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The occurrence of chemicals of emerging concern in samples of surface water and wastewater collected in Kraków, Poland

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ABSTRACT

The presence and fate of chemicals of emerging concern (CECs) in the natural freshwater resources are of great interest. It is therefore essential to evaluate the efficiency of the removal of CECs in commonly used treatments and long-term adverse health effects on humans and wildlife. The occurrence and removal of fifteen representatives CECs and selected metabolites (salicylic acid, carboxyibuprofen and 4′-hydroxydiclofenac), in two urban wastewater treatment plants (WWTPs), were investigated. The presence of target compounds in the surface water – sources of drinking water for Kraków – was also the subject of this study. Results obtained in this study showed that in the wastewater influents, all target compounds except (estrone, 17β-estradiol and estriol), were routinely detected with a concentration of up to 12.7 μg L^{-1}. The highest concentrations were observed for salicylic acid, caffeine, ibuprofen and its metabolite, carboxyibuprofen. The average efficiency of removal in terms of the total reduction of the concentration of CECs between the influent and effluent ranged from 15% (diclofenac) to 100% (acetaminophen and estrogens) at both WWTPs. Ten compounds were detected at levels above the method quantification limits in surface water. Salicylic acid and caffeine were detected in all the tested samples of surface water. The highest concentrations were observed for caffeine. In the current study from medium to high environmental risk levels for carbamazepine, triclosan and diclofenac were noted.

Keywords: Pharmaceuticals; Endocrine-disrupting chemicals; Municipal wastewater; Metabolites; Surface water; Gas chromatograph coupled with Ion Trap Mass Spectrometer (GC-(IT)MS/MS)

1. Introduction

In recent years, more and more attention has been devoted to the presence of non-regulated chemicals of emerging concern (CECs) in various environmental components and their potential impact on organisms and humans involved in their life cycle [1–7]. Recently, some attention was devoted to metabolites and products of metabolism of pharmaceuticals in the environment, which may prove to be more toxic than the original forms of drugs, or which may revert to their original form as a result of various processes that take place in the environment [8,9].

The occurrence of CECs in effluents, surface water, groundwater, soil and tap water is a result of extensive use and not complete removal in wastewater treatment plants

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(WWTPs) or direct emission to the environment [10,11]. Processes used in conventional WWTPs are not designed for the elimination of emerging contaminants and the efficiency of their removal requires further investigation in order to be clearly understood [12,13]. This problem is also reported from Polish wastewater treatment plants, where the emission of CECs to the environment is noted at the concentration level from ng L^{-1} to μg L^{-1} [14–16]. Caffeine showed the highest concentrations determined in Wisła River, over 360 ng L^{-1} [17]. The average concentrations of anti-inflammatory drugs reported for Wisła River were 58 and 175 nL^{-1} for ibuprofen and diclofenac, respectively [17]. Triclosan and bisphenol A were determined at the average concentrations of 45 and 23 ng L^{-1}, respectively [17]. These values were consistent with those observed in the UK [7]. Carbamazepine concentrations in surface waters in Portugal were ranged from 25 to 214 ng L^{-1} [18]. Naproxen was determined in Spain at the average level of 278 ng L^{-1} [19], but in Poland, this value was almost eight times lower and amounted to 37.7 ng L^{-1} [20]. Ketoprofen concentrations in surface waters near the Vistula Estuary to the Baltic Sea were ranged from 16 to 58.8 ng L^{-1} [20].

Pharmaceuticals such as antibiotics or analgesics are ubiquitous active compounds frequently detected in surface waters at concentrations reaching μg L^{-1} [21–24]. The environmental persistence, wide distribution and bioaccumulation of these pollutants vary depending on their chemical properties and environmental conditions [25–27]. Their continuous input into the environment may lead to ecotoxicological effects [28–33]. Recent studies have reported that a mixture of different compounds may have a synergistic effect leading to unexpected adverse effects on humans and other organisms [34,35]. Non-steroidal anti-inflammatory drugs (NSAIDs) are of great concern due to their wide application and the largest group of over-the-counter drugs sold worldwide [13,36]. The other groups which attract considerable attention are antibiotics and hormones widely used in human and veterinary medicine due to their potential role in the development of resistant mechanisms by bacteria and the effects on the endocrine system of organisms, respectively [27,36–38]. Under Decision 2015/495/EU a Watch List of substances for Union-wide monitoring in the field of water policy, containing 17 organic compounds called contaminants of emerging concern, for which wide monitoring data is needed [36,39] was published. The Watch List includes, among other things, five pharmaceuticals, diclofenac, the macrolide antibiotics, as well as synthetic estrogen 17-α-ethinylestradiol (EE2), as well as natural estrogens, estrone (E1) and 17β-estradiol (E2).

Existing data have provided fundamental knowledge for the understanding of CEC removal in the WWTP. However, the acquisition of more information about the contamination of CECs in different areas is still an urgent task and the continuous entering of these substances into the natural environment makes them a pseudo-persistent pollutant [27,34,40]. The consolidated data on the presence the pharmaceuticals and endocrine disruptors in the aquatic environment are crucial for the development of efficient treatment solutions in order to avoid or reduce their release into the environment.

This study was focused on the occurrence of NSAIDs (ibuprofen, diclofenac, ketoprofen, naproxen and acetaminophen) and related metabolites (salicylic acid, carboxyibuprofen and 4′-hydroxydiclofenac) and other chemicals of emerging concern (carbamazepine, caffeine, triclosan, estrone, β-estradiol, estriol, bisphenol A) in wastewater treatment plants and surface waters. The concentrations of xenobiotics in surface water – sources of drinking water at Kraków metropolitan area in South Poland and also their concentration, loads and removal in two WWTPs in Kraków were determined. A further aim was to correlate the presence of selected CECs detected during the study with potential associated environmental risks. This is the first study on the effects of this broad set of compounds on the Kraków metropolitan area environment.

2. Materials and methods

2.1. Chemicals and materials

All reference standards were of >98% purity and were purchased from Sigma-Aldrich (Saint Louis, USA). Surrogate/internal standards (Table S1) were purchased from Sigma-Aldrich (Saint Louis, USA). Standards, which were used as surrogate standards, were added to the samples before extraction and were also used for the quantification of the samples. The Derivatization Reagent BSTFA + 1% TMCS was purchased from Supelco (Bellefonte, USA). Methanol of GC grade and HCl (35%) were purchased from POCH (Gliwice, Poland). All compounds studied in this work are presented in a summary manner in Table S1. Solutions of the compounds both individually and as mixtures were prepared in methanol and stored in the dark at 4°C. Mixed standard solutions were prepared at 10 mg L^{-1} in methanol and diluted as necessary to prepare working solutions. Deionized water (<0.07 S cm^{-1}) used to prepare samples for the solid-phase extraction (SPE) was obtained from a HLP5 pure water system (Hydrolab, Gdańsk, Poland).

2.2. Acquisition of samples

Sampling sites were located along the Wisła River (Fig. 1), at four rivers which are sources of drinking water and two WWTPs which process Kraków wastewater. The most basic drinking water sources in Kraków are the surface water intakes, operating on Raba, Rudawa, Dłubnia and Sanka Rivers. The Dobczyce Reservoir is located 270 m above sea level on the Raba River at 60 km from the source. The Bielany surface water intake operates on the Sanka River. The main contaminants of rivers originate from agriculture and municipal activities with smaller industrial contributions [41]. The waters of Rudawa and Sanka Rivers are characterized by the highest total organic carbon (4.7 and 4.6 mg L^{-1}) among tested rivers (Table S2). Samples were collected two times at each sampling location except for the Dłubnia River site in November 2015. Sampling locations of surface water and wastewaters are presented in Fig. 1 and the technological capacity of water and wastewater treatment plants are presented in Tables S2 and S3, respectively. Samples of

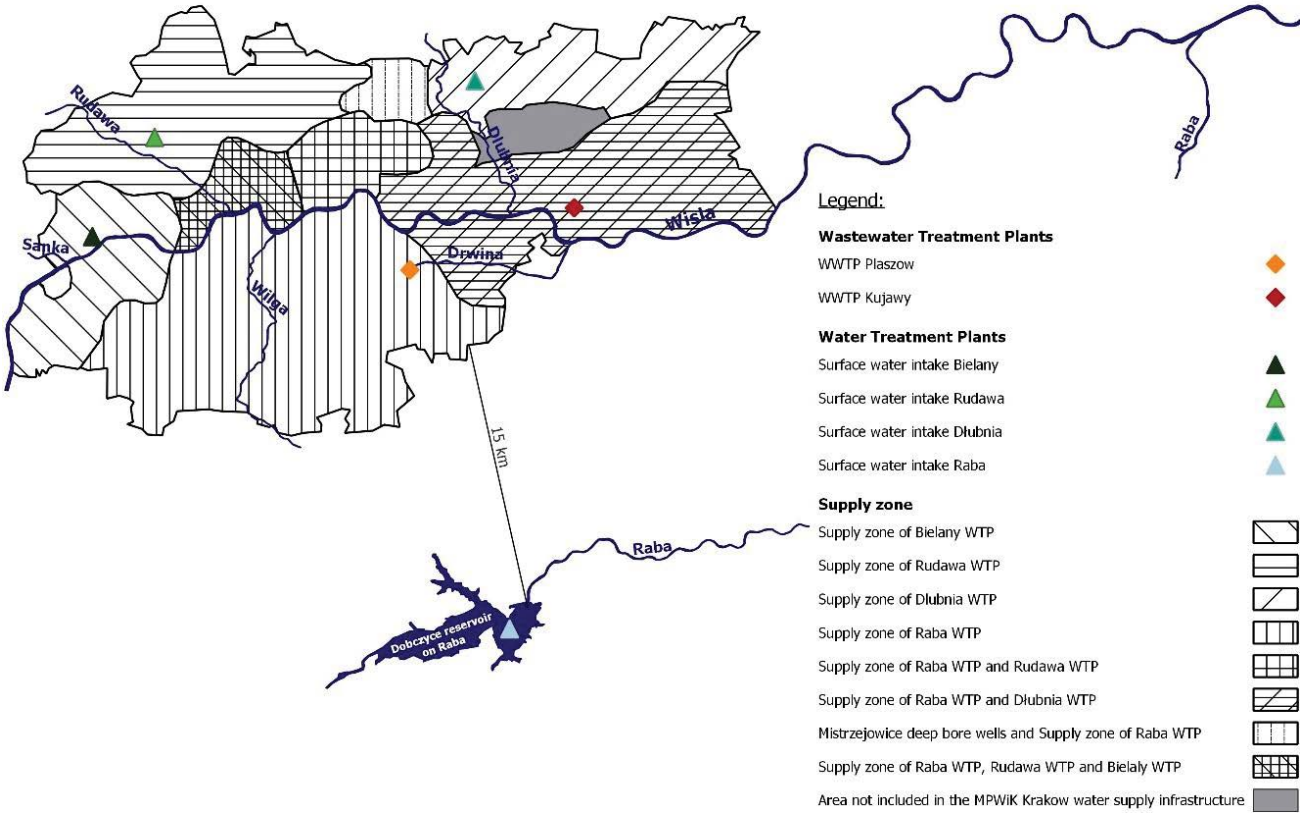

Fig. 1. A map of sampling locations of surface waters and wastewaters. Parts of Kraków city supplies of water sources.

raw surface water (5 L) were collected in water treatment intakes and transported back to the laboratory in a dark and iced cool box.

2.5 L of wastewater influent and effluent were collected at the Płaszów and Kujawy WWTPs (Fig. 1). Composite sewage samples (24 h) collected in May and in July 2015 in Kujawy WWTP and in June and July in Płaszów WWTP. The Płaszów WWTP in Kraków is the largest one in the city and the third in the country, which treats over 70% of Kraków wastewater from over 680 thousands of inhabitants from the central part of the city with an average capacity of 165,000 m^3 d^{-1}. The Kujawy WWTP is the second plant in terms of size in Kraków, located in the east part of the city (high contribution of industry). It treats around 52,000 m^3 d^{-1} of wastewater from the 250 thousands of inhabitants of Nowa Huta. The general treatment of the sewage in these plants is consists of mechanical pretreatment by screening coarse particles, an aerated grit-removal tank, a primary clarifier and biological stages with activated sludge and a denitrification stage. After the settling of sludge, the water is discharged into the receiving river, from the Kujawy WWTP to the Wisła River and from the Płaszów WWTP to Drwina River.

All samples were collected in amber bottles and transported back to the laboratory in a dark and iced cool box. Surface water and wastewater samples were vacuum filtered firstly through MN GF-4 (1.4 μm, 47 mm) glass fiber filter and subsequently through MN GF-5 (0.4 μm, 47 mm) glass fiber filter purchased from Macherey-Nagel (Düren, Germany). A Vacuum Filtration System (Alltech, USA) was used for the filtration of samples. After filtration, samples were acidified with HCl to pH = 2. Samples were stored in the dark at 4°C and extracted within 20 h.

2.3. Solid-phase extraction

SPE was carried out using HLB (water-wettable polymer with a unique Hydrophilic-Lipophilic Balance) (60 mg, 3 mL waters) cartridges to extract the organic substances from the samples after filtration. In order to carry out the extraction, 250 and 500 mL of wastewater from the influents and effluents, respectively, were taken. For the extraction of surface water, 1,000 mL of samples were taken. Acidified samples were spiked with 200 ng of each of the deuterated CEC internal standards and then passed through the SPE cartridges at a rate of 6 mL min^{-1}. The cartridges were conditioned using 3 mL of methanol and 3 mL of pure water (Hydrolab, Gdańsk, Poland) acidified to pH = 2 with HCl. After the extraction, the cartridges were dried under full vacuum for 20 min and then eluted with 2 mL × 2 mL of methanol. The extracts were completely dried under argon at 35°C and then dissolved in 50 μL of the derivatization reagent. The silylation process with BSTFA + 1% TMCS was carried out at 65°C for 35 min in a thermo-block. AccuBlock Labnet Digital Dry Bath (Woodbridge, USA). Solutions were then analyzed by gas chromatograph coupled with Ion Trap Mass Spectrometer (GC-(IT)MS/MS). A similar procedure was discovered by Migowska et al. [42] and Nosek et al. [43].

The absolute recovery was calculated using the following equation:

$$\mathrm{AR} = \frac{P_{\mathrm{extr}} - P_0}{P_{\mathrm{st}}} \times 100 \quad (1)$$

where P_{extr} is the peak area obtained for the sample spiked with the analyte prior to extraction minus P_0 the peak area of the analyte obtained for the non-spiked sample and P_{st} is the peak area of the analyte obtained for the standard solution.

Different water and wastewater samples were used for the development and validation of the method. Recoveries were determined by spiking samples of wastewater and surface water with standard solutions of target compounds at concentration 1,000 ng L^{-1}.

2.4. GC-(IT)MS/MS analysis

The analysis was carried out by means of a Thermo Scientific GC TRACE 1300 (GC-(IT)MS/MS) and a TriPlus RSH Autosampler. The flow of helium through a GC column was constant and was set at 1 mL min^{-1}. The programmable temperature of the vaporization injector and the transfer line were maintained at 250°C. The MS was operated with the ion source at 250°C. The injector was operated at splitless conditions for 1 min, then turned to the split mode at the ratio of 50:1. The volume of injections was 1 μL. All the separations of compounds were performed on a TG-SQC capillary column from Thermo Scientific that had 30 m × 0.25 mm inner diameter and a film thickness of 0.25 μm (5% phenyl 95% dimethylpolysiloxane). The temperatures program was as follows: initial temperature of 70°C for 2 min. followed by a temperature ramp of 20°C min^{-1} to 320°C at and finally 5 min at 320°C.

The analyses were performed in positive mode with an electron energy of 70 eV and an emission current of 250 μA. Helium (99.999%) was used as a collision gas with a flow of 0.3 mL min^{-1}. The choice of fragmentation products for each substance was based on the most intense signal. For the acquisition of the MRM transitions, the analytical run was split into time windows according to the expected retention time of the selected compounds. Data acquisition, processing and quantification were performed using Xcalibur® software. The quantification of organic substances was carried out using the highest characteristic precursor ion/product ion transitions and characteristic ions (Table S4). Specific and intense product-ions of each target analyte were used for quantification, and a secondary product-ion was used as a qualifier ion for confirmatory purposes. Deuterated internal standards were added prior to SPE extraction in order to compensate for losses or enhancement of compounds during both the sample preparation procedure and resulting from matrix effects. The corresponding isotope labeled surrogate standards were used for quantification whenever possible. The most suitable surrogate standard was selected according to the retention time and structural analogy. Where no labeled compounds were available quantification was performed with another labeled standard (Table S4).

The validation parameters of the SPE-GC-(IT)MS/MS method used for the determination of target compounds in surface water and wastewater samples is presented in Table S5.

2.5. Calculations

The mass loads of target analytes at both the influent and effluent of WWTP during the sampling period (g d^{-1}) were determined using the equation:

$$\text{Mass loads} = \frac{\text{Infl or Effl} \times \text{FR} \times 1}{1{,}000} \quad (2)$$

where Infl and Effl refer to the concentration in ng L^{-1} of the analytes determined at the influent and effluent wastewater samples and FR refers to the mean flow rate of the plant (ML d^{-1}) during the sampling day.

The ability of the WWTP to remove the determined analytes in the liquid phase were determined by calculating the percentage removal efficiency (RE %) between influent and effluent wastewater during the sampling period by means of the following equation:

$$\mathrm{RE}(\%) = \frac{(\text{Infl} - \text{Effl})}{\text{Infl}} \times 100 \quad (3)$$

where Infl refers to mass loads (g d^{-1}) of the analytes determined at the influent sample and Effl refers to the mass loads (g d^{-1}) of the analytes determined at the effluent of WWTP [37].

The risk quotient (RQ) values were calculated for each compound detected in wastewater effluents by dividing the measured environmental concentration in μg L^{-1} by a predicted no-effect concentration (PNEC, μg L^{-1}) using the equation:

$$\mathrm{RQ} = \frac{\mathrm{MEC}}{\mathrm{PNEC}} \quad (4)$$

PNECs (acute or chronic lethal toxicity outcomes) values obtained in tests of cyanobacteria, invertebrates, algae and fish reported in the literature were used [9,37,44–46] and presented in Table 1.

If the RQ values are below 0.1 no adverse effect is expected. If RQ values are between 0.1 and 1. The risk is low but the potential for adverse effects should be considered. If the RQ values are between 1.0 and 10 some adverse effect or moderate risk is probable. Finally, if the calculated RQ values are above 10 a high risk is anticipated [47].

3. Results and discussion

3.1. Xenobiotics concentrations and loads in WWTPs

The results of the analysis of xenobiotics in wastewater are presented in Table 2. Average concentrations of compounds were estimated at the level ranging from a few ng L^{-1} to μg L^{-1}. The highest concentrations in the influent, up to 12.7 μg L^{-1}, were noted in reference to the salicylic

Table 1
The value of PNEC – predicted no-effect concentration (acute or chronic lethal toxicity outcomes)

Compound	PNEC (μg L^{-1})	Reference
Ibuprofen (IBF)	7.10	[46]
Diclofenac (DCF)	0.10	[46]
Ketoprofen (KTP)	3.10	[46]
Naproxen (NPK)	3.30	[46]
Acetaminophen (ATP)	0.24	[46]
Carbamazepine (CBZ)	2.5	[45]
Caffeine (CAF)	87.0	[47]
Salicylic acid (SAL)	43.1	[47]
Carboxyibuprofen (CBXIBF)	2260	[9]
4′-Hydroxydiclofenac (OHDCF)	185	[9]
Triclosan (TCS)	0.069	[46]
Bisphenol A (BPA)	1.0	[38]
Estrone (E1)	0.10	[47]
β-Estradiol (E2)	0.01	[47]
Estriol (E3)	1.52	[47]

acid (SAL) at both WWTPs. In addition, ibuprofen (IBF), naproxen (NPK), acetaminophen (ATP), carbamazepine (CBZ), caffeine (CAF) and carboxyibuprofen (CBXIBF), also manifested higher concentrations in the influent, with the average concentrations of more than 1 μg L^{-1}. The concentrations observed for estrogens in the influent ranged from 42 to 150 ng L^{-1}. In the effluent, the highest concentrations were observed for caffeine, with the average concentrations more than 1 μg L^{-1}. The carbamazepine and carboxyibuprofen, were the most concentrated analytes in all WWTP effluents. Estrogens (E1, E2, E3) and acetaminophen were not detected in the effluent.

The concentrations of selected compounds observed in this study represented a similar range of concentrations observed in the previous research concerning wastewater samples collected at Płaszów WWTP in 2012. Influent-abundant compounds included salicylic acid (12.8 μg L^{-1}) and ibuprofen (2.5 μg L^{-1}), whereas the highest concentrations found in effluents corresponded to carbamazepine (2.9 μg L^{-1}) [43]. In the previous study, the concentrations of diclofenac (DCF), ketoprofen (KTP), naproxen, bisphenol A (BPA) and triclosan (TCS) ranged from 249 ng L^{-1} (diclofenac) to 1,393 ng L^{-1} (bisphenol A) in the influent and ranged from 313 ng L^{-1} (naproxen) to 813 ng L^{-1} (bisphenol A) in the effluent. Carbamazepine was the most abundant one among the tested compounds. The occurrence of this antiepileptic drug has been reported frequently in the influent and the effluent of WWTPs, where it proved to be highly recalcitrant [2,48,49]. The concentrations of estrogens, bisphenol A and triclosan observed in this study were similar to concentrations reported in the literature [1].

The anti-inflammatory/analgesic drugs occurred at the highest concentrations in raw wastewater and these compounds are among the most consumed drugs in Poland [50].

The data collected by the Polish Ministry of Health, in cooperation with the World Health Organization Regional Office for Europe, showed that the level of consumption of pharmaceuticals in Poland is one of the highest in Europe [51]. Every year, the number of non-prescription pharmaceuticals increases, especially antitussive, analgesic and anti-inflammatory drugs [21].

Ibuprofen is transformed in the human body or by microorganisms present in the WWTPs and in the natural environment into metabolites, which together with the basic form occur in water and sewage may increase the probability of their environmental presence [1]. Carboxyibuprofen was identified as a major metabolite under both oxic and anoxic conditions [52]. The concentrations of target metabolite of ibuprofen in the influents ranged from 1,005 to 7,202 ng L^{-1}, from 59 to 943 ng L^{-1} in the effluents, and in many cases, they were at least two times higher than the concentrations of the parent compound. The ratio between carboxyibuprofen to ibuprofen varied between 0.69 and 4.56 with an average value of 2.27. Data reported in the literature demonstrated that hydroxyibuprofen and carboxyibuprofen have been detected both in WWTPs and surface water in concentrations higher than the mother compound, which was also observed in the results obtained in this study [53–56]. The ratios between carboxyibuprofen and ibuprofen were 2.9 and 2.4 reported by Ferrando-Climent et al. [52], Buser et al. [53], Weigel et al. [54] and Dvořáková Březinova et al. [55], respectively.

The biotransformation of diclofenac in humans produces a range of detectable metabolites that are mainly hydroxylated, methoxylated, and acyl glucuronide conjugates in plasma and/or urine. The hydroxylated metabolites, such as 4′-hydroxydiclofenac and 5-hydroxydiclofenac, have been identified as predominant metabolites of diclofenac in humans [34].

In this study, the concentrations of diclofenac were 488 and 562 ng L^{-1} in influent and 46 and 223 ng L^{-1} in effluents at Kujawy WWTP. The concentrations of diclofenac in Płaszów WWTP were 387 and 905 ng L^{-1}, 560 and 113 ng L^{-1}, in the influent and effluent, respectively. In some samples, the concentrations of 4′-hydroxydiclofenac (OHDCF) in the influent and effluent were higher than the concentrations of diclofenac. The concentrations of 4′-hydroxydiclofenac ranged from 179 to 747 ng L^{-1} and from 120 to 247 ng L^{-1}, in the influents and effluents of the WWTPs which were tested, respectively. The ratio between 4′-hydroxydiclofenac and diclofenac varied between 0.31 and 1.93 with the average value of 0.94 in WWTPs was investigated. One may observe significant variation as far as the metabolite to parent compound ratio reported in the literature is concerned. The ratios between 4′-hydroxydiclofenac and diclofenac ranged from 1.87 to 6.76, and the average value of 4.18 was reported by Kołecka et al. [14]. The ratios reported by Stülten et al. [57], ranged from 0.085 to 0.35, and the average value was 0.21. The concentrations of ibuprofen, diclofenac and their selected metabolites were found to be relatively similar to those found in WWTPs in Germany, Spain, Poland and other countries [34,52–56].

The data which were observed indicate that not only diclofenac and ibuprofen but also its metabolites are globally entering the aqueous environment. In view of the toxic effects of diclofenac on several water organisms, it seems highly probable that the metabolites also initiate objectionable reactions in other organisms and require strict

Table 2
Concentrations (average ± stdev) of xenobiotics determined for samples collected in Płaszów and Kujawy WWTPs

	Kujawy WWTP				Płaszów WWTP			
	May		July		June		July	
	Inf.	Effl.	Inf.	Effl.	Inf.	Effl.	Inf.	Effl.
Compound	ng L^{-1}							
Ibuprofen (IBF)	2,099 ± 172.3	137.1 ± 0.2	1,579.5 ± 67.2	81.1 ± 10.2	3,427 ± 197	48 ± 10	1,451.9 ± 70.2	42.4 ± 0.3
Diclofenac (DCF)	562.0 ± 7.3	45.8 ± 5.3	487.8 ± 5.7	222.9 ± 5.1	387 ± 21	568 ± 25	905.7 ± 15.2	113.6 ± 21.2
Ketoprofen (KTP)	631.1 ± 0.2	<MQL	698.9 ± 21.7	29.4 ± 2.7	553.3 ± 43.7	354.9 ± 92.3	490.1 ± 9.2	74.2 ± 16.3
Naproxen (NPK)	1,401.1 ± 38.0	47.5 ± 7.1	1,286.6 ± 20.8	11.4 ± 0.6	722 ± 112	51 ± 11	1,176.2 ± 18.3	28.5 ± 0.0
Acetaminophen (ATP)	2,862.3 ± 208.6	16.5 ± 0.4	982.9 ± 26.2	7.3 ± 0.6	1,783 ± 6	<MQL	1,772.1 ± 23.2	<MQL
Carbamazepine (CBZ)	816.8 ± 109.5	17.4 ± 0.0	854.0 ± 20.4	672.3 ± 21.8	666 ± 85	512 ± 82	3,069.9 ± 132.5	625.5 ± 46.4
Caffeine (CAF)	2,334.3 ± 719.5	1,419.9 ± 152.3	2,556.6 ± 216.8	267.1 ± 37.1	6,768 ± 450	1,432 ± 180	4,325.1 ± 163.3	<MQL
Salicylic acid (SAL)	8506.1 ± 501.9	21.1 ± 0.5	12,790.3 ± 96.1	1.1 ± 0.2	8,779 ± 303	50 ± 12	11,094.6 ± 159.3	<MQL
Carboxyibuprofen (CBXIBF)	4,706.1 ± 457.6	171.2 ± 21.4	7,202.0 ± 777.7	226.9 ± 21.2	5,382 ± 487	59 ± 11	1,004.9 ± 42.7	94.2 ± 59.4
4′-Hydroxydiclofenac (OHDCF)	179.7 ± 24.4	120.7 ± 10.0	463.3 ± 14.2	183.8 ± 3.9	747 ± 55	247 ± 50	519.7 ± 13.5	195.5 ± 5.5
Triclosan (TCS)	167.1 ± 0.1	<MQL	199.4 ± 27.1	108.9 ± 2.5	198.7 ± 15.5	35.2 ± 14.5	222.6 ± 6.7	39.9 ± 1.2
Bisphenol A (BPA)	51.3 ± 1.9	<MQL	303.5 ± 2.8	42.4 ± 3.0	605.7 ± 87.0	42.2 ± 4.7	583.7 ± 9.2	<MQL
Estrone (E1)	<MQL	<MQL	67.0 ± 6.2	<MQL	62 ± 8	<MQL	150.1 ± 1.6	<MQL
β-Estradiol (E2)	<MQL	<MQL	56.4 ± 1.0	<MQL	72 ± 16	<MQL	<MQL	<MQL
Estriol (E3)	68.3 ± 5.9	<MQL	42.1 ± 2.1	<MQL	97 ± 12	<MQL	55.4 ± 2.0	<MQL

surveillance in both toxicological and environmental monitoring experiments [57,58].

Average daily loads of the target compounds were calculated in reference to the wastewater, and the contribution of classes of CECs in total amounts of target compounds contained to the influent and effluent was estimated using mass balance. The calculated loads of all xenobiotics in the influent and effluent of WWTPs are presented in Fig. 2.

A total of 15 xenobiotics were detected in the wastewater influent, and 12 xenobiotics in effluent samples, which represented seven classes of CECs, anti-inflammatory/analgesics, an antiepileptic drug, human indicator, disinfectant, plasticizers, estrogens and metabolites (Fig. 3). As shown in Fig. 2, the total loads found in the survey in reference to the various WWTPs ranged from 1,325 to 1,364 g d^{-1} for influents and from 82 to 99 for effluents sampled in the Kujawy WWTP and in the Płaszów WWTP from 3,630 to 3,790 g d^{-1} for influents and from 167 to 415 g per day for effluents. The Płaszów WWTP, which serves 70% of the Kraków area received the highest load of target xenobiotics and, consequently, it was responsible for the highest loads in the effluents that are afterward discharged into the receiving waters.

The contribution of the classes of CECs to the total amounts of target compounds, calculated for Płaszów WWTP ranged from 0.7% (estrogens) to 48% (metabolites) in the influent and from 0.7% (plasticizers) to 33% (metabolites) in the effluent (Table 3). In the Kujawy WWTP profile of contributions of the classes of CECs ranged from 0.4% (estrogens) to 62% (metabolites) in the influent and from 1% (plasticizers) to 43% (human indicators) in the effluent. In the Kujawy WWTP, the average contribution of metabolites decreased from 62% to 19%, while the contribution of the human indicator (caffeine) increased from 10% to 43%. It should be noted that in the group of metabolites emitted with effluents in the investigated WWTPs, over 85% of the total amount were metabolites of ibuprofen and diclofenac. The average contributions of anti-inflammatory/analgesics were on a similar level at both WWTPs, about 20%, in the influent as well as the effluent. The average contribution of an antiepileptic drug (carbamazepine) increased in the effluent from 3% to 19%, and from 7% to 34%, in the Kujawy WWTP and the Płaszów WWTP, respectively.

The results clearly showed that WWTP effluents discharged into the rivers constitute the main source of environmental contamination. The comparison of loads of xenobiotics in two WWTPs confirms the former discussion. The Płaszów WWTP, a large wastewater plant serving over 680,000 inhabitants, receives sewage with higher loads of xenobiotics than those of the Kujawy WWTP. However, the higher values of removal efficiency characterized the Płaszów WWTP, resulting in higher loads of xenobiotics entering the river water. The highest contributions in the discharged effluents were registered for metabolites and the human indicator, followed by antiepileptic and anti-inflammatory drugs.

3.2. Removal of xenobiotics in WWTPs

Fig. 3 shows the values of the removal efficiency of each xenobiotics after passing through all the wastewater treatment processes in both WWTPs. The WWTPs sampled in the current study showed varying levels of removal efficiency of the detected xenobiotics and conforms to similar removal data of CECs reported in other studies [37,59].

More than 99% of the salicylic acid which was present at the highest concentrations in the influent was removed in the WWTPs. Although acetaminophen concentrations in the initial influent were within the μg L^{-1} level, the concentrations were below the method quantification limit (MQL) in the effluent. The good values of removal efficiency were found also for estrogens, although it should be noted that their concentrations in the effluent were also below the MQL. The stable and high values of the removal efficiencies were observed for ibuprofen, naproxen and bisphenol A at both WWTPs and were 94%–98%, 93%–99%, 86%–100%, respectively. Moderately reduced values were observed for diclofenac, ketoprofen, carbamazepine, caffeine, 4′-hydroxydiclofenac and triclosan. Their removal efficiencies varied not only in WWTPs but also between days. The removal rates for diclofenac were 54%–93% and 26%–87% at Kujawy and Płaszów WWTPs, respectively. The low removal of diclofenac can be explained by its resistance and another possible reason was that it entered the WWTP as conjugates and was then subsequently back-transformed into the parental compound during treatment, leading to an apparent increase in concentrations of diclofenac during the treatment processes [43,56,60,61].

The removal of ketoprofen was higher than 95% at Kujawy WWTP, while its removal rates in the Płaszów WWTP were 31% and 84%.

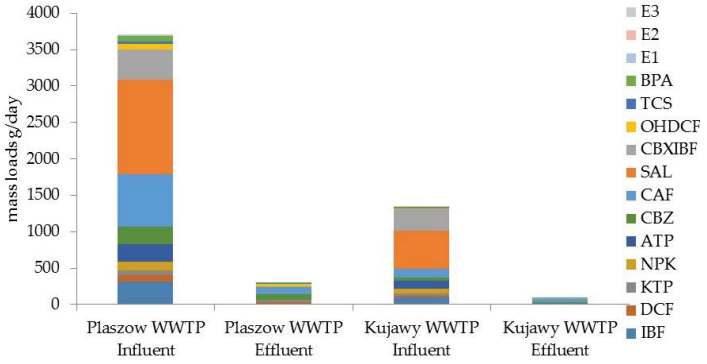

Fig. 2. Average mass loads of xenobiotics in the sewage influent and effluent in Płaszów and Kujawy WWTPs.

Table 3
Contribution (%) of various groups of xenobiotics in total loads (g d^{-1}) of the influent and effluent of WWTPs

	Płaszów WWTP		Kujawy WWTP	
	Influent	Effluent	Influent	Effluent
Compound	%			
Anti-inflammatory/analgesics	22.2	21.3	20.7	15.1
Antiepileptic drug	6.8	22.7	3.3	18.7
Human indicators	19.3	21.1	9.6	43.1
Metabolites	48.2	32.9	64.7	19.0
Disinfectant	0.7	1.5	0.7	3.0
Plasticizers	2.1	0.6	0.6	1.1
Estrogens	0.8	–	0.4	–

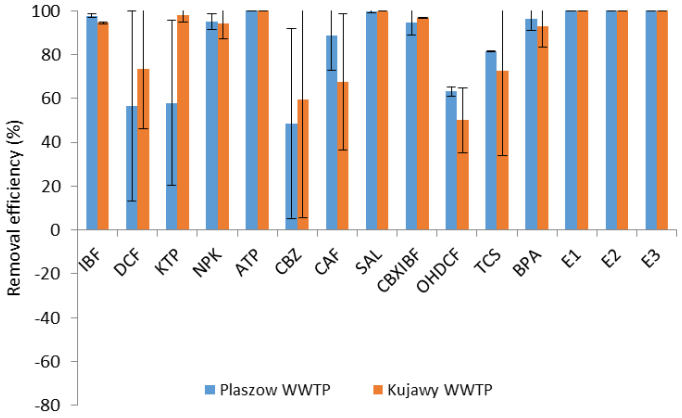

Fig. 3. Efficiency of the removal of xenobiotics during wastewater treatment for Płaszów and Kujawy WWTPs. Bars represent standard deviations between sampling days.

The removal efficiencies of carbamazepine were from 21% and 98%, 17% and 79% at the Kujawy and Płaszów WWTPs, respectively. Previous studies showed that the removal of carbamazepine was consistently low, could be below 30%, and similar variations of its removal were observed in the present study [62]. Its low removal rate is explained by persistent properties and water-soluble nature (18 mg L^{-1}) [63]. Also for caffeine, the values of removal efficiency varied from 45% to 100% in the Kujawy WWTP and the Płaszów WWTP, respectively.

The removal efficiencies of carboxyibuprofen were at a similar level, over 96% at the Kujawy WWTP, and over 90% in the Płaszów WWTP. Weigel et al. [54] and Dvořáková Březinova et al. [55] also reported high values of the removal of carboxyibuprofen at conventional wastewater treatment plants in wetlands treating municipal sewage. The reduction of 4′-hydroxydiclofenac was a maximum of 64% almost at all tested samples, except one day, when it approached 40%, in the Kujawy WWTP. According to [14], metabolites of diclofenac are more likely to manifest better removal efficiency compared to its parent compound, which was confirmed in the present study. The average efficiency of removal observed for 4′-hydroxydiclofenac in this study was 50% and 63% at the Kujawy and the Płaszów WWTPs, respectively.

A variation in the removal of triclosan was also observed. Higher values of the efficiency of the removal of triclosan were found in Płaszów WWTP, which were over 80%, whereas the values of its removal in the Kujawy WWTP were 45% and 100%. Previous studies reported removal of triclosan in the range of 50%–100% [64–67].

3.3. Occurrence of xenobiotics in surface water

Surface water samples were collected at four locations of water intake points. Measured concentration values in surface water samples are presented in Table 4. Of the targeted 15 compounds, 10 compounds were detected at levels above the MQL in surface water. Salicylic acid and caffeine were detected above the MQL in all the tested samples of surface water. The highest concentrations were observed for caffeine – from 230 to 1,198 ng L^{-1}. The concentrations of salicylic acid ranged between 11 and 57 ng L^{-1}. Salicylic acid is a phytohormone and therefore it is ubiquitous in plants and fruits. It is used in cosmetic products as a denaturant, a hair and skin conditioning agent, an exfoliant, an anti-acne cleansing agent, an anti-dandruff agent and a product preservative. It is also used as a preservative in food, as a chemical raw material for the synthesis of dyes and aspirin, and as an antiseptic

Table 4
Concentrations (average ± stdev) of xenobiotics in surface waters used as drinking water sources

Compound	Bielany 1	Bielany 2	Rudawa 1	Rudawa 2	Raba 1	Raba 2	Dłubnia
				ng L^{-1}			
IBF	38.4 ± 0.5	7.5 ± 0.2	32.5 ± 2.8	41.6 ± 1.9	7.4 ± 0.2	<MQL	9.7 ± 0.7
DCF	<MQL	<MQL	32.1 ± 4.1	<MQL	<MQL	<MQL	<MQL
KTP	62.6 ± 6.4	34.3 ± 3.2	87.8 ± 4.3	21.3 ± 1.1	45.0 ± 2.8	<MQL	24.3 ± 2.7
NPK	<MQL	<MQL	421.8 ± 25.6	41.8 ± 1.1	54.3 ± 2.5	40.3 ± 5.8	<MQL
ATP	<MQL	<MQL	28.2 ± 2.3	<MQL	<MQL	<MQL	<MQL
CBZ	40.3 ± 1.5	<MQL	58.1 ± 7.3	39.1 ± 7.2	63.5 ± 1.9	22.5 ± 1.7	46.7 ± 7.2
CAF	1,198.0 ± 93.6	550.2 ± 33.9	625.6 ± 36.9	230.8 ± 51.6	382.8 ± 14.8	563.6 ± 48.7	369.8 ± 40.2
SAL	17.0 ± 0.0	57.2 ± 2.3	11.0 ± 2.9	47.6 ± 2.0	32.9 ± 3.2	14.1 ± 1.6	39.8 ± 8.7
CBXIBF	16.9 ± 2.4	<MQL	95.2 ± 1.8	70.7 ± 4.9	12.3 ± 0.7	<MQL	<MQL
OHDCF	<MQL	<MQL	10.5 ± 0.4	9.8 ±1.7	9.7 ± 1.6	12.4 ± 0.6	<MQL
BPA	<MQL	<MQL	<MQL	8.6 ± 0.2	<MQL	<MQL	<MQL

and antifungal agent by topical application in veterinary medicine. Aspirin is metabolized to salicylic acid in the human body. Therefore, we concluded that many sources could be responsible for the observed occurrence of salicylic acid in the river systems [1,68]. An unusually high concentration of naproxen – 421 ng L^{-1} – was detected in one sample for Rudawa 1. Diclofenac was detected only in one sample, also in Rudawa 1, at a concentration level of 32 ng L^{-1}. Concentrations of estrogens and triclosan were below the MQL in all tested samples. The results obtained in this study can be compared with data representing similar investigations for Poland and other countries.

Caffeine has been used by various authors as a tracer for all waters influenced by human domestic emissions [55,69–71]. Despite the efficient removal in most WWTPs, caffeine was one of the compounds most frequently reported in the literature and found at the highest concentrations – up to 129,585 ng L^{-1} (Monjolinho River, Brazil) [36,71]. Research conducted by Loos et al. [72] for over 100 European rivers from 27 European countries demonstrated that caffeine, benzotriazole and carbamazepine were the most frequently detected and at the highest concentrations, with the average concentrations of 963, 493 and 248 ng L^{-1}, respectively [73]. In that study, maximum concentrations of ibuprofen, diclofenac, ketoprofen, naproxen and caffeine were 31,323, 247, 239, 2,027 and 7,997 ng L^{-1}. The analysis of drug residues in samples from lakes and rivers in North Poland and the Gulf of Gdańsk (South Baltic) confirmed the presence of ibuprofen and diclofenac in the range from 55 to 170 ng L^{-1} and from 300 to 528 ng L^{-1}, respectively [75]. In an analysis conducted for the Warta river, the concentrations for diclofenac were 17–486 ng L^{-1}, ibuprofen: 12–76 ng L^{-1}, ketoprofen: 6–47 ng L^{-1} and naproxen: 25–87 ng L^{-1} [51]. In the research conducted by Migowska et al. [42], in reference to the Wierzyca River, the concentration of ketoprofen was found to be 25 ng L^{-1}. Further analysis of 17 pharmaceuticals (six NSAIDs, three estrogenic hormones, six β-blockers and two β-agonists) in surface and groundwater of North Poland conducted by Buser et al. [53] demonstrated the presence of only NSAIDs in tested samples. In all samples, acetaminophen was detected at a level of 71–172 ng L^{-1}. The concentrations of ibuprofen, naproxen, ketoprofen and diclofenac were between 12 and 71 ng L^{-1} [53]. The metabolites of ibuprofen have been detected in surface water in concentrations higher than the mother compound [56,76]. The low values of the partitioning coefficients of bisphenol A, ketoprofen, diclofenac and carbamazepine except for triclosan, obtained in the sorption studies to sediments collected from the Dobczyce drinking water reservoir suggest that micropollutants are predominantly freely dissolved, which can have an adverse effect on the quality of drinking water [42]. Sorption to sediments was suggested to play a role in determining the fate of CECs in an aquatic environment; there are no detailed studies addressing the behavior and dynamics of CECs in freshwater systems [1]. On the basis of the results obtained in this study and the comparison with data representing other investigations around the world one may assume that water in the sources of drinking water for Kraków is not highly polluted by CECs However, this simple comparison is just an approximation of the problem, and a more in-depth analysis of the occurrence of CECs in the aquatic environment is required.

3.4. Environmental risk of the detected pollutants

CEC residues transported with effluents to receiving water bodies can represent a potential risk for aquatic life [77–80]. The risk quotients (RQ) were calculated for both effluents and river waters, using the conventional methods for environmental risk assessment (ERA), based on the measured concentrations of each of the CECs and by comparing them with the PNEC. ERA includes acute- and/or chronic toxicity data based on the most sensitive organism or a combination of organisms within a given ecosystem to determine the PNEC of a compound. According to the literature, if the exposure concentration exceeds the effect concentration, then the ecological risk is suspected. According to the approach that was embraced, an estimation of the aquatic risk for the target CECs is shown in Tables 5 and 6.

The RQ values calculated for rivers were consistently below 0.1, except for diclofenac (RQ = 0.3), detected in

Table 5
Environmental risk calculation of the determined xenobiotics based on conventional environmental risk assessment (ERA) for surface water samples

Compound	Bielany 1	Bielany 2	Dłubnia	Rudawa 1	Rudawa 2	Raba 1	Raba 2
IBF	0.005	0.001	0.001	0.005	0.006	0.001	–
DCF	–	–	–	0.3	–	–	–
KTP	0.02	0.01	0.008	0.03	0.007	0.01	–
NPK	–	–	–	0.1	0.01	0.01	0.01
ATP	–	–	–	0.1	–	–	–
CBZ	0.01	–	0.02	0.02	0.02	0.02	0.009
CAF	0.01	0.006	0.004	0.007	0.003	0.004	0.006
SAL	0.0004	0.001	0.001	0.0003	0.001	0.001	0.0003
CBXIBF	0.00001	–	–	0.00004	0.00003	0.00001	–
OHDCF	–	–	–	0.0001	0.0001	0.0001	0.0001
TCS	–	–	–	–	–	–	–
BPA	–	–	–	–	0.009	–	–

Table 6
Environmental risk calculation of the determined CECs based on conventional environmental risk assessment (ERA) for effluent samples

	Kujawy WWTP		Płaszów WWTP	
Compound	May 2015	July 2015	June 2015	July 2015
IBF	0.02	0.01	0.01	0.01
DCF	0.5	**2.2**	**5.7**	**1.1**
KTP	–	0.01	0.1	0.02
NPK	0.01	0.003	0.02	0.01
CBZ	0.01	0.3	0.2	0.3
CAF	0.02	0.003	0.02	–
SAL	0.0005	–	0.001	–
CBXIBF	0.0001	0.0001	0.00003	0.0004
OHDCF	0.001	0.001	0.001	0.001
TCS	–	**1.6**	0.5	0.6
BPA	–	0.04	0.04	–

RQ > 1 is indicated in bold and reflects that the CECs are of environmental concern.

one sample. It could be concluded that at the concentrations found in the sampling sites, the individual CECs could pose an environmental risk from minimal to zero, while in the effluents, diclofenac and triclosan posed a risk of a medium to a high level, with RQ values from 0.5 to 5.7 and from 0.5 to 1.6, respectively. Carbamazepine was found to represent a medium risk level (RQ in the range 0.2–0.3), except one day, when RQ was 0.01. For all other compounds in the effluents, the RQ values were lower than 0.1, corresponding to a minimal or zero risks. Diclofenac, triclosan and carbamazepine appeared to be the more hazardous components because of their low PNEC compared to the high concentrations detected (Table 1), reflecting their potential to cause ecological effects. Apart from some discrepancies, the results indicate that target CECs are present in effluents at concentrations high enough to generate chronic effects and to pose a potential risk to the aquatic environment. However, it should be mentioned that RQs were estimated for individual compounds, while CECs are usually present in the environment as mixtures. In addition, dilution, diffusion and degradation processes should be considered when CECs are discharged with the effluents into an aquatic environment.

4. Conclusions

The current study aimed to evaluate the occurrence and concentration of fifteen compounds from the CECs group and related metabolites in WWTPs and in rivers being sources of drinking water in Kraków (South Poland). The highest concentrations in the influents have been found for salicylic acid, caffeine, ibuprofen and its metabolite, carboxyibuprofen, up to 12,790 ng L^{-1}. While, in the effluents, caffeine and carbamazepine were present at the highest levels, up to 1,432 and 672 ng L^{-1}, respectively. The concentration of carboxyibuprofen was two time higher

than concentration of the parent compound in many sampling days, with the average ratio value of 2.27. The average ratio 4′-hydroxydiclofenac to diclofenac was 0.94. The results of the study confirmed that not only diclofenac and ibuprofen but also their metabolites entering to the aquatic environment. The removal efficiencies were more than 95% for most target compounds except of diclofenac, carbamazepine, caffeine, triclosan and 4′-hydroxydiclofenac. Although most CECs were shown to be notably reduced in WWTPs, some persisted, and were even detected at higher concentrations in the effluent. In the effluents, the contribution of metabolites and human indicators was dominated. Caffeine was the only compound that has been determined in each of the surface water samples. Regardless of where it was tested, it achieved the highest concentrations compared to the other CECs, but no adverse environmental effect is expected. The presence of caffeine and another target micropollutants like carbamazepine, anti-inflammatory drugs and their metabolites indicate direct discharge and/or illegal dumping of sewage into tested rivers. The simple estimation regarding the health impact which these pollutants may cause when entering environmental waters demonstrated a medium to high risk level only in reference to three compounds (carbamazepine, diclofenac and triclosan). It should be considered that these pollutants are present in complex mixtures with varying physicochemical properties and affinities to modulate molecular and cellular pathways in organisms, therefore more eco-toxicological studies are required.

Authors' contributions

K.S. designed the conception, performed the experiments, wrote the manuscript and discussed the results. J.D. assisted in the visualization and the preparation of the manuscript. A.M. assisted in the acquisition of WWTP samples and analysis of basic parameters. T.B. and T.Ż. managed the collection of samples and assisted in the discussion of the results.

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References

[1] A.J. Ebele, M. Abou-Elwafa Abdallah, S. Harrad, Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment, Emerging Contam., 3 (2017) 1–16.
[2] Y. Yang, Y.S. Ok, K.-H. Kim, E.E. Kwon, Y.F. Tsang, Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: a review, Sci. Total Environ., 596–597 (2017) 303–320.
[3] Q. Sui, X.Q. Cao, S.G. Lu, W.T. Zhao, Z.F. Qiu, G. Yu, Occurrence, sources and fate of pharmaceuticals and personal care products in the groundwater: a review, Emerging Contam., 1 (2015) 14–24.
[4] W.C. Li, Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil, Environ. Pollut., 187 (2014) 193–201.
[5] K. Styszko, U.E. Bollmann, K. Bester, Leaching of biocides from polymer renders under wet/dry cycles – rates and mechanisms, Chemosphere, 138 (2015) 609–615.
[6] D.R. Baker, B. Kasprzyk-Hordern, Spatial and temporal occurrence of pharmaceuticals and illicit drugs in the aqueous environment and during wastewater treatment: new developments, Sci. Total Environ., 454–455 (2013) 442–456.
[7] B. Petrie, R. Barden, B. Kasprzyk-Hordern, A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring, Water Res., 72 (2015) 3–27.
[8] M.D. Celiz, J. Tso, D.S. Aga, Pharmaceutical metabolites in the environment: analytical challenges and ecological risks, Environ. Toxicol. Chem., 28 (2009) 2473–2484.
[9] E.J. Han, D.S. Lee, Significance of metabolites in the environmental risk assessment of pharmaceuticals consumed by human, Sci. Total Environ., 592 (2017) 600–607.
[10] C.G. Daughton, Pharmaceuticals and the Environment (PiE): evolution and impact of the published literature revealed by bibliometric analysis, Sci. Total Environ., 562 (2016) 391–426.
[11] R. Naidu, V.A. Arias Espana, Y.J. Liu, J. Jit, Emerging contaminants in the environment: risk-based analysis for better management, Chemosphere, 154 (2016) 350–357.
[12] T.A. Larsen, J. Lienert, A. Joss, H. Siegrist, How to avoid pharmaceuticals in the aquatic environment, J. Biotechnol., 113 (2004) 295–304.
[13] D. White, D.J. Lapworth, W. Civil, P. Williams, Tracking changes in the occurrence and source of pharmaceuticals within the River Thames, UK; from source to sea, Environ. Pollut., 249 (2019) 257–266.
[14] K. Kołecka, M. Gajewska, P. Stepnowski, M. Caban, Spatial distribution of pharmaceuticals in conventional wastewater treatment plant with Sludge Treatment Reed Beds technology, Sci. Total Environ., 647 (2019) 149–157.
[15] K. Nosek, K. Styszko-Grochowiak, J. Gołaś, Determination of acidic pharmaceuticals in municipal wastewater by using solid-phase extraction followed by gas chromatography-mass spectrometry, Geomatics Environ. Eng., 6 (2012) 45, doi: 10.7494/geom.2012.6.3.45.
[16] U. Kotowska, J. Kapelewska, J. Sturgulewska, Determination of phenols and pharmaceuticals in municipal wastewaters from Polish treatment plants by ultrasound-assisted emulsification-microextraction followed by GC-MS, Environ. Sci. Pollut. Res., 21 (2014) 660–673.
[17] K. Styszko, K. Proctor, E. Castrignanò, B. Kasprzyk-Hordern, Occurrence of pharmaceutical residues, personal care products, lifestyle chemicals, illicit drugs and metabolites in wastewater and receiving surface waters of Krakow agglomeration in South Poland, Sci. Total Environ., 768 (2021) 1–18.
[18] P. Paíga, L.H.M.L.M. Santos, S. Ramos, S. Jorge, J.G. Silva, C. Delerue-Matos, Presence of pharmaceuticals in the Lis river (Portugal): sources, fate and seasonal variation, Sci. Total Environ., 573 (2016) 164–177.
[19] E. Carmona, V. Andreu, Y. Picó, Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: from waste to drinking water, Sci. Total Environ., 484 (2014) 53–63.
[20] M. Caban, E. Lis, J. Kumirska, P. Stepnowski, Determination of pharmaceutical residues in drinking water in Poland using a new SPE-GC-MS(SIM) method based on Speedisk extraction disks and DIMETRIS derivatization, Sci. Total Environ., 538 (2015) 402–411.
[21] J. Rossmann, S. Schubert, R. Gurke, R. Oertel, W. Kirch, Simultaneous determination of most prescribed antibiotics in multiple urban wastewater by SPE-LC-MS/MS, J. Chromatogr. B, 969 (2014) 162–170.
[22] O. Golovko, V. Kumar, G. Fedorova, T. Randak, R. Grabic, Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant, Chemosphere, 111 (2014) 418–426.
[23] H. Zhang, Y. Zhou, Y. Huang, L. Wu, X. Liu, Y. Luo, Residues and risks of veterinary antibiotics in protected vegetable soils following application of different manures, Chemosphere, 152 (2016) 229–237.
[24] P. Verlicchi, E. Zambello, Pharmaceuticals and personal care products in untreated and treated sewage sludge: occurrence

and environmental risk in the case of application on soil – a critical review, Sci. Total Environ., 538 (2015) 750–767.
[25] K. Styszko, K. Nosek, M. Motak, K. Bester, Preliminary selection of clay minerals for the removal of pharmaceuticals, bisphenol A and triclosan in acidic and neutral aqueous solutions, C.R. Chim., 18 (2015) 1134–1142.
[26] J.C. Durán-Álvarez, B. Prado, A. Ferroud, N. Juayerk, B. Jiménez-Cisneros, Sorption, desorption and displacement of ibuprofen, estrone, and 17β estradiol in wastewater irrigated and rainfed agricultural soils, Sci. Total Environ., 473–474 (2014) 189–198.
[27] M. Mezzelani, S. Gorbi, F. Regoli, Pharmaceuticals in the aquatic environments: Evidence of emerged threat and future challenges for marine organisms, Mar. Environ. Res., 140 (2018) 41–60.
[28] R. de S. Pohren, J.A.V. Rocha, K.A. Leal, V.M.F. Vargas, Soil mutagenicity as a strategy to evaluate environmental and health risks in a contaminated area, Environ. Int., 44 (2012) 40–52.
[29] M. Stuart, D. Lapworth, E. Crane, A. Hart, Review of risk from potential emerging contaminants in UK groundwater, Sci. Total Environ., 416 (2012) 1–21.
[30] E.J. Rosi-Marshall, D. Snow, S.L. Bartelt-Hunt, A. Paspalof, J.L. Tank, A review of ecological effects and environmental fate of illicit drugs in aquatic ecosystems, J. Hazard. Mater., 282 (2015) 18–25.
[31] M.S. El-Shahawi, A. Hamza, A.S. Bashammakh, W.T. Al-Saggaf, An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants, Talanta, 80 (2010) 1587–1597.
[32] A. Rivas, N. Olea, F. Olea-Serrano, Human exposure to endocrine-disrupting chemicals: assessing the total estrogenic xenobiotic burden, TrAC, Trends Anal. Chem., 16 (1997) 613–619.
[33] K. Fent, A.A. Weston, D. Caminada, Ecotoxicology of human pharmaceuticals, Aquat. Toxicol., 76 (2006) 122–159.
[34] P. Sathishkumar, R.A.A. Meena, T. Palanisami, V. Ashokkumar, T. Palvannan, F.L. Gu, Occurrence, interactive effects and ecological risk of diclofenac in environmental compartments and biota – a review, Sci. Total Environ., 698 (2020) 134057, doi: 10.1016/j.scitotenv.2019.134057.
[35] D.J. Caldwell, F. Mastrocco, L. Margiotta-Casaluci, B.W. Brooks, An integrated approach for prioritizing pharmaceuticals found in the environment for risk assessment, monitoring and advanced research, Chemosphere, 115 (2014) 4–12.
[36] J.C.G. Sousa, A.R. Ribeiro, M.O. Barbosa, M.F.R. Pereira, A.M.T. Silva, A review on environmental monitoring of water organic pollutants identified by EU guidelines, J. Hazard. Mater., 344 (2018) 146–162.
[37] E. Archer, B. Petrie, B. Kasprzyk-Hordern, G.M. Wolfaardt, The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters, Chemosphere, 174 (2017) 437–446.
[38] A. Hartmann, A.C. Alder, T. Koller, R.M. Widmer, Identification of fluoroquinolone antibiotics as the main source of *umuC* genotoxicity in native hospital wastewater, Environ. Toxicol. Chem., 17 (1998) 377–382.
[39] K. Vorkamp, R. Bossi, K. Bester, U.E. Bollmann, S. Boutrup, New priority substances of the European Water Framework Directive: biocides, pesticides and brominated flame retardants in the aquatic environment of Denmark, Sci. Total Environ., 470–471 (2014) 459–468.
[40] Q. Sun, Y. Li, M. Li, M. Ashfaq, M. Lv, H.J. Wang, A. Hu, C.-P. Yu, PPCPs in Jiulong River estuary (China): Spatiotemporal distributions, fate, and their use as chemical markers of wastewater, Chemosphere, 150 (2016) 596–604.
[41] K. Styszko, Sorption of emerging organic micropollutants onto fine sediments in a water supply dam reservoir, Poland, J. Soils Sediments, 16 (2016) 677–686.
[42] N. Migowska, M. Caban, P. Stepnowski, J. Kumirska, Simultaneous analysis of non-steroidal anti-inflammatory drugs and estrogenic hormones in water and wastewater samples using gas chromatography-mass spectrometry and gas chromatography with electron capture detection, Sci. Total Environ., 441 (2012) 77–88.
[43] K. Nosek, K. Styszko, J. Golas, Combined method of solid-phase extraction and GC-MS for determination of acidic, neutral, and basic emerging contaminants in wastewater (Poland), Int. J. Environ. Anal. Chem., 94 (2014) 961–974.
[44] J.G.B. Ferrari, R. Mons, B. Vollat, B. Fraysse, N. Paxeus, R. Lo Giudice, A. Pollio, environmental risk assessment of six human pharmaceuticals: are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment?, Environ. Toxicol. Chem., 23 (2004) 1344–1354.
[45] A. Bergmann, R. Fohrmann, F.-A. Weber, U. Des, Zusammenstellung von Monitoringdaten zu Umweltkonzentrationen von Arzneimitteln (Compilation of monitoring data on environmental concentrations of pharmaceuticals), Umweltbundesamt, 66 (2011) 1–108.
[46] J. Martín, M.D. Camacho-Muñoz, J.L. Santos, I. Aparicio, E. Alonso, Distribution and temporal evolution of pharmaceutically active compounds alongside sewage sludge treatment. Risk assessment of sludge application onto soils, J. Environ. Manage., 102 (2012) 18–25.
[47] A. Mendoza, J.L. Rodríguez-Gil, S. González-Alonso, N. Mastroianni, M. López de Alda, D. Barceló, Y. Valcárcel, Drugs of abuse and benzodiazepines in the Madrid Region (Central Spain): seasonal variation in river waters, occurrence in tap water and potential environmental and human risk, Environ. Int., 70 (2014) 76–87.
[48] K.O. K'oreje, F.J. Kandie, L. Vergeynst, M.A. Abira, H. Van Langenhove, M. Okoth, K. Demeestere, Occurrence, fate and removal of pharmaceuticals, personal care products and pesticides in wastewater stabilization ponds and receiving rivers in the Nzoia Basin, Kenya, Sci. Total Environ., 637–638 (2018) 336–348.
[49] Y. Luo, W. Guo, H.H. Ngo, L.D. Nghiem, F.I. Hai, J. Zhang, S. Liang, X.C. Wang, A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment, Sci. Total Environ., 473–474 (2014) 619–641.
[50] B. Kasprzyk-Hordern, A. Dabrowska, N. Vieno, L. Kronberg, J. Nawrocki, Occurrence of acidic pharmaceuticals in the Warta River in Poland, Chem. Anal. (Warsaw), 53 (2008) 289–303.
[51] A. Sagan, D. Panteli, W. Borkowski, M. Dmowski, F. Domanski, M. Czyzewski, P. Gorynski, D. Karpacka, E. Kiersztyn, I. Kowalska, M. Ksiezak, K. Kuszewski, A. Lesniewska, I. Lipska, R. Maciag, J. Madowicz, A. Madra, M. Marek, A. Mokrzyck, Poland health system review, Health Syst. Transit., 13 (2011) 1–193.
[52] L. Ferrando-Climent, N. Collado, G. Buttiglieri, M. Gros, I. Rodriguez-Roda, S. Rodriguez-Mozaz, D. Barceló, Comprehensive study of ibuprofen and its metabolites in activated sludge batch experiments and aquatic environment, Sci. Total Environ., 438 (2012) 404–413.
[53] H.R. Buser, T. Poiger, M.D. Müller, Occurrence and fate of the pharmaceutical drug diclofenac in surface waters: rapid photodegradation in a lake, Environ. Sci. Technol., 32 (1998) 3449–3456.
[54] S. Weigel, U. Berger, E. Jensen, R. Kallenborn, H. Thoresen, H. Hühnerfuss, Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromsø/Norway with emphasis on ibuprofen and its metabolites, Chemosphere, 56 (2004) 583–592.
[55] T. Dvořáková Březinova, J. Vymazal, M. Koželuh, L. Kule, Occurrence and removal of ibuprofen and its metabolites in full-scale constructed wetlands treating municipal wastewater, Ecol. Eng., 120 (2018) 1–5.
[56] D. Rozman, Z. Hrkal, M. Váňa, J. Vymazal, Z. Boukalová, Occurrence of pharmaceuticals in wastewater and their interaction with shallow aquifers: a case study of Horní Beřkovice, Czech Republic, Water, 9 (2017) 1–15.

[57] D. Stülten, S. Zühlke, M. Lamshöft, M. Spiteller, Occurrence of diclofenac and selected metabolites in sewage effluents, Sci. Total Environ., 405 (2008) 310–316.
[58] V. Osorio, J. Sanchís, J.L. Abad, A. Ginebreda, M. Farré, S. Pérez, D. Barceló, Investigating the formation and toxicity of nitrogen transformation products of diclofenac and sulfamethoxazole in wastewater treatment plants, J. Hazard. Mater., 309 (2016) 157–164.
[59] Q. Sun, M. Lv, A. Hu, X. Yang, C.P. Yu, Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in a wastewater treatment plant in Xiamen, China, J. Hazard. Mater., 277 (2014) 69–75.
[60] N.M. Vieno, H. Härkki, T. Tuhkanen, L. Kronberg, Occurrence of pharmaceuticals in river water and their elimination in a pilot-scale drinking water treatment plant, Environ. Sci. Technol., 41 (2007) 5077–5084.
[61] H.J. Lee, E. Lee, S.H. Yoon, H.R. Chang, K. Kim, J.H. Kwon, Enzymatic and microbial transformation assays for the evaluation of the environmental fate of diclofenac and its metabolites, Chemosphere, 87 (2012) 969–974.
[62] K.M. Onesios, J.T. Yu, E.J. Bouwer, Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review, Biodegradation, 20 (2009) 441–466.
[63] S.K. Behera, H.W. Kim, J.E. Oh, H.S. Park, Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea, Sci. Total Environ., 409 (2011) 4351–4360.
[64] J. Heidler, R.U. Halden, Mass balance assessment of triclosan removal during conventional sewage treatment, Chemosphere, 66 (2007) 362–369.
[65] J. Heidler, A. Sapkota, R.U. Halden, Partitioning, persistence, and accumulation in digested sludge of the topical antiseptic triclocarban during wastewater treatment, Environ. Sci. Technol., 40 (2006) 3634–3639.
[66] N. Nakada, T. Tanishima, H. Shinohara, K. Kiri, H. Takada, Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment, Water Res., 40 (2006) 3297–3303.
[67] G. Kor-Bicakci, T. Abbott, E. Ubay-Cokgor, C. Eskicioglu, Occurrence of the persistent antimicrobial triclosan in microwave pretreated and anaerobically digested municipal sludges under various process conditions, Molecules, 25 (2020) 310, doi: 10.3390/molecules25020310.
[68] C.L.G.U. Bernauer, L. Bodin, Q. Chaudhry, P.J. Coenraads, M. Dusinska, J. Ezendam, E. Gaffet, A.B. Granum, E. Panteri, V. Rogiers, C. Rousselle, M. Stepnik, T. Vanhaecke, S. Wijnhoven, W.U. Koutsodimou, A. Simonnard, Opinion on Salicylic Acid (CAS 69-72-7) Submission I, SCCS/1601/18 – Final Opin., 2018, pp. 1–70.
[69] C. Potera, Caffeine in wastewater is a tracer for human fecal contamination, Environ. Health Perspect., 120 (2012) 108–109.
[70] Z. Chen, P. Pavelic, P. Dillon, R. Naidu, Determination of caffeine as a tracer of sewage effluent in natural waters by on-line solid-phase extraction and liquid chromatography with diode-array detection, Water Res., 36 (2002) 4830–4838.
[71] I.J. Buerge, T. Poiger, M.D. Müller, H.R. Buser, Caffeine, an anthropogenic marker for wastewater contamination of surface waters, Environ. Sci. Technol., 37 (2003) 691–700.
[72] R. Loos, B.M. Gawlik, G. Locoro, E. Rimaviciute, S. Contini, G. Bidoglio, EU-wide survey of polar organic persistent pollutants in European river waters, Environ. Pollut., 157 (2009) 561–568.
[73] J. Debska, A. Kot-Wasik, J. Namiesnik, Determination of nonsteroidal antiinflammatory drugs in water samples using liquid chromatography coupled with diode-array detector and mass spectrometry, J. Sep. Sci., 28 (2005) 2419–2426.
[74] H.R. Buser, T. Poiger, M.D. Muller, Occurrence and environmental behavior of the chiral pharmaceutical drug ibuprofen in surface waters and in wastewater, Environ. Sci. Technol., 33 (1999) 2529–2535.
[75] M. Crane, C. Watts, T. Boucard, Chronic aquatic environmental risks from exposure to human pharmaceuticals, Sci. Total Environ., 367 (2006) 23–41.
[76] F. Stuer-Lauridsen, M. Birkved, L.P. Hansen, H.-C. Holten, L. Utzhùft, B. Halling-Sùrensen, environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use, Chemosphere, 40 (2000) 783–793.
[77] V.L. Cunningham, S.P. Binks, M.J. Olson, Human health risk assessment from the presence of human pharmaceuticals in the aquatic environment, Regul. Toxicol. Pharm., 53 (2009) 39–45.
[78] L. Patrolecco, S. Capri, N. Ademollo, Occurrence of selected pharmaceuticals in the principal sewage treatment plants in Rome (Italy) and in the receiving surface waters, Environ. Sci. Pollut. Res., 22 (2015) 5864–5876.

Supplementary information

Method detection limits, MDL (ng L^{-1}) and method quantification limits, MQL (ng L^{-1}) were calculated using the following equations:

$$\mathrm{MDL} = \frac{\mathrm{IDL} \times 100}{\mathrm{AR} \times \mathrm{CF}} \tag{S1}$$

$$\mathrm{MQL} = \frac{\mathrm{IQL} \times 100}{\mathrm{AR} \times \mathrm{CF}} \tag{S2}$$

where IDL is the instrumental detection limit (ng L^{-1}) and IQL is the instrumental quantification limit (ng L^{-1}), AR the absolute recovery of the analyte (%) and CF is the concentration factor, which is 1,250 for influent, 2,500 for effluent and 5,000 for surface water samples. The instrumental quantification limit was calculated as the lowest point on the calibration curves obtained with the precision of 10% RSD and accuracies between 80% and 120%. The instrumental detection limit IDL was calculated as the IQL divided by three [35].

Table S1
Chosen PPCPs and their properties

Group	Compound	CAS No	Molecular formula	MW	pK_a	$\log K_{OW}$
Anti-inflammatory/ analgesics	Ibuprofen (IBF)	15687-21-1	$C_{13}H_{18}O_2$	206.28	4.9	3.5–4.0
	Diclofenac (DCF)	15307-79-6	$C_{14}H_{10}Cl_2NNaO_2$	318.10	4.2	4.2–4.5
	Ketoprofen (KTP)	22071-15-4	$C_{16}H_{14}O_3$	254.28	4.5	3.1–3.6
	Naproxen (NPK)	22204-53-1	$C_{14}H_{14}O_3$	230.26	4.2	3.2–3.3
	Acetaminophen (ATP)	103-90-2	$C_8H_9NO_2$	151.16	9.4	0.5–0.9
Antiepileptic drug	Carbamazepine (CBZ)	298-46-4	$C_{15}H_{12}N_2O$	236.27	13.9	2.4–2.9
Stimulant	Caffeine (CAF)	58-08-2	$C_8H_{10}N_4O_2$	194.20	10.4	–0.07
Metabolites	Salicylic acid (SAL)	69-72-7	$C_7H_6O_3$	138.12	3.0	2.3–2.4
	Carboxyibuprofen (CBXIBF)	15935-54-3	$C_{13}H_{16}O_4$	236.26		
	4′-Hydroxydiclofenac (OHDCF)	64118-84-9	$C_{14}H_{11}Cl_2NO_3$	312.15		
Disinfectant	Triclosan (TCS)	3380-34-5	$C_{12}H_7Cl_3O_2$	289.54	7.7	4.8
Estrogens	Estrone (E1)	53-16-7	$C_{18}H_{22}O_2$	270.37	10.3	3.1
	β-Estradiol (E2)	50-28-2	$C_{18}H_{24}O_2$	272.39	10.3	4.0
	Estriol (E3)	50-27-1	$C_{18}H_{24}O_3$	288.39	10.3	2.5
Plasticizers	Bisphenol A (BPA)	80-05-7	$C_{15}H_{16}O_2$	228.29	10.1	3.3
Surrogate/internal standards	Ibuprofen-D_3	121662-14-4	$C_{13}D_3H_{15}O_2$	209.30		
	Diclofenac-$^{13}C_6$	1261393-73-0	$^{13}C_6C_8H_{10}Cl_2NNaO_2$	405.16	–	–
	Caffeine-$^{13}C_3$	78072-66-9	$^{13}C_3C_5H_{10}N_4O_2$	197.17		
	Bisphenol A-D_{16}	96210-87-6	$C_{15}D_{16}O_2$	244.38		
	17β-Estradiol D_5	221093-45-4	$C_{18}H_{19}O_2D_5$	277.41		
	Acetaminophen-D_4	64315-36-2	$C_8D_4H_5NO_2$	155.19		
	Carbamazepine-D_{10}	132183-78-9	$C_{15}D_{10}H_2N_2O$	246.33		

PPCPs – Pharmaceuticals and personal care product.

Table S2
Technological ability of water treatment plants and the annual average values of basic parameters*

Water treatment plants	Technological ability ($m^3\ d^{-1}$)	Population served	Average annual temp. (°C)	Total organic carbon (mg L^{-1})	pH
Raba	110,000	ca. 350,000	20.0	3.1	8.2–8.7
Rudawa	22,000	ca. 200,000	12.1	4.7	7.9–8.2
Dlubnia	20,000	ca. 200,000	11.5	3.5	8.0–8.2
Sanka	12,000	ca. 150,000	10.1	4.6	7.1–8.1

*Assessment of the status of river water bodies and dam reservoirs in the years 2010–2015, report, The Chief Inspector of Environmental Protection (in Polish).

Table S3
The volume of sewage per day and average values of basic parameters in WWTPs

	Kujawy WWTP				Plaszow WWTP			
	May 2015		July 2015		June 2015		July 2015	
Parameter	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
Suspended solids, mg L^{-1}	360	16	350	6.4	230	<2.0	400	2.0
Biochemical oxygen demand, mg L^{-1}	410	2.6	350	2.3	320	1.7	220	3.8
Chemical oxygen demand, mg L^{-1}	900	21.8	830	25.5	585	19.9	510	22.0
Total organic carbon, mg L^{-1}	192	11.2	120	7.7	78.5	10.1	71.2	7.38
Total nitrogen, mg L^{-1}	88.9	10.0	67.3	5.2	48.7	7.05	46.5	8.26
Total phosphorus, mg L^{-1}	8.20	0.389	7.55	0.633	6.02	0.377	6.43	0.470
pH	7.6	7.7	7.4	7.5	7.6	7.6	7.5	7.1
Volume of sewage, m^3	55.948	50.072	44.844	44.849	125.300	133.910	135.380	137.940

Table S4
Retention times (RT), mass spectrometer (MS/MS) parameters for the analysis of the chosen PPCPs by GC-(IT)MS/MS

Compound	RT (min)	Precursor ion (m/z)	Product ions quantification/ confirmation (m/z)	Internal standard
Salicylic acid	8.27	267	209/249	Ibuprofen-D_3
Ibuprofen	8.96	160	145/117	Ibuprofen-D_3
Ibuprofen-D_3	8.96	163	148/119	
Acetaminophen	9.04	280	206/–	Acetaminophen-D_4
Acetaminophen-D_4	8.98	284	210/–	
Caffeine	10.48	194	165/109	Caffeine-$^{13}C_3$
Caffeine-$^{13}C_3$	10.49	197	168/111	
Carboxyibuprofen	11.05	218	147/-	Ibuprofen-D_3
Naproxen	11.49	185	170/153	Diclofenac-$^{13}C_6$
Triclosan	11.76	347	200/312	Bisphenol A-D_{16}
Bisphenol A-D_{16}	12.01	368	197/277	
Bisphenol A	12.04	357	191/267	Bisphenol A-D_{16}
Ketoprofen	12.10	282	253/266	Diclofenac-$^{13}C_6$
Carbamazepine	12.42	193	165/191	Carbamazepine-D_{10}
Carbamazepine-D_{10}	12.41	203	175/200	
Diclofenac	12.49	214	179/151	Diclofenac-$^{13}C_6$
Diclofenac-$^{13}C_6$	12.50	220	185/157	
4′-Hydroxydiclofenac	13.60	330	258/302	Diclofenac-$^{13}C_6$
Estrone (E1)	13.95	342	257/–	β-Estradiol-D_5
β-Estradiol (E2)	14.07	416	285/326	β-Estradiol-D_5
β-Estradiol-D_5	14.04	421	287/331	
Estriol (E3)	14.79	324	309/295	β-Estradiol-D_5

Table S5
Selected validation data of solid phase extraction and GC-MS/MS method for analysis of analytes in surface water and wastewater samples

Compound	Linearity range (ng L^{-1})	Correlation coefficient (R^2)	MQL (ng L^{-1})			MDL (ng L^{-1})			AR (%)		
			Influent	Effluent	Surface water	Influent	Effluent	Surface water	Influent	Effluent	Surface water
IBF	8–10,000	0.992	8.4	7.9	7.4	2.8	2.6	2.5	76 ± 9	81 ± 12	86 ± 11
DCF	12–10,000	0.993	12.5	11.1	11.4	4.2	3.7	3.8	64 ± 10	72 ± 9	70 ± 5
KTP	82–10,000	0.997	24.6	22.2	20.3	8.2	7.4	6.8	65 ± 12	72 ± 26	79 ± 4
NPK	45–10,000	0.998	10.7	10.3	8.9	3.6	3.4	3.0	60 ± 22	62 ± 16	72 ± 9
ATP	41–8,000	0.999	41.6	50.8	40.5	13.9	16.9	13.5	77 ± 1	63 ± 1	79 ± 0.5
CBZ	44–10,000	0.990	14.3	11.8	10.8	4.8	3.9	3.6	56 ± 12	68 ± 18	74 ± 8
CAF	150–10,000	0.995	96.4	89.9	74.8	32.1	30.0	24.9	83 ± 18	89 ± 27	107 ± 13
SAL	4–10,000	0.993	5.4	4.8	3.8	1.8	1.6	1.3	59 ± 11	67 ± 12	85 ± 3
CBXIBF	11–10,000	0.995	13.8	13.1	10.7	4.6	4.4	3.6	58 ± 8	61 ± 2	75 ± 5
OHDCF	48–10,000	0.998	11.0	10.0	9.6	3.7	3.3	3.2	58 ± 18	64 ± 4	67 ± 7
TCS	6–10,000	0.998	6.4	6.0	5.6	2.1	2.0	1.9	75 ± 2	80 ± 11	86 ± 7
BPA	9–10,000	0.996	8.2	8.8	8.2	2.7	2.9	2.7	97 ± 22	91 ± 8	98 ± 23
E1	22–10,000	0.999	28.6	24.6	21.6	9.5	8.2	7.2	56 ± 9	65 ± 12	74 ± 13
E2	46–10,000	0.989	43.6	42.1	45.3	14.5	14.0	15.1	55 ± 10	57 ± 12	53 ± 11
E3	35–10,000	0.989	39.2	37.0	34.5	13.1	12.3	11.5	51 ± 11	54 ± 4	58 ± 16