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Occurrence, distribution and environmental risk of selected non-steroidal anti-inflammatory drugs, their metabolites and metformin in European and Balkan surface waters

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ABSTRACT

Pharmaceutical residues in surface waters represent a growing environmental concern due to their persistence and ecological impacts. This review summarizes recent European and Balkan studies (2013–2024) on the occurrence, distribution, and environmental risks of widely used non-steroidal anti-inflammatory drugs (NSAIDs), metformin, and key metabolites such as 2-hydroxyibuprofen. Data from 308 samples across 70 monitoring sites reveal widespread contamination, with diclofenac and ibuprofen detected at up to several µg/L, particularly near wastewater treatment plant discharges. Metabolites frequently exceeded parent compound concentrations, highlighting monitoring gaps. Risk assessments indicate potential harm to aquatic ecosystems, with hazard quotients often surpassing safety thresholds, especially under low-flow conditions. This review underscores the need for enhanced monitoring frameworks incorporating pharmaceutical metabolites. Strengthening regulatory compliance, particularly in Balkan countries with insufficient treatment infrastructure, is critical for safeguarding surface water quality. Integrating pharmaceutical contamination data into ecological engineering practices offers pathways for effective pollution mitigation and sustainable water resource management.

Keywords: NSAIDs, Metformin, surface water, occurrence, contamination, Balkan, Europe.

INTRODUCTION

Pharmaceuticals and personal care products (PPCPs) have become common contaminants in freshwater worldwide due to continuous release from various human activities, although ongoing efforts are being made to mitigate their impact. Among these contaminants, non-steroidal anti-inflammatory drugs (NSAIDs), including Diclofenac (DCF), Ibuprofen (IBU), Naproxen (NPX), and Ketoprofen (KTF), are among the frequently detected pharmaceuticals due to their extensive use as both prescription and over-the-counter medicines. These compounds are chemically stable and relatively persistent in aquatic environments (Boxall, 2004; Fent et al., 2006; Lalithambika et al., 2024). Similarly, metformin, one of the most widely prescribed drugs for type 2 diabetes, has been identified as an environmental contaminant due to its high consumption and predominant excretion unchanged by the human body.

Pharmaceuticals enter rivers and lakes primarily through wastewater treatment plant (WWTP) effluents, which represent the major pathway for their release into aquatic environments (Heberer, 2002; Radjenović et al., 2007; Vieno and Sillanpää, 2014). Additional sources include the improper disposal of unused medicines (such as flushing them down household drains where this practice is not prohibited), direct discharges of untreated wastewater, and diffuse inputs from veterinary pharmaceuticals (OECD, 2022; Styszko et al., 2022; UNEP, 2019). These multiple pathways contribute to the continuous introduction

of pharmaceuticals into surface waters, making them pseudo-persistent contaminants despite their relatively short individual residence times. Conventional WWTPs are not specifically designed to remove pharmaceutical compounds and therefore often fail to eliminate NSAIDs and Metformin completely. Removal efficiencies vary considerably among compounds because of differences in their physicochemical properties and the treatment technologies employed, allowing substantial quantities to persist in treated effluents and contaminate downstream aquatic ecosystems (Heberer, 2002; Radjenović et al., 2007; Vieno & Sillanpää, 2014). Environmental conditions further influence the occurrence and persistence of pharmaceuticals in surface waters. Seasonal variations, drought, river hydrology, and changes in stream flow strongly affect dilution capacity and contaminant transport. During periods of low river discharge, pharmaceutical concentrations typically increase, resulting in greater exposure of aquatic organisms and elevated ecological risks (Malaj et al., 2014; Palma et al., 2019; Sousa et al., 2018).

The Balkan Peninsula, including Albania, is particularly vulnerable to pharmaceutical contamination because of limited wastewater treatment infrastructure, insufficient enforcement of environmental regulations, and widespread self-medication practices that increase pharmaceutical loads entering river systems. Although monitoring data from this region remain scarce, recent studies indicate contamination levels comparable to those reported in other European countries undergoing similar socioeconomic transitions, highlighting an important but insufficiently investigated environmental concern (Jaku et al., 2025; Wilkinson et al., 2022). Despite the growing body of evidence on pharmaceutical contamination of surface waters, important knowledge gaps remain regarding their temporal and spatial distribution, particularly in Eastern and Southeastern Europe, where monitoring studies are considerably less abundant than in Western Europe and North America (Ene et al., 2025; Wilkinson et al., 2022). Furthermore, ecological risk assessments that consider pharmaceutical mixtures, chronic exposure, and realistic ecosystem-level effects remain less developed than assessments for many other classes of environmental contaminants (Backhaus and Faust, 2018; Guo et al., 2016). Even at environmentally relevant concentrations, pharmaceuticals may adversely affect aquatic organisms because they are specifically designed to produce biological activity at very low doses, sometimes at part-per-trillion levels (Mozaz et al., 2010). NSAIDs such as diclofenac, ibuprofen, naproxen, and ketoprofen, together with metformin, have been detected in surface waters at concentrations ranging from nanograms per liter (ng/L) to micrograms per liter (µg/L). Exposure to these compounds has been associated with oxidative stress, DNA damage (genotoxicity), endocrine disruption, impaired growth and development, reproductive abnormalities, and physiological alterations in fish, invertebrates, and other aquatic organisms (Ferrari et al., 2004; Fent et al., 2006; Lalithambika et al., 2024; Mezzelani et al., 2018; Richmond et al., 2017). Diclofenac has received particular attention because of its high toxicity, with studies linking environmental exposure to organ damage, fish mortality, and population declines, although responses vary among species and exposure conditions. NSAIDs have also been reported to impair mitochondrial function and blood flow during oxidative stress, negatively affecting reproduction, whereas metformin has been shown to alter morphology and physiology in fish and zooplankton populations (EMA; Zheng et al., 2024). These findings emphasize the importance of continuous environmental monitoring to support ecological risk assessment and mitigation strategies.

An additional challenge is the presence of pharmaceutical metabolites, many of which remain biologically active and environmentally persistent. Among these, 2-hydroxyibuprofen is often detected as a transformation product in wastewater-impacted rivers and has been proposed as a biomarker of human wastewater contamination (Celiz et al., 2009; Nguyen et al., 2021; Paíga et al., 2025). Because most monitoring programs focus primarily on parent compounds, the occurrence and ecological significance of metabolites may be underestimated (Mozaz and Weinberg, 2010; Rutere et al., 2020). Moreover, increasing evidence indicates that chronic exposure to mixtures of NSAIDs and their metabolites may produce additive or synergistic effects, including endocrine disruption, altered reproductive performance, and multigenerational impacts on vertebrates, suggesting that mixture toxicity is considered an important aspect of pharmaceutical pollution (Backhaus and Faust, 2018; Brion et al., 2019; Hodkovicov et al., 2022; Mezzelani et al., 2018; Philibert et al., 2023; Richmond et al., 2017).

The European Union Water Framework Directive (WFD; Directive 2000/60/EC) requires Member States to monitor emerging contaminants and achieve good chemical status in surface waters. Nevertheless, pharmaceutical concentrations can exceed predicted no-effect concentrations at times, indicating that current wastewater management and mitigation measures may sometimes be insufficient to adequately protect aquatic ecosystems (Chan et al., 2024; European Parliament and Council, 2000, 2013; Feng et al., 2023). This review aims to compile and critically evaluate published data from 2013 to 2024 on the occurrence, distribution, and ecological risks of selected NSAIDs, metformin, and their metabolites in the surface waters of Europe, with particular emphasis on the Balkan region. In addition, it summarizes current analytical approaches, contamination patterns, the influence of wastewater treatment processes, and the implications of these findings for environmental monitoring and policy development, particularly in vulnerable river basins of Southeastern Europe.

PHARMACEUTICALS MONITORING

The review compiled data from 308 surface water samples collected at 70 monitoring sites across nine European countries, including several Balkan river basins. Sampling intensity varied considerably among the selected studies, ranging from two to nineteen monitoring locations per investigation. Despite methodological differences, all reviewed studies fulfilled the predefined inclusion criteria and provided quantitative data suitable for comparative analysis. Overall, the reviewed dataset demonstrated the widespread occurrence of the selected pharmaceuticals in European surface waters. Ibuprofen, diclofenac, naproxen, ketoprofen, 2-hydroxyibuprofen, and metformin were all detected, although their occurrence, concentration ranges, and spatial distribution differed substantially among river basins. Table 1 summarizes the regional characteristics of the analyzed studies.

Ibuprofen and diclofenac exhibited the highest detection frequencies (89%), indicating their widespread occurrence in European surface waters. In contrast, metformin showed the lowest detection frequency (22%), reflecting both differences in environmental behavior and the more limited number of studies specifically targeting highly polar antidiabetic compounds. Detection frequencies for 2-hydroxyibuprofen, naproxen, and ketoprofen were 56%, 33%, and 33%, respectively. Descriptive statistical analysis demonstrated considerable spatial variability across compounds and monitoring locations. Concentration distributions were strongly right-skewed, with several localized contamination hotspots associated with wastewater treatment plant discharges and densely populated urban areas. Diclofenac and 2-hydroxyibuprofen displayed the highest concentration variability, with maximum values exceeding median concentrations, indicating episodic or site-specific contamination events. The median detection frequency across all compounds was 56%, highlighting heterogeneous contamination patterns driven by differences in pharmaceutical consumption, wastewater treatment efficiency, hydrological conditions, and monitoring intensity among European regions (Table 2).

Statistical evaluation of concentration data revealed substantial heterogeneity among compounds and monitoring sites. Diclofenac concentrations ranged from 38 to 4.806 ng/L, while ibuprofen concentrations varied between 45 and 3.161 ng/L. The metabolite

Table 1. Summary of pharmaceutical occurrence across reviewed European regions

Region	No. of Studies	No. of Sampling Sites	Main compounds	Representative concentrations (ng/L)	Dominant sources
Danube/Balkan	4	32	Diclofenac, Ibuprofen, Naproxen	DCF: 0.8–132; IBU: 45–4048; NPX: 75–12029	WWTP discharge, urban runoff
Mediterranean	2	15	Diclofenac, Ibuprofen	DCF: 38–4806; IBU: 45–3161	Drought conditions, WWTPs
Central Europe	3	14	Ibuprofen, Carbamazepine, Naproxen	IBU: 45–4048; NPX: up to 12029	Municipal wastewater
Polish Waters	1	9	Naproxen, Diclofenac	Influent wastewater: NPX up to 551,960	Wastewater influent
Albanian Rivers	2	8	Ibuprofen, Diclofenac, Naproxen	Limited quantitative data available	Untreated urban discharge

Table 2. Occurrence of pharmaceutical compounds in analyzed sample

Compound	Minimum (ng/L)	Maximum (ng/L)	Studies reporting detection	Detection frequency
Ibuprofen	45	4048	8	89%
Diclofenac	38	4806	8	89%
Naproxen	75	12029	3	33%
Ketoprofen	98	160	3	33%
2-Hydroxyibuprofen	40	5623	5	56%
Metformin	110	110	2	22%

2-hydroxyibuprofen exhibited the widest concentration range, reaching a maximum value of 5.623 ng/L in Croatian surface waters. Mean concentrations were consistently higher than median values for most compounds, indicating the presence of extreme values and non-normal distributions associated with localized contamination sources. Spatial variability was particularly pronounced in river systems receiving untreated or partially treated wastewater effluents. Monitoring stations located downstream of wastewater treatment plants generally exhibited significantly higher pharmaceutical concentrations than upstream reference sites. This pattern was consistently observed across Danube Basin studies, Mediterranean catchments, and Balkan river systems. Due to methodological heterogeneity among studies, including differences in sampling frequency, analytical instrumentation, seasonal coverage, and reporting formats, advanced inferential statistical comparisons and formal meta-analysis were not considered methodologically appropriate. Consequently, results were synthesized using descriptive statistical approaches and comparative regional analysis.

Analytical approaches for pharmaceutical determination

Pharmaceuticals include hundreds of chemicals and their metabolites, posing a major challenge for standardizing reliable sampling and analytical methods with adequate quality control (Mozaz and Weinberg, 2010). The primary analytical techniques in the reviewed literature were solid-phase extraction (SPE) followed by HPLC-MS/MS or UHPLC-MS/MS (Hoi et al., 2021; Kafeenah et al., 2018; Fatta et al., 2007). These hyphenated methods are the gold standard for environmental pharmaceutical analysis due to their sensitivity and selectivity, permitting detection at ppb or ppt levels. GC-MS has been used as an alternative but requires derivatization of polar pharmaceuticals, which is a drawback (Fatta et al., 2007). HPLC-PDA and LC-ESI-MS/MS were also reported (Herghelegiu et al., 2023; Shipingana et al., 2022).

Detection in European surface waters has largely relied on advanced chromatographic–mass spectrometric approaches developed over the last decade. SPE combined with HPLC-MS/MS or UHPLC-MS/MS is dominant (Ene et al., 2025), providing chromatographic separation plus mass-based confirmation via fragmentation patterns. Mixed-mode SPE cartridges (e.g., Oasis HLB, Nexus Bond Elut) are commonly used to extract compounds across polarities, yielding recoveries typically between 60–120% depending on analyte and matrix. Multi-step extraction protocols with washing and conditioning steps reduce matrix interferences and optimize recovery (Kafeenah et al., 2018). LOD values have improved with instrumental advances, with modern approaches reaching 0.01–10 ng/L depending on the analyte, matrix, and detection system (Ene et al., 2025). For metformin, specialized extraction and preconcentration have enabled detection limits around 0.16 µg/L using HPLC-DAD combined with optimized dSPE (Sekopelo et al., 2024). Electrospray ionization in positive and negative modes with multiple reaction monitoring (MRM) facilitates simultaneous analysis across compounds with diverse ionization efficiencies (Zhuo et al., 2025). Matrix-matched calibration is often employed to account for matrix effects. The evolution and validation of these methods have been crucial in advancing pharmaceutical monitoring in European waters. As shown in Table 3. UHPLC-MS/MS is currently considered the top-tier method, with LODs down to 0.01–2 ng/L (Bczkowska et al., 2025),

Table 3. Comparison of analytical methods for trace contaminant determination

Analytical method	LOD (ng/L)	LOQ (ng/L)	Selectivity	Matrix effects	Cost	Typical use
HPLC-MS/MS	0.5–5	2–15	Excellent	Present but manageable	High	Research, Monitoring
UHPLC-MS/MS	0.01–2	0.05–5	Excellent	Present but manageable	Very High	Advanced monitoring
LC-ESI-MS/MS	0.1–3	0.5–10	Excellent	Present but manageable	High	Environmental monitoring
GC-MS	01–10	5–30	Very Good	Minimal	Medium	Complementary method

but it requires substantial investment and expertise. HPLC-MS/MS achieves acceptable performance at lower cost. (Gomez-Regalado et al., 2022). The selected studies were analyzed for detection methodology, instrumentation specs, sample preparation, LOD/LOQ, site characteristics, temporal patterns, concentration ranges, metabolites identified, WWTP influence, and risk assessments (Hoi et al., 2021; Zhuo et al., 2025). Study quality was evaluated against analytical method validation standards (e.g., FDA, EMA), acceptable recovery rates (70–120%), precision (RSD), matrix effects, environmental context, sampling frequency, and statistical treatment of data (ICH Q2(R2), 2023).

Occurrence of pharmaceuticals in European surface waters

Danube basin

As it is shown in the Figure 1. The Danube River Basin was the most extensively investigated river system among the reviewed studies and consistently exhibited elevated pharmaceutical contamination associated with municipal wastewater discharges. Diclofenac, ibuprofen, naproxen, and ketoprofen were the most frequently detected NSAIDs, with detection frequencies generally ranging from 50% to 100% depending on the sampling campaign and river section. Spatial variability was strongly influenced by proximity to wastewater treatment plant (WWTP) discharges, hydrological conditions, and urban population density. Recent monitoring campaigns conducted in the lower Danube and Black Sea basin of southeastern Romania identified 19 emerging contaminants among 30 target compounds using SPE-LC-HRMS analysis. Diclofenac was one of the most frequently detected pharmaceuticals, occurring at 87% of sampling sites with maximum concentrations reaching 132 ng/L. Ketoprofen and naproxen were also widely detected, while correlations between pharmaceutical occurrence and total coliform counts indicated insufficiently treated municipal wastewater as the principal contamination source (Ene et al., 2025). Additional investigations in the Hungarian section of the Danube demonstrated considerable spatial variability in pharmaceutical concentrations. Naproxen reached average concentrations of 12,029 ng/L at heavily impacted locations, while ibuprofen reached approximately 4.048 ng/L. Carbamazepine and diclofenac were detected at all investigated sites, confirming the widespread distribution of pharmaceuticals throughout this river section. Variations among sampling sites suggested localized point-source inputs associated with wastewater discharges and river hydrodynamics (József et al., 2023).

Further evidence from the Mureș River Basin in Romania confirmed the occurrence of both parent pharmaceuticals and transformation products. Ibuprofen, carbamazepine, and metabolites including 1-hydroxyibuprofen, 2-hydroxyibuprofen, and carboxyibuprofen were detected above the analytical quantification limits at multiple locations. Concentrations were consistently highest downstream of wastewater treatment plant effluents and lowest at upstream reference sites, further supporting WWTPs as the dominant source of pharmaceutical contamination. The simultaneous occurrence of parent compounds and metabolites also highlights the importance of considering transformation products during environmental monitoring and ecological risk assessment (Burcea et al., 2020). Large-scale monitoring initiatives further confirmed the regional significance of pharmaceutical contamination within the Danube Basin. The Joint Danube Survey detected pharmaceuticals in more than 80% of investigated samples, while passive sampling campaigns identified numerous contaminants exceeding ecological risk thresholds. Together, these findings demonstrate that pharmaceutical occurrence within the Danube Basin is primarily controlled by municipal wastewater inputs, transboundary river transport, and incomplete removal during conventional

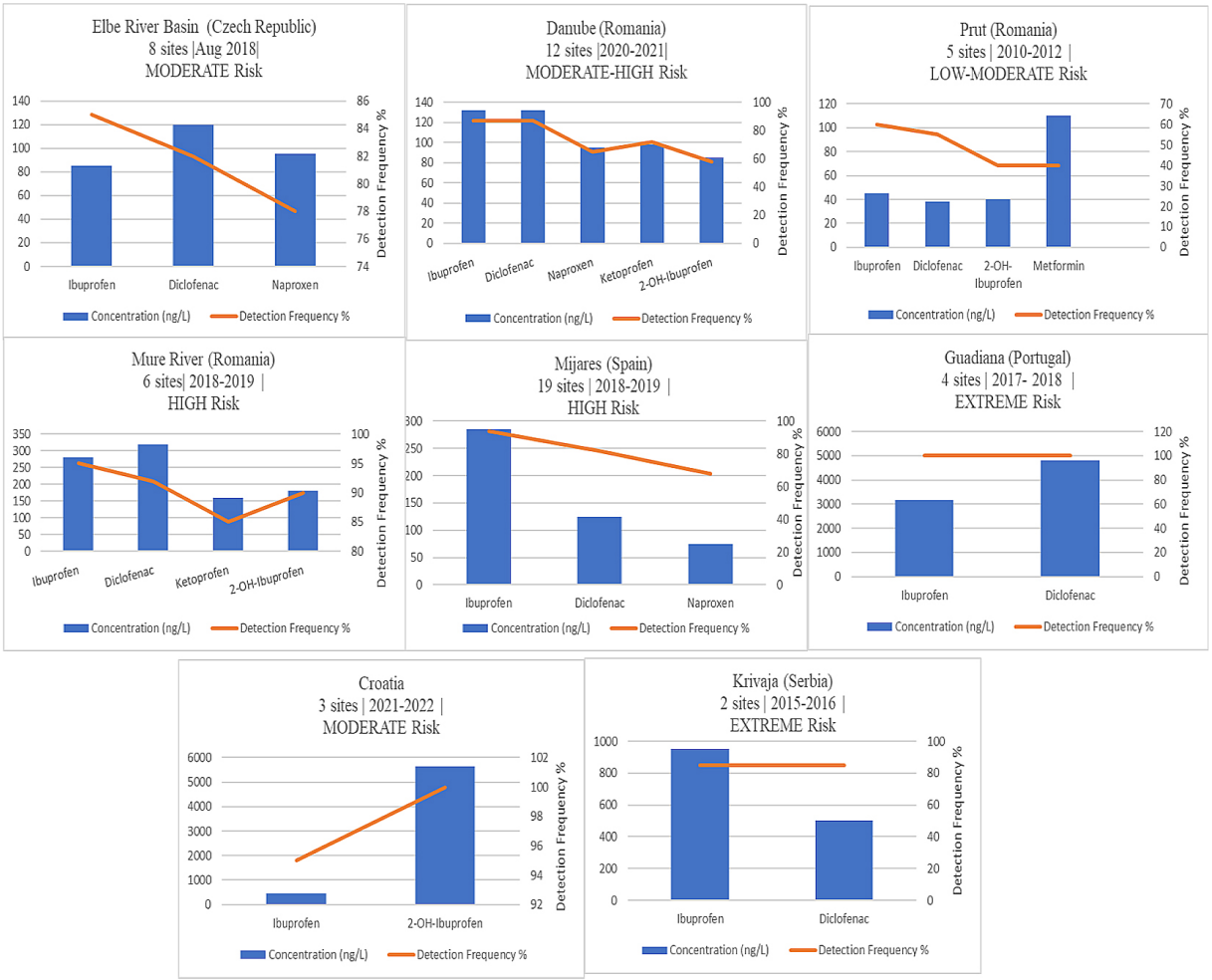

Figure 1. Distribution of monitoring sites by river system. Own data elaboration from 8 major monitoring studies conducted across Central Europe, Eastern Europe, Mediterranean, and Balkan regions

wastewater treatment, making the basin one of the major pharmaceutical contamination hotspots in Central and Eastern Europe.

Mediterranean region

The Mediterranean region exhibited some of the highest pharmaceutical concentrations reported among the reviewed studies, largely due to hydrological conditions that reduce river dilution capacity during prolonged dry periods. Seasonal drought, low river discharge, and continuous wastewater inputs were consistently identified as the principal factors influencing pharmaceutical accumulation in surface waters. The Guadiana River Basin in Portugal represents one of the best-documented examples of hydrological influence on pharmaceutical occurrence. Monitoring campaigns conducted during both drought (2017) and post-drought (2018) periods detected elevated concentrations of several pharmaceuticals using SPE coupled with HPLC-MS/MS. Diclofenac reached a maximum concentration of 4.806 ng/L, while ibuprofen reached 3.161 ng/L. Other pharmaceuticals, including hydrochlorothiazide (2,726 ng/L) and carbamazepine (3.223 ng/L), were also frequently detected. Concentrations were consistently higher during drought periods, reflecting reduced dilution capacity and increased persistence of pharmaceuticals in river water (Palma et al., 2019; Sousa et al., 2018). Overall, Mediterranean studies demonstrate that climatic conditions represent an important driver of pharmaceutical contamination. Unlike the Danube Basin, where urban wastewater inputs dominate contamination patterns, Mediterranean river systems are additionally influenced by seasonal hydrological variability, which substantially increases contaminant concentrations and ecological risks during periods of low flow.

Central European rivers

Central European rivers generally exhibited widespread pharmaceutical occurrence, although concentration ranges varied according to population density, wastewater treatment efficiency, and local hydrological conditions. Monitoring studies from Austria, the Czech Republic, Germany, and Poland consistently identified NSAIDs among the most frequently detected pharmaceutical groups.

Poland has contributed extensive monitoring data on pharmaceuticals in rivers, lakes, and wastewater systems. A comprehensive review by Isarczyk et al. (2021) reported widespread occurrence of NSAIDs, particularly naproxen and diclofenac, in both surface waters and wastewater. Extremely high concentrations of naproxen (up to 551,960 ng/L) and diclofenac (108,340 ng/L) were reported in wastewater influents, highlighting untreated municipal wastewater as the primary contamination source. Although concentrations measured in receiving rivers were substantially lower because of dilution and natural attenuation processes, pharmaceuticals remained consistently detectable, demonstrating their persistence in aquatic environments. Across Central Europe, pharmaceutical occurrence reflects differences in consumption patterns, wastewater management practices, and analytical monitoring intensity. Countries with more developed monitoring programmes generally reported higher detection frequencies, whereas concentration hotspots were typically associated with densely populated urban catchments and wastewater treatment plant discharges.

Balkan rivers

Compared with Central and Western Europe, pharmaceutical monitoring in the Balkan region remains relatively limited despite increasing evidence of contamination in major river systems. Available studies indicate that NSAIDs are consistently detected in rivers receiving untreated or partially treated municipal wastewater, highlighting the need for more comprehensive monitoring programmes throughout Southeastern Europe. Croatian and Romanian investigations have demonstrated the occurrence of both parent pharmaceuticals and transformation products, confirming that pharmaceutical contamination extends beyond the major Danube corridor. The detection of metabolites, including 2-hydroxyibuprofen, further indicates active biotransformation processes within wastewater treatment systems and receiving aquatic environments. Although pharmaceutical monitoring in Albania is still emerging, recent investigations provide the first quantitative evidence of pharmaceutical residues in Albanian surface waters. A seasonal survey conducted in the Ishëm River Basin (2023–2024) identified twelve pharmaceutical compounds, including ibuprofen, diclofenac, and naproxen, in both water and sediment samples, demonstrating contamination levels comparable to those reported elsewhere in Europe (Peqini et al., 2025). Earlier studies of the Osumi and Erzeni–Ishëm river basins focused primarily on physicochemical water quality and therefore did not include pharmaceutical contaminants (Shegani, 2016; Gjeci et al., 2025). Recent monitoring programmes covering the Ishëm, Erzeni, and Shkumbini river basins further support the occurrence of contaminants of emerging concern in Albanian rivers (Jaku et al., 2025). Although the available dataset remains limited, current evidence suggests that pharmaceutical contamination is already widespread and justifies the inclusion of pharmaceuticals within routine national water-quality monitoring programmes in accordance with the objectives of the European Water Framework Directive.

Occurrence of pharmaceutical metabolites

Among the pharmaceutical transformation products identified in the reviewed studies, 2-hydroxyibuprofen was the most frequently reported metabolite and exhibited the greatest variability in environmental concentrations. In several monitoring campaigns, metabolite concentrations exceeded those of the parent compound, indicating that environmental biotransformation processes may substantially contribute to the overall pharmaceutical burden in surface waters. The highest concentration of 2-hydroxyibuprofen reported in the reviewed literature reached 5.623 ng/L in Croatian surface waters, exceeding the concentration of ibuprofen measured at the same location (Paíga et al., 2025). Similarly, wastewater investigations in Portugal detected 2-hydroxyibuprofen at concentrations up to 639 µg/L, confirming extensive biotransformation during wastewater treatment and subsequent release into receiving waters (Paíga et al., 2024). The widespread occurrence of 2-hydroxyibuprofen reflects both human metabolism and microbial transformation of ibuprofen within wastewater treatment plants

and aquatic environments (Nguyen et al., 2021). Because these metabolites may retain biological activity and persistence comparable to their parent compounds, monitoring programmes focused exclusively on parent pharmaceuticals are likely to underestimate the actual pharmaceutical burden and associated ecological risks (Rutere et al., 2020).

Occurrence of metformin in European surface waters

Metformin has emerged as one of the most frequently detected antidiabetic pharmaceuticals in aquatic environments owing to its extensive consumption and limited human metabolism. Although fewer studies have investigated metformin compared with NSAIDs, available evidence confirms its widespread occurrence in European surface waters. Monitoring studies identified metformin in several European river basins, particularly those influenced by municipal wastewater discharges. In transboundary river systems of Spain and Portugal, metformin was detected in approximately 40% of river samples, confirming its persistence in wastewater-impacted aquatic environments (R et al., 2023). Recent analytical developments, including dispersive solid-phase extraction coupled with HPLC, have substantially improved the detection of this highly polar compound (Sekopelo et al., 2024). Unlike NSAIDs, metformin is excreted largely unchanged by the human body and therefore represents a useful indicator of wastewater-derived contamination. Although most environmental risk assessments reported hazard quotient (HQ) values below unity, recent ecotoxicological studies suggest that chronic exposure may affect fish physiology and metabolic processes at environmentally relevant concentrations (Zheng et al., 2024). These findings indicate that, despite its comparatively lower acute toxicity, metformin should be considered an important contaminant of emerging concern and included in future environmental monitoring programmes.

Environmental risk assessment

Environmental risk assessment provides an essential framework for evaluating the ecological implications of pharmaceutical contamination beyond concentration measurements alone. Most of the reviewed studies assessed ecological risk using the risk quotient (RQ) approach, calculated as the ratio between the measured environmental concentration (MEC) and the Predicted No-Effect Concentration (PNEC). Among the investigated pharmaceuticals, diclofenac consistently represented the highest ecological concern because of its widespread occurrence, persistence, and documented toxicity to aquatic organisms. Reported concentrations ranged from 38 ng/L in the Prut River (Romania) to 4,806 ng/L in the Guadiana River Basin (Portugal), illustrating substantial spatial variability across European river systems. The highest concentrations were generally associated with densely populated catchments, wastewater treatment plant (WWTP) discharges, and reduced river flow during drought periods. Several studies reported RQ values exceeding 1.0, particularly for diclofenac and, in some cases, ibuprofen, indicating a potential risk to aquatic ecosystems (Palma et al., 2019). Experimental evidence demonstrates adverse effects on fish, algae, and aquatic invertebrates, including oxidative stress, reproductive impairment, immune dysfunction, and reduced photosynthetic activity. These findings support the prioritization of diclofenac as one of the pharmaceuticals of greatest environmental concern in European surface waters. Although metformin generally exhibited lower individual hazard quotients than NSAIDs, recent ecotoxicological studies suggest that long-term exposure may affect fish physiology and metabolic regulation, indicating that chronic toxicity should not be overlooked. Similarly, the occurrence of transformation products, particularly 2-hydroxyibuprofen, highlights the need to incorporate pharmaceutical metabolites into future ecological risk assessments.

An additional limitation identified across the reviewed studies is the assessment of pharmaceuticals as individual substances. In natural aquatic environments, pharmaceuticals occur as complex mixtures, and increasing evidence indicates that combined exposure may produce additive or even synergistic toxic effects that are not adequately represented by single-compound risk assessments. Consequently, future environmental risk evaluations should integrate mixture toxicity, pharmaceutical metabolites, and long-term exposure scenarios to provide a more realistic assessment of ecological impacts.

CONCLUSIONS

Pharmaceutical contamination, particularly by NSAIDs such as diclofenac and ibuprofen, their metabolites like 2-hydroxyibuprofen, and metformin, is widespread in European surface waters, with notable severity in the Balkans and Southeastern Europe. Limited wastewater treatment infrastructure, high pharmaceutical consumption, seasonal droughts, and inadequate disposal practices exacerbate contamination in these vulnerable regions. Southern European rivers often exhibit pharmaceutical concentrations 5 to 40 times higher than those in Northern Europe, largely due to hydrological, climatic, and anthropogenic factors affecting dilution and removal efficiency. While large rivers like the Danube have been extensively studied, smaller and often more impacted rivers in the Balkans remain under-monitored, leaving critical knowledge gaps. Emerging evidence highlights that pharmaceutical metabolites contribute significantly to environmental burdens and may exceed parent compound concentrations, emphasizing the urgent need to include metabolites and mixture toxicity in routine monitoring and ecological risk assessments. Current monitoring efforts are hindered by inconsistent methodologies, limited geographic coverage, and a lack of standardized protocols, particularly in Southeastern Europe. To address these challenges, future research should focus on expanding harmonized monitoring frameworks with standardized sampling and analytical methods across the Balkans. Advanced wastewater treatment technologies such as ozonation, activated carbon adsorption, and membrane filtration should be implemented to improve pharmaceutical removal efficiencies. Additionally, policy actions must strengthen pharmaceutical take-back programs, regulate disposal practices, and reinforce compliance with the EU Water Framework Directive and its Watch List obligations. Integrating environmental monitoring data with pharmaceutical usage and public health information will enhance understanding of contamination sources and support evidence-based water management strategies. Collectively, these measures are essential to mitigate pharmaceutical pollution, safeguard aquatic ecosystems, and achieve sustainable water quality across Europe.

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