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Removal of antibacterial drugs in urban wastewater treatment plants

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ABSTRACT

The aim of the study was to detect and quantify selected antibacterial drugs in the raw and treated wastewater collected under various temperature conditions (i.e. winter and summer period) from two urban wastewater treatment plants (WWTPs), located in the northern and southern part of Poland. An additional goal of the study was to estimate the effectiveness of the removal of these antibacterial drugs in the above-mentioned conditions. For the study were selected: clarithromycin (CLA), erythromycin (ERY), roxithromycin (ROX), sulfamethoxazole (SMX), N-acetyl-sulfamethoxazole (N-Ac-SMX), sulfamethoxine (SMN), sulfamerazine (SMR), tiamulin (TIA), and trimethoprim (TRP). The study showed that CLA, ERY, ROX, SMX, TRP, and N-Ac-SMX (main SMX metabolite) were detected in all wastewater samples, regardless of the place of collection and the season of the sampling, while SMN, SMR, and TIA were not detected in the investigated wastewater. The studies have shown that N-Ac-SMX was the most efficiently removed compound at both treatment plants, regardless of the process temperature (over 95%), however, this substance may reversibly transform into the origin form of SMX under the conditions prevailing at WWTPs, which means that the removal can be only apparent. In the study, the temperature was an important parameter affecting the removal efficiency of CLA, ROX, and TRP. The removal efficiency of these substances always has been higher in summer than in the winter period. The process was also slightly more effective at WWTP 2. At WWTP 2, the average removal for CLA, ROX, and TRP in the summer period was equal to 76%, 47%, and 38%, respectively. Erythromycin, was a substance that was not degraded in any of the investigated technological systems and no effect of temperature on the removal efficiency was observed.

Keywords: Antibiotics; Antibacterial drugs; Micropollutants removal; Seasonal effect; Sulfonamides; Wastewater treatment plants

1. Introduction

The term "antibiotic" originally refers to only natural substances produced by bacteria or fungi, however, nowadays more often is also used to refer to both synthetic (or semi-synthetic) compounds (e.g. sulfonamides) and natural compounds (e.g. penicillins), which show any antibacterial activity [1]. Therefore, the terms "antibiotic" and "antibacterial drug" are very often used interchangeably and such terminology will be used later in this paper.

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Antibacterial drugs are used in large quantities in human and veterinary medicine, for treating microbial infection of various origins [2]. In the second decade of the 21st century, the global annual consumption of antibiotics reached approximately 70 billion standard units/year for human use [3], while in the case of livestock, antibiotic consumption has been estimated to be 63,151 ± 1,560 tons/y [4]. However, it should be emphasized that approximately 50% of these drugs were used in veterinary medicine and as growth promoters as well [5]. Admittedly, in the European Union and in Associated Countries (e.g. Switzerland), the use of antibiotics as growth promoters in animal farming has been forbidden in 2006, not all countries of the World Health Organization European Region comply with these recommendations [6]. In some countries, the use of antibiotics in veterinary medicine exceeds the consumption of human medicine, although the consumption in medicine (globally) is constantly increasing. Only between the years 2000 and 2010, the antibiotic consumption for human treatment has been found to increase by 36% [3]. In the year 2012, the total antibiotics consumption in EU countries in terms of outpatient health care reached 3,400 tons, and Poland in the context of antibiotic consumption took the fifth place behind Spain, Italy, Germany, and France [7].

Most of the antibacterial drugs are not completely metabolized by humans and animals after intake – about 25% to 75% of them are excreted via feces or urine in the unchanged form [8]. The main sources of antibiotics (and other pharmaceuticals) in the environment are wastewater treatment plants (WWTPs), pharmaceutical manufacturing plants, animal husbandry farms, and broadly understood agriculture (with particular emphasis on surface runoff from manure fertilized areas) [6,9]. The negative impact caused by the presence of antibiotics in the environment is associated not only with their direct effect on living organisms but also with their indirect action. It is assumed that the residues of antibiotics indirectly may contribute to the spread of the phenomenon of resistance among bacteria inhabiting various environmental niches. The presence of antibiotic-resistant bacteria in the environment results in a greater risk to humans and animals due to the increased number of infections that are very difficult to treat, the effects of which can even be fatal [1,10,11]. It is estimated that drug-resistant bacteria cause yearly about 700,000 deaths globally. Further predictions indicate that if this problem is not resolved (at least in part), then the number of deceases from such reasons could grow to 10 million per year by 2050 [12]. One of the concepts to prevent the spread of antibiotic resistance in the environment is to prevent the release of antibacterial drugs into the environment and to control this type of pollution "at source", including the treated urban wastewater. From the environmental point of view, most of the antibiotics are recalcitrant compounds, which can be stable in the environment for a long time. Due to their ineffective removal from wastewater in conventional WWTPs, the antibiotics have been detected worldwide in the various environmental matrices as raw and treated wastewater, activated sludge, surface and groundwater, soil, and sediment samples, and many other [6,8,13–20]. The concentrations of the antibacterial drugs in the environmental samples usually range from a few nanograms per liter to several micrograms per liter [16,17,20], depending on the type of matrix.

Even if the antibiotics and their transformation by-products are present in the environment as recalcitrant substances causing negative ecotoxicological effects [16,21], there are still not enough legal regulations, for example, on the maximum allowable concentrations in the environment or the required degree of removal at WWTPs. Three macrolides (i.e. erythromycin, clarithromycin, azithromycin) were included in the first Watch List of priority substances for Union-wide monitoring in the framework of water policy established by European Commission [22]. After the revision of the first EU Watch List [20], the European Commission, in the year 2018, established the second Watch List. In addition to the macrolides mentioned above, new antibiotics have appeared on the second Watch List, that is, amoxicillin (β-lactam antibiotic) and ciprofloxacin (from the group of fluoroquinolones) [23].

Due to lack of legal regulations on the maximum allowable concentrations of the antibiotics in the environment, as well as global regulations related to their required degree of the removal in WWTPs, providing new information is important to be able to effectively counteract threats arising from the presence of antibiotics in the environment. Although more and more information is available on this subject, there are areas where there is much less of such data compared to other regions (e. g. region of Central and Eastern Europe). Therefore, the aim of the study was to detect and quantify selected antibacterial drugs in the raw and treated wastewater collected under various temperature conditions (i.e. winter and summer period) from two municipal WWTPs, located in the northern and southern part of Poland. The following antimicrobials were selected for the study: clarithromycin (CLA), erythromycin (ERY), roxithromycin (ROX), sulfamethoxazole (SMX), N-acetyl-sulfamethoxazole (N-Ac-SMX), sulfamethoxine (SMN), sulfamerazine (SMR), tiamulin (TIA), and trimethoprim (TRP).

2. Materials and methods

2.1. Sampling procedure and WWTPs description

The wastewater samples for the analysis were collected from two Polish municipal WWTPs at distinct locations: southern (WWTP 1) and the northern part of Poland (WWTP 2). WWTP 1 is located in the area of Upper Silesia – the most urbanized region of Poland. The treated wastewater is introduced into the small river which is a tributary of the second-largest river in Poland (the Oder river). The WWTP 1 serves a population equivalent of 248 000 inhabitants (Fig. 1a). The maximum flow rate is at the level of 63 300 m^3 d^{-1}. WWTP 2 is situated at the delta of the Vistula River (the largest river in Poland) and the wastewater after the treatment process is directly introduced into the Gdansk Bay which is a south-eastern bay of the Baltic Sea. The WWTP 2 has a population equivalent of 700 000 inhabitants and the max flow rate is equal to 95 000 m^3 d^{-1} (Fig. 1b).

The samples were taken in six sampling series – three of them in the winter period (February–March) and the remaining of them in the summer period (June). The main wastewater characteristics of WWTP 1 and WWTP 2 are shown in Table 1.

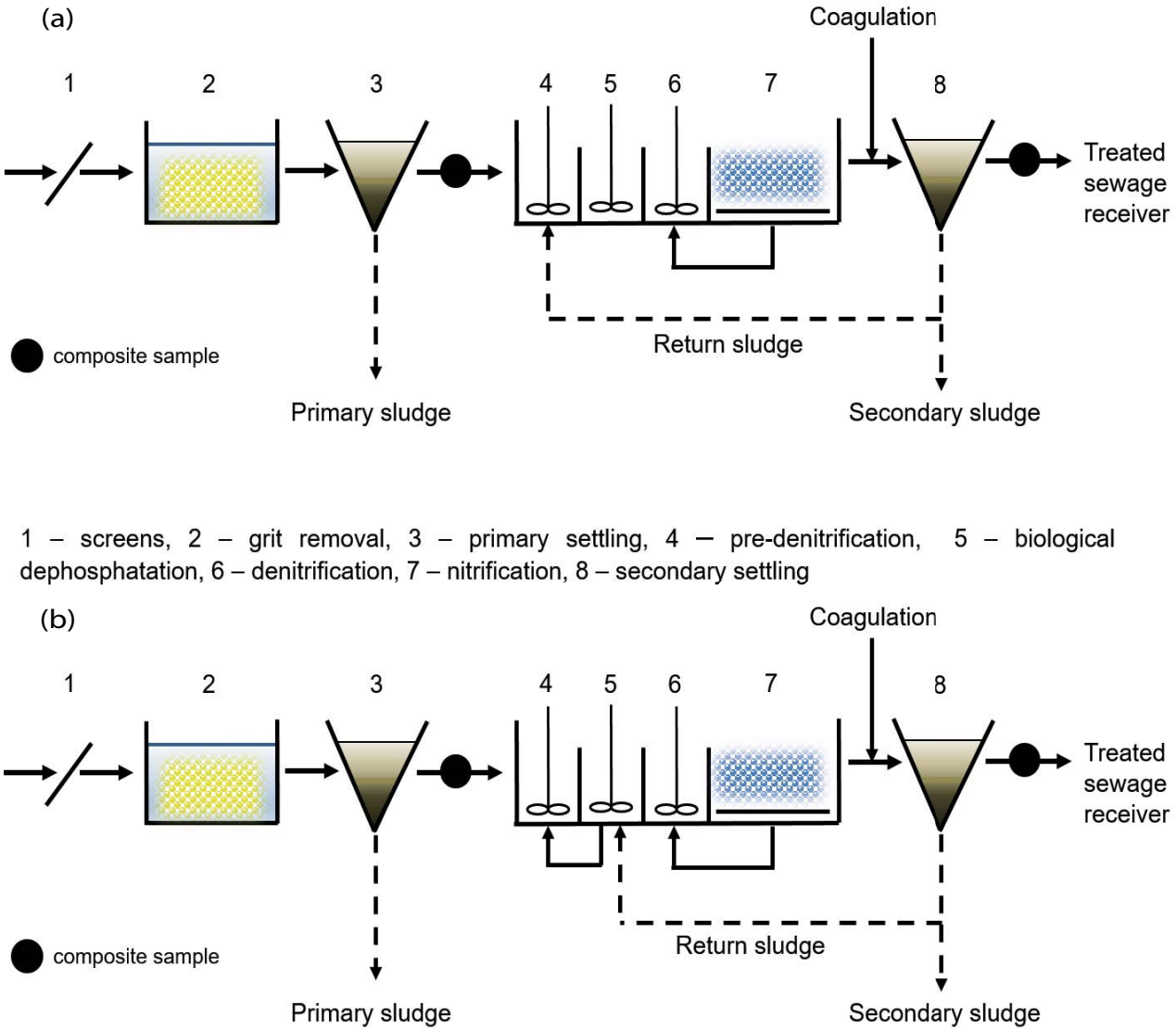

Fig. 1. Technological scheme of: (a) WWTP 1 and (b) WWTP 2.

For each plant, the composite 24 h samples of influents (after primary settling tanks) and effluents were obtained by combination wastewater collected every 20 min by automatic sampling device. The effluent samples were collected with consideration of hydraulic retention time of the wastewater in the WWTPs. The wastewater samples were frozen and stored in dark glass bottles at – 20°C until analysis.

2.2. Wastewater samples preparation–solid-phase extraction

All wastewater samples were filtered through glass fiber filters with a pore size of <1 μm and diameter 55 mm (Schleicher and Schuell, Dassel, Germany). The 100 mL of influent and 200 mL of effluent samples were spiked with a mixture of internal standards (IS). The pH of all samples was adjusted to 7.5. As the internal standards the following substances were used: sulfamerazine-d4 (IS for sulfonamides and trimethoprim), sulfamethoxazole-d4 (IS for sulfamethoxazole), N-acetyl-sulfamethoxazole-d5 (IS for N-acetyl-sulfamethoxazole) and (E)-9-[O-(2-methyloxime)]-erythromycin (IS for macrolides and tiamulin). Sulfamerazine-d4, sulfamethoxazole-d4, N-acetyl-sulfamethoxazole-d5 were purchased from Sigma-Aldrich and (E)-9-[O-(2-methyloxime)]-erythromycin was synthesized according to the procedure described [24,25].

The solid-phase extraction (SPE) of the wastewater samples was performed by means of Oasis HLB (200 mg, 6 ml) cartridges (Waters, Milford, MA, USA). Before the extraction, the cartridges were conditioned by flushing them with 1 mL × 2 mL *n*-heptane, 1 mL × 2 mL acetone, 3 mL × 2 mL methanol and 4 mL × 2 mL water (pH adjusted to 7.5). The flow rate of the samples through the cartridges have not exceeded the value of 20 mL min^{-1}. After the SPE, the cartridges were dried with nitrogen for 2 h and eluted with 4 mL × 2 mL acetone. The eluates were concentrated to the volume of 200 μL, diluted with 250 μL of methanol and evaporated under a gentle nitrogen stream to the volume of 100 μL. Then the extracts were re-dissolved in 400 μL Milli-Q water. The next step was the chromatographic analysis.

Table 1
Characteristics of raw wastewater collected from municipal wastewater treatment plants WWTP 1 and WWTP 2

Parameter	WWTP 1		WWTP 2	
	Min.	Max.	Min.	Max.
COD, mg O_2 L^{-1}	305 ± 11	810 ± 17	786 ± 28	1212 ± 37
BOD, mg O_2 L^{-1}	190 ± 14	506 ± 34	320 ± 17	422 ± 13
$N–NH_4^+$, mg L^{-1}	44.4 ± 2.3	89.6 ± 4.1	47.8 ± 1.3	68.2 ± 1.6
P_{total}, mg L^{-1}	11.2 ± 0.4	14.9 ± 0.6	9.1 ± 0.3	11.4 ± 0.3

2.3. Chromatographic analysis

All the samples were analyzed by reversed-phase liquid chromatography-tandem mass spectrometry (HPLC-MS-MS). The HPLC system consisted of autosampler (type G1313A), quaternary HPLC pump (type G1311A), and degasser (type G1379A) - all devices were from Agilent (Waldbronn, Germany). Additionally, the HPLC system was provided with the CTO-10A column oven and the SCL-10A system controller (all from Shimadzu, Duisburg, Germany). The detection was performed on an API 4000 mass spectrometer (Applied Biosystems, Foster City, CA, USA). Parameters such as declustering potential (DP), collision energy (CP), and cell exit potential (CXP) were optimized in the auto-tuning program. For all compounds two multiple reaction monitoring (MRM) transitions were monitored for identification and quantification of the analytes. More details concerning chromatographic analysis performance are presented in other publications [26].

3. Results and discussion

The presence of nine selected antibacterial drugs was monitored in the raw and treated wastewater samples, collected from WWTP 1 and WWTP 2. Six antibiotics namely: CLA, ROX, ERY, SMX, TRP, and N-Ac-SMX (main SMX metabolite) were detected in all investigated wastewater samples. However, SMR, SMN, and TIA have been not found in any wastewater sample. The data concerning concentrations of target antibiotics in the influent and effluent collected from WWTP 1 and WWTP2 are summarized in Table 2.

Table 2
Concentration of selected antibiotics in raw and treated wastewater collected from WWTP 1 and WWTP 2 in winter (T = 10°C) and summer (T = 20°C) period

Substance	Temperature	WWTP 1		WWTP 2	
		Influent, ng L^{-1}	Effluent, ng L^{-1}	Influent, ng L^{-1}	Effluent, ng L^{-1}
CLA	T = 10°C	1,836 ± 435	928 ± 65	1,416 ± 401	761 ± 106
	T = 20°C	1,329 ± 243	457 ± 82	904 ± 433	185 ± 32
SMX	T = 10°C	1,745 ± 253	1,003 ± 146	1,464 ± 203	508 ± 25
	T = 20°C	1,778 ± 274	1,226 ± 205	1,225 ± 173	642 ± 114
N-Ac-SMX	T = 10°C	3,349 ± 719	196 ± 47	1,763 ± 470	16 ± 3
	T = 20°C	2,933 ± 429	39 ± 18	1,358 ± 224	ND
TRP	T = 10°C	400 ± 22	369 ± 29	482 ± 116	445 ± 72
	T = 20°C	364 ± 60	283 ± 49	441 ± 81	269 ± 14
ROX	T = 10°C	116 ± 11	101 ± 12	161 ± 0	132 ± 14
	T = 20°C	68 ± 23	47 ± 13	105 ± 17	55 ± 6
ERY	T = 10°C	ND	16 ± 2	ND	17 ± 5
	T = 20°C	ND	17 ± 2	ND	14 ± 5
SMN	T = 10°C	ND	ND	ND	ND
	T = 20°C	ND	ND	ND	ND
SMR	T = 10°C	ND	ND	ND	ND
	T = 20°C	ND	ND	ND	ND
TIA	T = 10°C	ND	ND	ND	ND
	T = 20°C	ND	ND	ND	ND

ND – not detected

The studies have shown that the highest concentration was observed in the raw wastewater for SMX main metabolite, that is, N-Ac-SMX (3,349 ± 719 ng L^{-1}). The concentration was observed in the winter season, for WWTP 1.

Although the highest concentration of the above-mentioned metabolite was observed in the winter season, in the summer season its concentration in raw sewage was at a comparable level. In fact, even for other tested drugs, no significant differences in quantified concentrations were observed during winter and summer. It is theoretically assumed that in the winter season the concentration of antibiotics may be higher because of the greater likelihood of various types of infections. However, it should be noted that the research was conducted in a relatively large catchment area, in which the daily and seasonal fluctuations are not as large as in the case of small WWTPs and averaging effect of the medium can be observed [17,18].

The studies have shown that SMX together with its derivative (N-Ac-SMX) were also determined in all wastewater samples collected from WWTP 1 and WWTP 2 (Table 2). In the case of SMX, its removal efficiency should be calculated taking into account the concentration of its main metabolite (N-Ac-SMX). N-Ac-SMX is generated in a reaction of SMX acetylation in the human (or animal) body. However, the cleaving back of the acetyl derivate to SMX is possible under the environmental condition [6,16,17]. Under the conditions prevailing at biological WWTPs, N-Ac-SMX has been found to transform into the origin form of SMX, and the process is not dependent on the temperature. Therefore, the removal efficiency of SMX (along with its main derivative) was at a similar level in the summer, and in the winter as well (Fig. 2). Verlicchi et al. [27] obtained 52% removal of SMX (initial concentration equaled to 440 ng L^{-1}) in full-scale WWTP treated mix of municipal and hospital wastewater. In other studies, SMX (at its initial concentration 1,172 ng L^{-1}) was efficiently removed in the CAS system to 311.3 ng L^{-1} and in the MBR system to 336.0 ng L^{-1} as well [15]. However, other authors [28] reported that SMX was not removed from wastewater in four full-scale WWTP based on integrated fixed-film activated sludge, where the median removal efficiency equals to –44.3% (it probably resulted from the fact that the derivatives of SMX have not been analyzed). Therefore, for calculations on the removal efficiency of SMX in the conditions of WWTPs, it is very important to take into account concentrations of its main derivative (N-Ac-SMX), because the lack of such actions may affect the unwitting falsification of the experimental results.

The study has also shown, that CLA has been found to be incompletely removed from wastewater in WWTP 1 and its removal efficiency depends on the season (i.e. ambient temperature). In the winter, the removal efficiency of CLA equals to 47%, while an increase of the temperature in the summer improved average removal to 66% (Fig. 2a). The same trend and similar removal efficiency were obtained in the case of WWTP 2, where CLA removal in winter and summer period equals to 43% and 76% (Fig. 2b), respectively, even if the initial concentration of CLA was higher in WWTP 1 compared to WWTP 2. Tran et al. [15] observed that CLA was removed more effectively in membrane bioreactors (MBR) than in conventional systems based on activated sludge (CAS). In the above-mentioned studies, a decrease

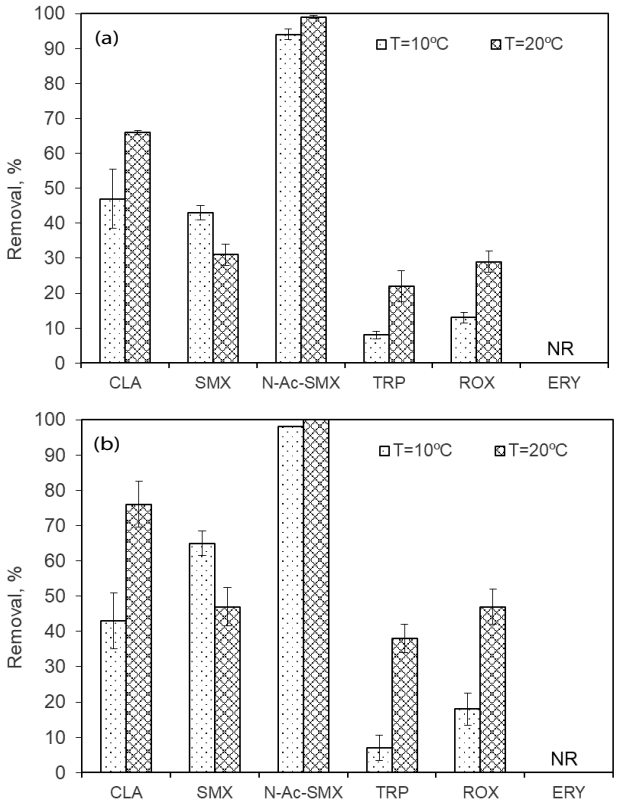

Fig. 2. Removal of selected antibiotics from municipal wastewater during winter (T = 10°C) and summer (T = 20°C) (a) WWTP 1 and (b) WWTP 2.

CLA – clarithromycin; SMX – sulfamethoxazole; N-Ac-SMX – N-acetyl-sulfamethoxazole; TRP – trimethoprim; ROX – roxithromycin; ERY – erythromycin; NR – not removed.

in CLA concentration from 1,497 to 425.0 and 531.7 ng L^{-1} was observed for MBR and CAS, respectively. It means that the average removal efficiency in MBR was equal to 71.6% while for CAS 64.5%. In other studies, CLA has been found as a recalcitrant compound to conventional biological wastewater treatment and its removal was negligible [16,17,29], however, the studies do not indicate the temperature range in which that removal was observed. The results obtained in this investigation suggest that temperature may be one of the factors influencing the effectiveness of clarithromycin removal. Therefore, in our opinion, the removal effectiveness of this substance should be discussed in relation to the temperature of the process.

TRP, a substance used in medicinal preparations in combination with SMX (and other sulfonamides) was poorly removed from wastewater in both investigated WWTPs. However, higher TRP removal efficiency was observed in the summer period (22% and 38% for WWTP 1 and WWTP 2, respectively) compared to the winter period (8% and 7% for WWTP 1 and WWTP 2, respectively) (Fig. 2). Generally, TRP is considered to be low to moderately degradable substance [15,29,30], however, some authors indicate its satisfactory removal, even at a level of up to 90% [31]. Considering such discrepancies in observations regarding the effectiveness of trimethoprim removal, some authors suggest that

temperature is a key parameter affecting the efficiency of this process [31], which can be also confirmed by the results presented in the study. Incomplete removal of trimethoprim at the level of WWTPs makes it one of the most frequently detected substances in the aquatic environment [31,32].

Regarding the efficiency of removal in winter and summer conditions, a very similar trend as in the case of trimethoprim was observed for ROX, a popular macrolide antibiotic. In the summer period the removal efficiency of this substance in WWTP 1 and WWTP 2 equaled to 29% and 47%, respectively, while in the winter period only 13% and 18%, respectively (Fig. 2). This allows us to classify this compound also to the group of low to moderately degradable in biological processes. Very similar observations can be seen in the work of Verlicchi et al. [27] in which was reported moderate removal (54%) of the target compound, wherein the concentration of ROX in the raw wastewater was twice lower than in the present study. There is also no information about the process temperature, which seems to have an impact on the degradation efficiency of this compound.

Another macrolide antibiotic, namely ERY, was not detected in the raw wastewater, however, the substance was detected in the treated wastewater at the concentration not exceeding the value of 20 ng L^{-1} (Table 2). The increase of ERY concentration in treated wastewater may be explained by the occurrence of its derivatives, which under the conditions taking place at WWTP are converted into the origin compound. For example, ERY in the environmental condition may reversibly transform into its anhydrous form, that is, erythromycin-H_2O (ERY-H_2O) [33]. Additionally, ERY and its derivatives have a relatively strong affinity for the solid phase, including suspended solids occurring in the raw sewage. Due to the research methodology, the liquid samples had to be filtered before analysis. In practice, it means that part of the absorbed ERY and its potential derivatives could have been removed from the sample at the preparation stage (with the post-filter sludge). A similar phenomenon that the concentration of ERY after the treatment process is higher in the treated than in the raw wastewater was reported by the other authors [29]. This may support the hypothesis about the reversible transformation of ERY to ERY-H_2O under environmental conditions. However, generally, erythromycin is classified as poorly or moderately removable from the wastewater at WWTPs with the removal efficiency from 0% to 64% [15,27]. However, there are many indications that the removal effectiveness of this compound is not affected by the process temperature, but rather by the configuration of the technological system, including coagulation-flocculation processes located at the beginning of the technological system, as well as the type of coagulant itself [34].

4. Conclusions

The studies have shown that among the selected for the experiment antibacterial drugs (and the selected metabolite) in the wastewater samples were quantified: CLA, ERY, ROX, TRP, SMX, and N-Ac-SMX (main SMX metabolite) and these drugs were detected in all investigated samples, irrespective of the season and the place of collection. Of all the compounds tested, the substance for which the highest concentration was observed, was N-Ac-SMX (3,349 ± 719 ng L^{-1}). However, antibacterial drugs as SMN, SMR, and TIA were not detected in any investigated sample. The absence in the samples of these drugs can be explained by the fact that they are mainly used in veterinary medicine, and the catchments of both WWTPs from which the samples were taken, were located in highly urbanized areas, without a significant share of wastewater from agricultural areas (including framings).

The studies have confirmed that N-Ac-SMX, the main metabolite of SMX, should be taking into account to calculate the removal efficiency of SMX due to its ability to transform into the origin form (and conversely) under the conditions prevailing at WWTPs. The SMX derivative was also the most efficiently removed from wastewater, regardless of the season and technology used, but certainly, some of this substance was converted back to SMX.

In the present study, the temperature of the process was an important parameter affecting the removal efficiency of CLA, ROX, and TRP from wastewater – their removal efficiency was definitely higher in the summer in comparison to the winter period.

Erythromycin turned out to be the least effectively degraded drug – its concentration was higher in treated sewage than in raw wastewater. However, it can be assumed that in the case of ERY, not the process temperature, but the configuration of the technological system (including coagulation–flocculation processes) may be significantly important in removing this compound at the WWTPs.

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