toulouse (31,659 ng/L; T13), Benevento (7420 ng/L; BN2) and Coimbra (1690 ng/L; C12), as well as for acetaminophen in Ghent (16,789 ng/L; G6) and Oslo (788 ng/L; O9). The highest cumulative concentrations of all detected pharmaceuticals in a site were found in Toulouse

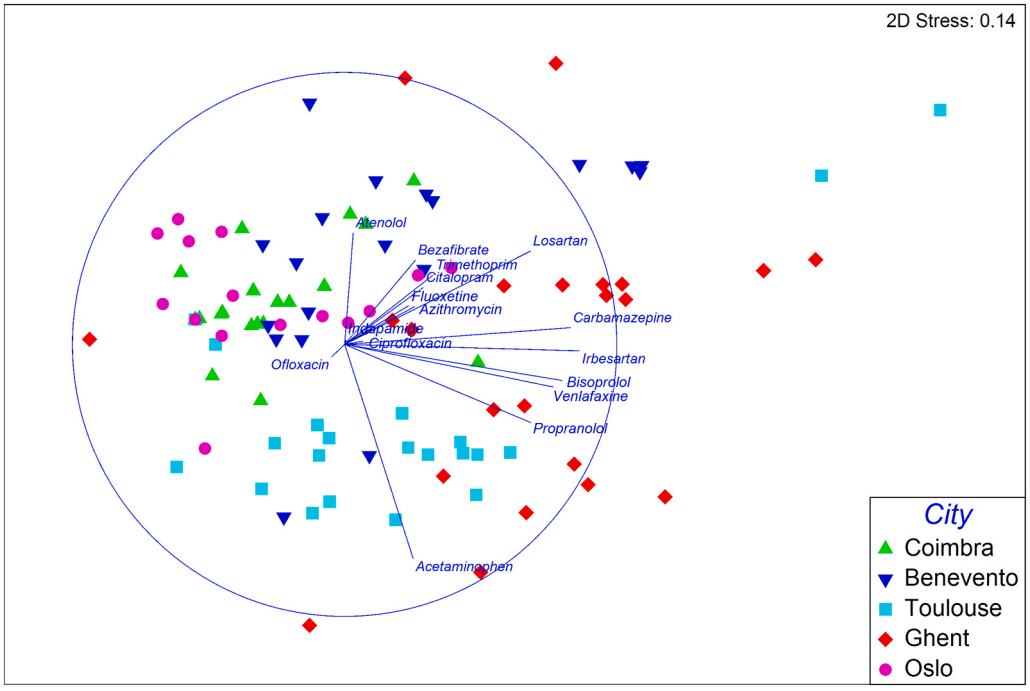

Fig. 3. Non-metric Multidimensional Scaling (nMDS; Euclidean distance) of the stream sites by city based on pharmaceutical concentrations, with overlaying vectors representing the pharmaceuticals correlated (Spearman) with the ordination space.

(37,128 ng/L – T13; 24,194 ng/L – T14), Ghent (18,262 ng/L – G6; 8181 ng/L – G10) and Benevento (9118 ng/L – BN2). The total pharmaceutical concentration was higher in Toulouse, followed by Ghent, Benevento, Coimbra and Oslo.

The PERMANOVA confirmed that there were statistical differences among the cities regarding the pharmaceuticals detected in the streams (Pseudo-F = 11.341, p = 0.0001, permutations = 9913). Those differences were significant for all pairs of cities (PERMANOVA pair-wise tests, $p < 0.05$), except between Coimbra and Oslo ($p = 0.4166$; Table S3).

According to the SIMPER analysis, eight pharmaceuticals that contributed the most to the within city similarity (> 10 %) were in common among the five cities: acetaminophen, irbesartan, atenolol (antihypertensive), losartan, carbamazepine, venlafaxine, bezafibrate and citalopram (Table S4). Irbesartan was the pharmaceutical with the highest contribution to the within city similarity for Coimbra, Toulouse and Oslo, while losartan and acetaminophen were the ones contributing the most to the within similarity of Benevento and Ghent sites. Losartan also had important contributions to the within similarity of Coimbra and Oslo sites, as well as acetaminophen for Benevento and Toulouse, carbamazepine for Benevento, Toulouse and Oslo, and atenolol for all cities.

The nMDS (Fig. 3) shows a segregation of the sites of Toulouse and part of Ghent (G4, G9 and G14) from the other three cities, influenced by higher concentrations of acetaminophen than in other cities. Oslo and part of the sites of Coimbra and one site of Ghent (G21) were segregated due to lower number and/or concentrations of pharmaceuticals. Some sites of Benevento were closer to this group (BN5, BN10, BN16, BN17 and BN19). Part of Ghent sites were also associated with higher concentrations of carbamazepine, and two sites of Toulouse (T13 and T14) and two of Ghent (G5 and G18) were associated with higher mixtures and concentrations of several pharmaceuticals, especially of losartan.

The nMDS bubble plots (Fig. S3), considering only the pharmaceuticals that had contribution within city to similarity > 10 % (from SIMPER analysis), highlight that irbesartan was widespread in similar concentrations throughout the studied streams, although absent from some sites of Oslo and Coimbra, and others of Toulouse and Ghent. The presence of acetaminophen characterised the sites of Toulouse. Pharmaceutical detections and concentrations were consistently lower in sites of Oslo. Losartan, carbamazepine and venlafaxine had similar distributions, mostly in Ghent and Benevento. Bezafibrate and citalopram were rarer but appeared in sites from different cites with a relatively high concentration.

3.3. Influence of urbanisation in the distribution of pharmaceuticals

The DistLM including all cities (Table 4) identified population density (PopD) and tree cover density (TCD) as the variables (individual results in Table S5) with the highest contribution to explain the concentration of pharmaceuticals in the urban streams ($p < 0.05$). Additionally, the Distance-based Redundancy analysis (dbRDA) (Fig. 4) shows that a higher TCD was associated with some Oslo sites, with dbRDA1 (axis 1) explaining 53.7 % of fitted models and 7.3 % of total variation.

In Coimbra, the DistLM (Table 4) showed that the concentration of pharmaceuticals is explained by the integrity of the riparian zone (RZ), imperviousness density (IMD) and the morphological condition of the streams (MC), in Benevento by RZ and in Ghent by water pollution (WP). Within Toulouse and Oslo, none of the tested variables significantly explained the variation in pharmaceuticals.

3.4. Comparison with concentrations reported in the literature

Fourteen out of the 20 pharmaceuticals presented higher

Table 4
Summary of Distance-based Linear Models (DistLM; Euclidean distance) showing the predictors of pharmaceutical concentrations in the five cities ("All cities") and by city (UL – urbanisation level; SC – stream connectivity; RZ – riparian zone; MC – morphological condition; WP – water pollution; PopD – population density; IMD – imperviousness density; and TCD – tree cover density). In **bold**, $p < 0.05$.

All cities				**Coimbra**			
Variable	R^2 (accumulated)	Pseudo-F	P	Variable	R^2 (accumulated)	Pseudo-F	P
PopD	**0.0407**	**4.1131**	**0.0041**	**MC**	**0.1491**	**3.1548**	**0.0155**
TCD	**0.0708**	**3.1124**	**0.0152**	**IMD**	**0.2700**	**2.8151**	**0.0160**
WP	0.0883	1.8200	0.1029	PopD	0.3493	1.9482	0.0820
IMD	0.1031	1.5583	0.1579	**RZ**	**0.4677**	**3.3386**	**0.0072**
RZ	0.1164	1.3997	0.1936	UL	0.5244	1.6670	0.1402
UL	0.1239	0.7885	0.5444	WP	0.5667	1.2719	0.2741
MC	0.1308	0.7207	0.6075	TCD	0.5992	0.9728	0.4457
SC	0.1361	0.5438	0.7641	SC	0.6088	0.2674	0.9500
Benevento				**Toulouse**			
Variable	R^2 (accumulated)	Pseudo-F	P	Variable	R^2 (accumulated)	Pseudo-F	P
PopD	0.1284	2.5040	0.0500	TCD	0.0795	1.5535	0.2068
RZ	**0.2503**	**2.6020**	**0.0412**	PopD	0.1623	1.6815	0.1528
IMD	0.3155	1.4285	0.2038	UL	0.2020	0.7958	0.5103
SC	0.3668	1.1345	0.3248	IMD	0.2364	0.6749	0.5689
WP	0.4080	0.9050	0.4628	SC	0.2679	0.6038	0.6300
TCD	0.4413	0.7154	0.5981	MC	0.3224	1.0459	0.3541
MC	0.4697	0.5889	0.6931	RZ	0.3339	0.2057	0.9351
UL	0.5021	0.6505	0.6368	WP	0.3425	0.1442	0.9651
Ghent				**Oslo**			
Variable	R^2 (accumulated)	Pseudo-F	P	Variable	R^2 (accumulated)	Pseudo-F	P
WP	**0.1158**	**2.3582**	**0.0462**	UL	0.0549	1.0458	0.3736
PopD	0.2109	2.0485	0.0735	RZ	0.1208	1.2743	0.2616
SC	0.2694	1.2803	0.2524	WP	0.1582	0.7101	0.6172
TCD	0.3256	1.2499	0.2705	SC	0.1876	0.5434	0.7654
RZ	0.3644	0.8546	0.4981	IMD	0.2269	0.7115	0.6046
UL	0.4130	1.0757	0.3701	TCD	0.2613	0.6048	0.6841
IMD	0.4822	1.6055	0.1602	MC	0.2940	0.5568	0.7243
MC	0.5037	0.4756	0.8210	PopD	No test	No test	No test

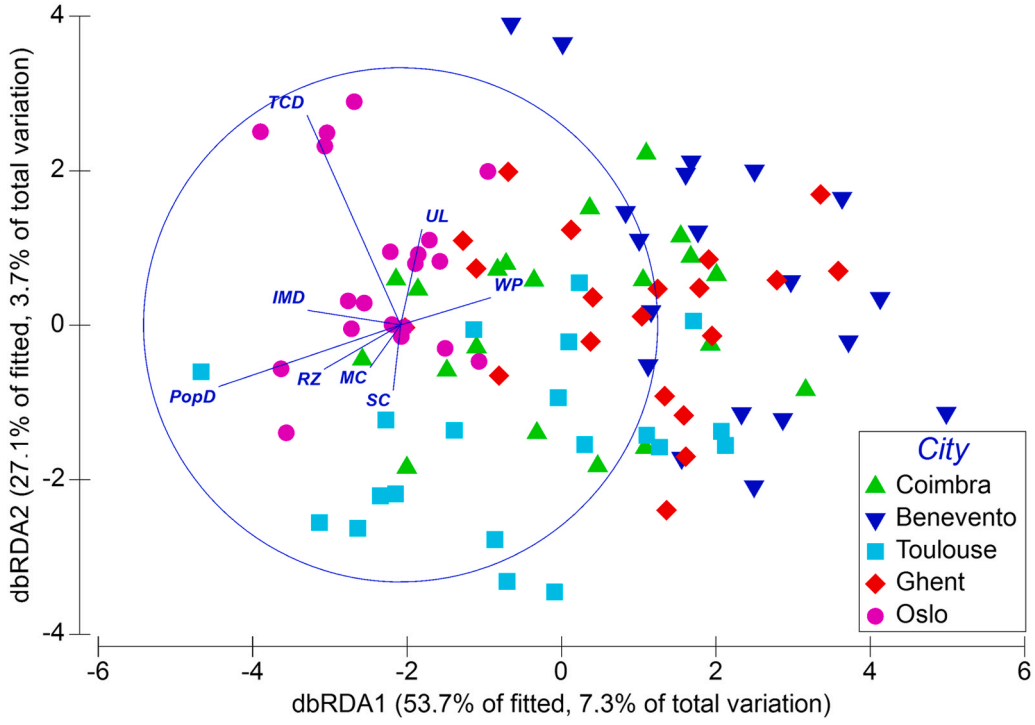

Fig. 4. Distance-based Redundancy analysis (dbRDA; Euclidean distance) of the stream sites by city based on pharmaceutical concentrations, with the vectors showing the predictive variables: UL – urbanisation level; SC – stream connectivity; RZ – riparian zone; MC – morphological condition; WP – water pollution; PopD – population density; IMD – imperviousness density; and TCD – tree cover density.

concentrations in this study compared to other previous studies (Table 5; Table S6). Compared to other urban streams, the average concentrations for azithromycin, trimethoprim, carbamazepine, acetaminophen, bezafibrate, bisoprolol, irbesartan, citalopram, fluoxetine and venlafaxine were higher than the global ones in at least one of the cities, while the maximum concentrations had higher values for the last five pharmaceuticals and also for ofloxacin. Higher average and maximum concentrations were also found for bezafibrate (35 and 53 times, respectively) and carbamazepine (6 and 10 times, respectively) compared to those ever detected in Italy, and for atenolol (43 and 9 times, respectively) and carbamazepine (10 and 6 times, respectively) in Norway. The average concentrations detected here were 13, 10 and 7 times higher for citalopram, fluoxetine (both in Toulouse) and bisoprolol (Ghent) and the maximum ones were 13, 12 and 6 times higher for bisoprolol (Ghent), irbesartan and fluoxetine (both in Toulouse), respectively.

Although carbamazepine had higher average concentrations compared to the global value for urban streams, a recent study [29] detected an average concentration 2 times higher. However, the same study had average and maximum concentrations higher than the global values for bisoprolol, but lower than some of those found here. This was also applied to another recent study [24], but regarding the maximum concentration for citalopram.

Concerning the maximum concentrations reported in the literature for urban rivers, ofloxacin, irbesartan, losartan, citalopram, fluoxetine and venlafaxine had higher values in the present study, particularly for bisoprolol rate that was 21 times higher. Additionally, the average and maximum concentrations in these reviewed studies were lower than those detected here for several pharmaceuticals. The concentrations (average and maximum, respectively) detected here for atenolol in Portugal were higher (33 and 49 times), while in Italy they occurred for carbamazepine (24 and 68 times), atenolol (17 and 28 times), trimethoprim (16 and 20 times), and acetaminophen (18 and 14 times). In the case of France, the maximum concentrations detected in this study were higher for azithromycin (470 times), propranolol (8 times) and bisoprolol (6 times), while both average and maximum values (respectively) were higher for venlafaxine (9 and 43 times), atenolol (24 and 38 times), carbamazepine (7 and 26 times) and losartan (17 and 10 times). On the other hand, for Belgium, the average and maximum concentrations (respectively) detected here were higher for acetaminophen (60 and 168 times) and citalopram (18 and 13 times), and also irbesartan had higher maximum concentration (49 times).

Indapamide was never detected in the reviewed studies on urban streams or rivers but this pharmaceutical was observed in this study. In addition, losartan was previously observed in urban rivers but the average and maximum concentrations detected in this study were higher in Benevento and Ghent. Losartan was also detected in a recent study on urban streams [29], but the average and maximum concentrations detected here were 10 and 2 times higher than those detected in that study.

Some pharmaceuticals had higher average and maximum concentrations in this study than in any other study identified in the literature, both for urban streams and urban rivers, namely irbesartan, losartan, and fluoxetine, *i.e.*, they had the highest concentration ever detected.

4. Discussion

4.1. Major findings on pharmaceutical occurrence

The present study showed a widespread contamination by pharmaceuticals of urban streams located in five European cities, with 91 % of detection in the 102 samples collected, and sixteen pharmaceuticals identified from six therapeutic groups. Antihypertensives and anticonvulsants were found in more than 50 % of the sites and fourteen pharmaceuticals presented higher concentrations than previously reported. The highest concentrations were of analgesics/antipyretics, with median values near 1 μg/L (considering all sites), followed by antihypertensives of which the highest concentration reached 31 μg/L.

Theoretically, pharmaceuticals should not reach streams because, according to best practices (*e.g.*, [53,54]), WWTPs should discharge only

Table 5
Average and maximum concentrations of the pharmaceuticals by therapeutic group detected in urban streams in this study and on a global scale, as well as maximum concentration in urban rivers. The concentrations in **bold** are higher than the global ones for urban streams and the ones in *italic* are higher than the maximum concentrations for urban rivers. The lines with grey background correspond to the pharmaceuticals detected in this study. "All cities" refers to concentrations considering all 102 sites; nd = not detected; "-" = not available.

Pharmaceutical	Urban streams Average Maximum (ng/L)							Urban rivers Maximum (ng/L)
	This study						World[7]	
	Coimbra	Benevento	Toulouse	Ghent	Oslo	All cities		
Azithromycin[1]	21	nd	**141**	58	nd	59	60	
	25	nd	141	135	nd	141	569	2819[8] (Portugal)
Ciprofloxacin[1]	4	nd	nd	nd	nd	4	66	
	5	nd	nd	nd	nd	5	304	1570[9] (Angola)
Ofloxacin[1]	17	nd	nd	1625	nd	821	-	
	17	nd	nd	***1625***	nd	***1625***	552	1213.6[10] (China)
Oxytetracycline[1]	nd	nd	nd	nd	nd	nd	8	
	nd	nd	nd	nd	nd	nd	8	680[11] (France)
Sulfadiazine[1]	nd	nd	nd	nd	nd	nd	-	
	nd	nd	nd	nd	nd	nd	-	1620[9] (Austria)
Tetracycline[1]	nd	nd	nd	nd	nd	nd	23	
	nd	nd	nd	nd	nd	nd	24	805[9] (Malaysia)
Trimethoprim[1]	1	**58**	**66**	**53**	46	48	48	
	1	73	80	98	88	98	690	4190[9] (Kenya)
Carbamazepine[2]	18	**236**	**247**	**489**	60	**250**	185	
	100	738	966	1218	94	1218	67,715	10,300[9] (Belgium)
Atenolol[3]	279	248	334	348	428	295	612	
	553	540	579	361	519	579	8199	3900[9] (Israel)
Bisoprolol[3]	6	**57**	**70**	**286**	26	**139**	41	
	11	***159***	***266***	***966***	44	***966***	75	45[12] (France)
Indapamide[3]	nd	32	nd	nd	nd	32	-	
	nd	41	nd	nd	nd	41	-	-
Irbesartan[3]	268	**1692**	**3602**	537	295	**1516**	1475	
	1690	***7420***	***31,659***	1610	641	***31,659***	2673	1930[13] (Germany)
Losartan[3]	58	435	226	427	344	348	-	
	307	*1079*	241	*1676*	398	*1676*	-	410[13] (Germany)
Propranolol[3]	nd	8	19	15	nd	16	34	
	nd	8	83	51	nd	83	161	459[9] (Kenya)
Acetaminophen[4]	60	1598	1031	**4609**	788	**1974**	1753	
	107	1945	3797	16,789	788	16,789	30,421	227,000[9] (Bolivia)
Bezafibrate[5]	26	**238**	97	nd	nd	**179**	109	
	26	534	97	nd	nd	534	628	1235[14] (Cyprus)
α-Hydroxy-alprazolam[6]	nd	nd	nd	nd	nd	nd	-	
	nd	nd	nd	nd	nd	nd	-	-
Citalopram[6]	80	nd	**1279**	**1082**	nd	**756**	96	
	168	nd	***1599***	**1434**	nd	***1599***	547	1510[9] (Germany)
Fluoxetine[6]	7	nd	**175**	nd	nd	**119**	17	
	7	nd	***222***	nd	nd	***222***	38	96.1[9] (Belgium)
Venlafaxine[6]	10	97	242	**309**	28	222	286	
	31	108	***1194***	*955*	28	***1194***	1003	681[9] (USA)

[1]Antibiotic; [2]Anticonvulsant; [3]Antihypertensive; [4]Analgesic/antipyretic; [5]Lipid regulator; [6]Psychopharmaceutical; [7][12]; [8][46]; [9][47]; [10][48]; [11][49]; [12][50]; [13][51]; [14][52].

treated sewage waters to larger water bodies like main rivers, to avoid high concentrations of pollutants in freshwaters. On the other hand, WWTPs are known pathways for the release of pharmaceuticals into the aquatic environment, and the results presented here must be interpreted in light of this well-established context. Some treatments were proposed for the removal of pharmaceuticals in WWTP, but they still need to be technically and financially viable. For some antibiotics, treatment with Photo-Fenton process [55] and metal-organic frameworks modified resin catalysts [56] were studied. Additionally, the removal of several pharmaceuticals is done successfully with TiO_2 catalysts [57]. Beyond the general inefficiency of the WWTPs to remove pharmaceuticals, many urban areas still use combined sewer systems, which bypass treatment facilities during storm events [58] and introduce large amounts of untreated wastewater and contaminants into streams and rivers [59]. This is expected to become an increasingly growing concern, since the climate change scenarios include stronger and more frequent extreme precipitation events [60].

As far as is known, this is the first large-scale study addressing these contaminants in European urban streams, with original data collected in a standardised way and that analysed the patterns within and among cities. A previous study [52], addressing a mixture of streams and rivers in Europe and covering fewer pharmaceuticals, indicated that the most common contaminant was carbamazepine, which is in agreement with the present results. A global study performed mostly in larger rivers [47], and not focused on cities, also showed widespread contamination in Europe. Other contaminants were tested but several important pharmaceuticals analysed here were not covered by that global study [47]: azithromycin, ofloxacin, bisoprolol, indapamide, irbesartan,

losartan, bezafibrate and α-hydroxyalprazolam.

4.2. Comparison with previous studies

Some pharmaceuticals (irbesartan, losartan, and fluoxetine) had higher average and maximum concentrations in this study than in any other study of the investigated literature, both for urban streams and urban rivers, revealing here the highest concentrations ever detected. It could be explained by the high consumption of these pharmaceuticals by the population of the studied cities. Toulouse was the city where the highest average and maximum concentrations were obtained for irbesartan, with a consumption of antihypertensives in France of 184.5 defined daily dose per 1000 inhabitants – DDD/1000 inhabitants [61]. In previous studies, the country with the highest reported average and maximum concentrations of the same pharmaceutical was Australia [62], with a consumption lower than in Toulouse, of 134.9 DDD/1000 inhabitants [63].

Moreover, the studies described in the literature with the highest average and maximum concentrations for irbesartan, losartan, and fluoxetine were conducted before the pandemic of Covid-19 (*e.g.*, [51, 62,29,47]; with samples collected in 2015, 2016, 2018 and 2018, respectively), while the samples of this study were collected after this event. Therefore, a change in the consumption pattern of these pharmaceuticals may have occurred towards an increase demand, especially for these therapeutic groups – antihypertensives and antidepressants [64–67].

The higher concentrations observed in this study compared to those reported in the literature could also be explained by the influence of methodological factors. Differences in sampling strategies, such as the timing and frequency of sample collection, can lead to variations in detected concentrations because pharmaceutical levels may fluctuate daily or seasonally. The selection of sampling sites near sources of contamination (WWTPs, for instance) is relevant as well. The choice of containers, preservation, filtration, and storage methods may also affect the stability of certain compounds, potentially causing degradation or loss before analysis. For example, among the studies described in the literature, some used amber borosilicate glass bottles, which avoid molecule degradation (*e.g.*, [29,47]). Others used high-density polyethylene bottles (*e.g.*, [51,10]), which are safer for freezing samples. Some studies used automatic water samplers (*e.g.*, [62]), and others used passive samplers (*e.g.*, [51]), instead of collecting spot samples.

Additionally, analytical methods including extraction techniques, instrument sensitivity, calibration procedures, and detection limits can significantly influence the reported concentrations. For instance, some of the studies previously reported used acetone as an extraction solvent complementary to methanol (*e.g.*, [51]). The instrument of analysis also varied among these studies, from HPLC-MS/MS (*e.g.*, [47]) to SFC-MS/MS – a supercritical fluid chromatography (*e.g.*, [29]). Even the selection of target pharmaceuticals and their respective analytical standards may introduce variability compared to other studies.

Other factors may have contributed to the higher concentrations presented here compared to those of previous studies, for instance, the ecological condition of the streams and their susceptibility to photodegradation and biodegradation. Meanwhile, many factors can act at the same time to the degradation (or not) of a pharmaceutical in the stream ecosystem, therefore, they should be analysed together for a better understanding of the occurrences in the water.

4.3. Pharmaceutical patterns in urban streams among cities

The analyses performed in this study showed that there were differences in water contamination by pharmaceuticals among cities, except for Coimbra and Oslo. Therefore, the first hypothesis is only partially confirmed. Toulouse and Ghent had higher levels of pharmaceutical contamination in streams, while Oslo, followed by Coimbra, were less contaminated. Only two publications were found regarding pharmaceuticals in the running waters of Oslo, but none for the other cities. In one of these studies [28], ten analgesics/antipyretics/anti-inflammatories, two antibiotics, two anticonvulsants, four antihypertensives, two psychopharmaceuticals and one radiocontrast agent were found in an urban stream of Oslo, including some also detected here (acetaminophen, carbamazepine, atenolol and venlafaxine). Another study [52] detected other pharmaceuticals in one of the Oslo streams, the Alna stream, but did not detect carbamazepine, the only pharmaceutical in common and detected in this study, suggesting an increase in the contamination of this stream over time.

The different patterns of pharmaceuticals found in the streams could not be associated with characteristics of the cities, since Coimbra and Oslo, the most distant cities (distinct climates), with different socio-economic, cultural and nature protection policies backgrounds had the most similar and lower water contamination. Moreover, Benevento and Coimbra, with more similar characteristics, were significantly different in pharmaceuticals. Some studies associated fluctuations in pharmaceuticals occurrence in streams/rivers with variations in seasonal climatic conditions (precipitation, temperature, sunshine duration, humidity) in the same locations (*e.g.*, [68,62,69]), which was not an aim here.

Similarities or differences among cities could also be due to differences in consumption or pharmaceuticals authorised/sold in the respective countries. In general, the patterns that were observed here by city corresponded to the available data on consumptions (DDD/1000 inhabitants; [61]), since some of the highest detected therapeutic groups also present high consumption rates in those countries. This is the case of the antihypertensives and anticonvulsants in Coimbra, Benevento, Ghent and Oslo, and the antihypertensives and analgesics/antipyretics in Toulouse. The consumption of acetaminophen in France has already been considered excessive compared to Portugal and Italy [70]. This may explain the higher detection frequency and concentration of this pharmaceutical in the sites of Toulouse (81 %), compared to the lower detection frequency in Benevento and Coimbra (11 % and 15 %, respectively). Yet, although lipid regulators had low detections and concentrations in this study, except for Benevento, a high consumption in Portugal, Italy, France, Belgium and Norway is attributed to this therapeutic group [61]. Its low detection here may have occurred because only one lipid regulator was evaluated and there are other pharmaceuticals of this group which are largely used in Europe, such as atorvastatin [71].

4.4. Pharmaceuticals and their environmental risks

The antihypertensive irbesartan was extensively distributed through the studied streams, with average concentration five times higher than most of the other pharmaceuticals. The highest concentration detected in this study was 12 times higher than that previously reported for an urban stream [62]. Losartan is also spread through many sites of Ghent, some of Benevento and a few of Coimbra, while it was almost absent from Toulouse, contributing to the different patterns in this city. Propranolol, an antihypertensive present in the current European Union Watch List [7], had high detection frequency in Ghent, but low concentrations in this city as well as in the others (Benevento and Toulouse). These results reflect the prevalence of high blood pressure and/or anginas in the European population, with larger consumption of irbesartan than of other antihypertensives [72], and can result in high risks for the aquatic biota, namely through bioaccumulation [20].

In the case of analgesics/antipyretics, acetaminophen was found widely in Toulouse sites but also in Ghent and other cities, however in concentrations lower than those found in an urban stream of São Paulo, Brazil [73]. This group of pharmaceuticals act on a wide number of common symptoms, like flues, fever, reactions to vaccination, migraines, headaches, toothaches, earaches, menstrual, traumatic, muscle and joint pain [74]. The analgesics and antipyretics were also found to bioaccumulate in aquatic biota [20].

The anticonvulsant carbamazepine was frequent in the sites (58 %), which agrees with its high European consumption [72], and had higher concentrations in the sites from central Europe (Toulouse and Ghent). The anticonvulsants treat certain types of seizures (epilepsy), neurological diseases (trigeminal neuralgia) and psychiatric conditions (episodes of bipolar mood disorders and some types of depression) [74]. This pharmaceutical is also considered persistent in freshwaters due to its difficult degradation by temperature [26], and commonly prescribed as an antipsychotic [10]. Nevertheless, another study [75] also found that acetaminophen and carbamazepine had much quicker in-stream attenuation rates to half of the concentration than irbesartan, which also contribute to explain the results of much higher concentrations to this last pharmaceutical. Carbamazepine can also bioaccumulate in the tissues of aquatic organisms [20]. Additionally, a mixture of carbamazepine with other four pharmaceuticals led to several effects on a model fish: reactions in liver; increase of vitellogenin immunostaining; and increased hepatic mass [76]. The carbamazepine concentration in the water that induced these effects on the fish was lower than several concentrations found in all the cities of the present study.

The psychopharmaceutical venlafaxine was in the previous Watch List [77] and was detected in all five cities, with high detection frequency and concentrations in Toulouse and Ghent. This pharmaceutical is used to treat depression, panic/anxiety disorders and nervous bulimia [74]. It increases serotonin and norepinephrine in the brain [78]. Fluoxetine, in the current Watch List [7], was found in two cities (Coimbra and Toulouse) with low detection frequencies. The concentrations of fluoxetine detected in Toulouse were higher than that reported in a previous study that related concentration in the water to changes in spawning of a bivalve [79]. Psychopharmaceuticals and their metabolites (fluoxetine, norfluoxetine, sertraline, desmethylsertraline and methylphenidate) were also found in the body of bivalves [20].

Four antibiotics found in the studied streams are used in the treatment of localised infections in various parts of the human body caused by bacteria [74]. Ofloxacin, detected in Coimbra and with a high concentration in a site of Ghent, is included in the current Watch List [7]. The Watch List of 2022 [77] already had ofloxacin but also trimethoprim, which was detected in all cities. A previous study [80] reported lower concentrations compared to a site of Ghent for ofloxacin, and to several sites of Benevento, Toulouse, Ghent and Oslo for trimethoprim. It also showed that ofloxacin and trimethoprim are bioaccumulated in biofilms [80]. Some effects on a model fish were also associated with a mixture containing trimethoprim [76] at a concentration lower than the one detected in a site of Ghent.

Two other antibiotics from previous Watch Lists were additionally detected in the urban streams: ciprofloxacin, detected only in low concentrations (< 10 ng/L) in Coimbra was on the Watch List of 2020 [81]; and azithromycin, frequent in Ghent and with a high concentration in Toulouse was on the Watch List of 2015 [82]. The exposure to ciprofloxacin significantly decreased gross primary production and biomass in biofilms [21]. Azithromycin is bioaccumulated in biofilms [80].

The high concentration and abundance of antibiotics in freshwaters is considered a serious threat for human health and to the stability of the ecosystem [83]. In the European Union, human consumption of antibiotics in 2022 and 2023 largely rebounded to pre-pandemic numbers, following a drop in their intake between 2019 and 2020 [84]. The antibiotics are considered pseudo-persistent and have an easy spread, thus, their presence can induce the occurrence of antibiotic-resistance genes (ARGs) and antibiotic-resistant bacteria (ARB) [85]. This can lead to serious therapeutic issues, such as infections that cannot be treated with antibiotics because the bacteria have become totally resistant. Therefore, dealing with antibiotics in freshwaters is a matter of urgency [86] and several studies have been addressing this concern (*e.g.*, [87–89]).

Besides the individual effect of pharmaceuticals, a mixture of multiple pharmaceuticals was observed in 79 % of the sites. Previous studies also indicate that smaller water bodies like streams may have a higher mixture risk compared to rivers [90,91]. The effect of such mixtures may be even more relevant for the ecosystems but with risks that are very difficult to predict, as they might be additive or synergistic [8]. Yet this remains largely understudied, especially regarding the mechanisms leading to impacts. Some evaluations of the pharmaceutical mixtures were performed in the laboratory in the context of Environmental Risk Assessments [3,90,91,10]. Also in laboratory experiments, water from streams and rivers contaminated by azithromycin, ciprofloxacin, trimethoprim, carbamazepine, atenolol, propranolol, paracetamol and bezafibrate caused algal toxicity, inhibition of reproduction or increase in mortality of cladoceran, and alterations in hatching and/or mortality of fish [17]. In addition, the possible impacts of metabolites and transformation products on the aquatic ecosystem have been neglected [12], which may be even worse than their parent pharmaceuticals.

4.5. Drivers of pharmaceutical contamination

The level of contamination of streams by pharmaceuticals was explained by the population density in the surroundings of the streams, which is not surprising and confirms the second hypothesis. Clear relationships were found between pharmaceuticals and the urbanisation gradients. In addition, tree cover density also explained the pharmaceutical patterns of the five cities, and riparian zone, morphological condition and water pollution were associated with the urbanisation gradients of three cities. Therefore, the third hypothesis was confirmed. Higher tree cover density was also associated with sites of Oslo, which was the city with lower number and concentrations of pharmaceuticals, although with the highest municipality population. This suggests that population *per se* is not the only cause of pharmaceutical occurrences in freshwaters and that there is a positive effect of the ecosystem integrity, namely of the riparian vegetation condition in the water contamination by pharmaceuticals. This is in agreement with studies showing that aquatic and riparian vegetations are prone to remove these contaminants from the water [92,31]. Yet, this capacity may be influenced by other factors acting simultaneously like water temperature, light, dissolved organic carbon, and turbidity [93,30,69], the most cited in the literature, especially light for inducing the photodegradation of pharmaceutical molecules. The diversity of habitats and different types of substrates (assessed here by the variable morphological condition) could also influence the retention of pharmaceuticals, as some types of sediments act as sinks or bioreactors [26,94,30].

Specific differences within city concerning the hydromorphology and ecological condition of the streams, as well as the urbanisation degree, may have contributed to the significant variation in concentrations of some pharmaceuticals at different sampling sites. Additionally, some potential reasons for the higher concentrations found in this study in comparison to those reported in the literature are: the consumption of some pharmaceuticals by the population of the studied cities could be higher; the influence of methodological factors used; the ecological condition of some of the studied streams could be poorer; the photodegradation and biodegradation in the streams could be hampered; and the urban pressure on some of the streams' basins and their water yields could be higher, including illegal sewage discharges and even contaminated underground waterways.

Despite the initial criteria established for the selection of pharmaceuticals, which aimed to cover the most consumed ones, this study likely underestimated the real contamination of urban streams. Firstly, the anti-inflammatories were not included here; secondly, the pharmaceuticals used for veterinary purposes were also not analysed (except for fluoxetine that is used for both animal and human health treatments). In the last case, veterinary pharmaceutical use was assumed to be less significant in urban areas but they could still be present due to the existence of many pets in cities or upstream rural areas.

Some pharmaceuticals are also subject to misuse or abuse, which may have significantly impact on the freshwater ecosystems, although this remains understudied. This is the case of antiretroviral drugs [95], synthetic androgens used to improve the performance of athletes [96],

as well as the anaesthetics morphine and ketamine [97]. In addition, others are becoming more relevant in human health treatments, such as the antineoplastic (anticancer) which should also be included in future wide-scale analyses on the aquatic ecosystems [3]. Since patients who take this medication can suffer relevant collateral effects, it is believed that their impact on aquatic organisms may also be significant, similarly to hormones [19].

4.6. Potential impacts on One Health

The main findings of this study on the overall presence of pharmaceuticals in urban streams have important consequences within the One Health context, which highlights the interconnection between environmental, human, and animal health. The occurrence of multiple pharmaceutical compounds indicates a risk of chronic environmental exposure in densely populated areas, with possible significant effects on ecosystem functioning and public health [98]. Specific findings in this study highlight these implications, showing that several compounds reached concentrations that overlap with effect thresholds reported in ecotoxicological studies. For instance, in Toulouse and Ghent, where concentrations were the highest, urban streams presented high levels of pharmaceuticals (*e.g.*, irbesartan, acetaminophen, and carbamazepine) previously associated with bioaccumulation in bivalves [20] and sublethal or toxic effects well known for causing reproductive and immunological effects on aquatic organisms such as fish [76].

In the urban streams investigated, a wide range of organisms could be subject to pharmaceutical contamination. Among them are aquatic macroinvertebrates, resident fish populations, microalgae, macrophytes, birds, amphibians, semi-aquatic mammals and even terrestrial insects [99,14,15,100]. Chronic exposure to the pharmaceuticals detected in this study can lead, for instance, to altered community structure [21] and to increased hepatic mass in fish [76]. The detection of multiple psychoactive compounds, cardiovascular drugs, and antibiotics (such as in T13 and T14 in Toulouse and G5, G6, G10, and G18 in Ghent) may amplify the risks compared to single-compound exposures. Yet, these effects remain poorly understood [8].

The presence of several antibiotics (azithromycin, ciprofloxacin, ofloxacin and trimethoprim) in the studied streams raises concerns about the potential development and spread of antibiotic resistance within aquatic microbial communities [83]. Given that many urban streams drain into larger water bodies, often used for recreation, drinking water abstraction, or fishing, this contamination may have indirect consequences for human health through the spread of antibiotic resistance to humans, posing risks to the populations of the cities studied. This exposure pathway includes not only the consumption of water and food contaminated by pharmaceuticals, but also by their metabolites and/or transformation products, whose effects are even less explored [12]. Another concern is the possible bloom of bacteria carrying antibiotic-resistant genes, which can be specially dangerous for the most vulnerable citizens, such as pregnant women, children, the elderly, patients in immunosuppressive therapy, cancer patients, and people with some chronic disease, like diabetes, aids or kidney disease [101–103].

These findings show that the contamination of urban streams by pharmaceuticals should not be considered just an environmental problem. In fact, it poses a complex issue that affects the ecosystem health, animal populations, and ultimately human well-being. Addressing this challenge needs integrated monitoring and mitigation strategies that explicitly recognize the interactions of these domains, in line with the One Health approach.

5. Conclusions

Pharmaceuticals from several different therapeutic groups were detected in the streams of the five European cities studied, with many sites presenting mixtures and high concentrations. Despite multivariate analyses involve some uncertainty, they showed that the urbanisation degree and the ecological condition of the streams had negative and positive influences, respectively, regarding pharmaceutical occurrences in urban streams. This is also important in a One Health perspective, since the occurrences point to wider implications, especially for the antibiotics detected, adding risks for the people using the streams, as well as possible impacts of mixtures on the ecosystem health.

The lack of appropriate urban sewage infrastructure and treatment, as well as the poor dilution in streams, may have influenced the occurrence and high concentrations of pharmaceuticals in the waters. It is necessary to develop new technology for pharmaceutical removal and avoid bypasses.

The restoration of freshwater ecosystems, such as streams and rivers in cities, particularly recovering riparian vegetation and improving stream morphology, may constitute a natural solution to decrease the concentrations of pharmaceuticals in the water. This measure would also result in the additional advantage of improving the health of citizens leading to a decrease in the consumption of pharmaceuticals, and therefore their entrance in the aquatic ecosystems. Such ecosystem-based solutions are well aligned with the One Health approach, as they support water quality, ecosystem services, and human well-being in an integrated manner.

Further studies should focus on the ecological effects of pharmaceutical mixtures (interactions) under environmentally relevant conditions. Also, a long-term monitoring to assess seasonal and interannual variability is needed, as well as a study on the sources of contamination related to sewage discharges.

Also, as preventive measures for mitigation of streams contamination by pharmaceuticals, new paradigms and regulations for treatments and pharmaceuticals production are urgent, for instance adapted doses and number of pills purchased according to the needs of the patients – personalised prescribing.

Finally, considering the results of this study, an addition of some pharmaceuticals to the future Watch List is suggested: carbamazepine, atenolol, losartan, irbesartan, acetaminophen and citalopram.

Environmental implications

This study is environmentally relevant because it showed the influence of urbanisation factors on the presence and concentration of pharmaceuticals in streams. The pharmaceuticals should be considered “hazardous materials”, since previous studies revealed that they can pose hazardous effects on stream ecosystem, consequently on humans, by contamination of the water. This study helps to address environmental problems based on the broad analysis and discussion of the case study gathering the chosen European cities. Additionally, our results show the importance of the conservation and restoration of the streams to mitigate the pharmaceutical effects and the risks for One Health.

CRediT authorship contribution statement

Fernanda Rodrigues: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ana R. Calapez**: Writing – review & editing, Methodology, Data curation. **André M.P.T. Pereira:** Writing – review & editing, Resources, Methodology. **Liliana J.G. Silva:** Writing – review & editing, Resources, Methodology. **Andreia Freitas:** Writing – review & editing, Resources, Methodology. **Rayan Bouchali:** Writing – review & editing, Data curation. **Andree De Cock:** Writing – review & editing, Data curation. **Marie Anne Eurie Forio:** Writing – review & editing, Data curation. **Peter Goethals:** Writing – review & editing, Data curation. **Silje H. Henni:** Writing – review & editing, Data curation. **Adeline Loyau:** Writing – review & editing, Data curation. **Anne Moen:** Writing – review & editing, Data curation. **Nadia Piscopo:** Writing – review & editing, Data curation. **Dirk S. Schmeller:** Writing – review & editing, Data curation. **Janine P. da Silva:** Writing – review & editing,

Methodology. **Luisa Durães:** Writing – review & editing, Supervision, Funding acquisition. **Nuno E.C. Simões:** Writing – review & editing, Supervision, Funding acquisition. **Maria J. Feio:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.139946.

Data availability

Data will be made available on request.

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