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Spatial distribution of pharmaceuticals in conventional wastewater treatment plant with Sludge Treatment Reed Beds technology

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Abstract

Pharmaceutical residues are an emerging environmental problem. It is strongly confirmed that pharmaceuticals are present in soils and environmental waters (surface, marine and even groundwater), and that wastewater treatment plant (WWTP) effluents are the main source of pharmaceuticals in the watershed. The aim of this study was to recognize the spatial distribution and seasonal changes of selected pharmaceuticals in conventional WWTP with Sludge Treatment Reed Beds (STRBs) technology used for dewatering and stabilization of sewage sludge, because these systems have never been studied in terms of pharmaceuticals distribution or removal potential.

The research was conducted in conventional WWTP in Gniewino, where raw wastewater was treated using mechanical, biological and chemical removal of the organic matter and nutrients, and sewage sludge was treated with STRB. Determinations of pharmaceuticals (non-steroidal anti-inflammatory drugs - ibuprofen, paracetamol, flurbiprofen, naproxen, diclofenac and its metabolites) and basic parameters were carried out in samples of influent and effluent from WWTP and in the liquid phase of surplus activated sludge (SAS) as well as reject water from STRB.

The potential of removal varied among target pharmaceuticals. Ibuprofen and naproxen were completely removed by the standard applied technology of the Gniewino WWTP. Diclofenac and its metabolites were the chemicals with the lowest removal potential in wastewater and the highest detection frequency. These pharmaceuticals were also detected in the liquid phase of SAS as well as in reject water. However, removal potential when using STRB was higher than 94 % (mostly higher than 99 %), independent of the season. Indeed, the STRB technology is not only efficient in sludge dewatering and nutrient removal (primary purpose), but also elimination of polar pollutants. Nevertheless, removal in STRB did not mean that pharmaceuticals were totally eliminated because these compounds could be “trapped and stored” in beds (by the process of sorption) or transformed into other products. This study is a starting point for further exploration of STRB technology for elimination of emerging pollutants.

Keywords: conventional wastewater treatment plant, sewage sludge processing, Sludge Treatment Reed Beds, pharmaceutical residues, non-steroidal anti-inflammatory drugs

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1. INTRODUCTION

Over the last decade, the scientific community has been focused on the presence of pharmaceuticals in the environment. Pharmaceuticals are a large group of bioactive chemical compounds used in veterinary medicine, animal farms and in medicine. They represent a diverse group of water pollutants that are not systematically monitored and can cause negative effects in the environment (Farré et al., 2008). The research indicates that pharmaceuticals are present in surface waters, sea water and even in groundwater (Borecka et al., 2015; Boxall et al., 2012; Caban et al., 2015; Farré et al., 2008; Watkinson et al., 2009), though the highest concentration of pharmaceuticals can be found in raw wastewater (inflow to municipal wastewater treatment plants - WWTPs) (Biel-Maeso et al., 2018; Sim et al., 2011; Verlicchi et al., 2012). Jarosova et al. (2012) undertook the investigation of the presence of pharmaceuticals in seven headwaters flowing through relatively unpolluted areas of the Czech Republic, a small country with a relatively low density of population (Jarosova et al., 2012). It was found that the WWTPs are the most significant source of pharmaceuticals in water bodies. Other sources were practically negligible. This was confirmed by many other research projects (Arlos et al., 2014; Zorita et al., 2009). It was also found that the distribution of the contaminations, including pharmaceuticals, was highly dependent on the treatment process and effluent quality (Arlos et al., 2014).

According to Verlicchi et al. (2013) the range of pharmaceutical concentrations in raw wastewater is from 10^{-3} to 10^{2} µg/l and even more, and common WWTPs are not able to efficiently remove all of them from liquid effluent as well as sludge. It was observed that removal efficiencies varied in a wide range for the different compounds, as well as for the same substance, due to the different chemical and physical characteristics and to operational conditions. Other research indicated that the total concentration of the individual pharmaceuticals (except carbamazepine and crotamiton) in the influent was efficiently removed by 80% during the biological treatment. It was also found that they total concentrations in the effluent from conventional activated sludge process was 1.5 times higher than that from biological nutrient removal process (Okuda et al., 2008). Many research confirms that ibuprofen is nearly completely removed from wastewater in conventional WWTPs (removal rates >90%) (Clara et al., 2005; Paxéus, 2004; Joss et al., 2005). The lower removal efficiency was found in case of naproxen (80%) and diclofenac (39%) (Clara et al., 2005). Other studies show the lower efficiency of pharmaceuticals removal in conventional activated sludge processes. According to Tiwari et al. (2017) the removal rates of ibuprofen and naproxen are common ranges between 75% and 85% and 50–60%, respectively. Diclofenac revealed low and varied removal rate ranging from 10 to 50%.

The processes occurring in Sludge Treatment Reed Beds (STRBs) are similar to those in constructed wetlands (CWs). According to Carvalho et al. (2017), CWs present similar or better removal of pharmaceuticals compared to conventional WWTP systems. The pharmaceuticals are removed mostly thanks to (i) degradation in a hydroponic medium vegetated by wetland plants, (ii) uptake by the wetland plants, and (iii) degradation in CW mesocosms.

Chen et. al. investigated pharmaceuticals in wastewater from rural areas treated in CWs located in the Czech Republic (Chen et al., 2016). The removal efficiencies of pharmaceuticals and personal care products (PPCPs) in the rural CWs exhibited large variability with 11-100% for anti-inflammatories, 37-99% for β-blockers and 18-95% for diuretics. The statistical results revealed significant correlations between removal efficiencies of some PPCPs and removal efficiencies for organic matter, ammonia and phosphorus (Chen et al., 2016). Other research (Vymazal et al., 2017) of wastewater treated in CW indicated wide variation in removal efficiency among systems as well as among pharmaceuticals. The highest average removal was found for paracetamol (91%). Moderate removal was found for ibuprofen. Diclofenac removal was insufficient and did not exceed 50%. Matamoros et al. also confirmed that diclofenac was not effectively removal in CWs (Matamoros et al., 2009). Although efficiency of pharmaceuticals removal in CWs is rather well know, their removal in STRBs has not been studied.

STRB technology offers simultaneous dewatering and stabilization of sewage sludge taken from conventional WWTPs. These systems are used for treatment of sludge from very small single-family

WWTPs (for a few persons) to big WWTP (for example Kolding STRB for 125,000 pe - personal equivalent), but mostly they are useful for medium-size WWTPs (Nielsen, 2003).

STRB technology is based on the same processes that occur in natural wetland ecosystems. STRBs are built as concrete constructions or as tight tanks placed in the ground. The whole system is divided into several beds planted with reeds. The long-term experiences indicate that in medium or big WWTPs the number of beds should be at least eight. STRB technology consists of periodical loading of sludge with low content of dry matter (0.5-1.5%) (Kołecka and Obarska-Pempkowiak, 2008; Nielsen, 2003). The time of loading typically takes about 3 - 7 days. After discharging of sludge onto a bed, time for its dewatering (so-called resting time) is needed, therefore sludge should be loaded onto another bed. The resting time is about 21-49 days (Brix, 2017). The sludge is stored in system for about 10-15 years. After this time it is removed from the system and can be used as fertilizer (Kołecka and Obarska-Pempkowiak, 2013; Nielsen, 2011).

STRBs are especially useful in rural areas and housing estates where economic considerations limit the use of expensive mechanical equipment. These systems can be established in any area and are simple to build and operate. Their low energy consumption is their main advantage. Additionally, they do not require addition of chemicals for improvement of dewatering capability (Kołecka et al., 2017).

Research shows that sludge dewatering efficiency in reed systems is comparable to that of mechanical equipment such as a filter press (content of dry matter can even reach up to 40%). It has also been proven that sludge after long-term treatment in STRBs is stabilized and has a chemical composition similar to that of humus. Additionally, it was proven that the obtained product is safe with regard to its microbiological characteristics (Nielsen, 2007).Unlike most other conventional methods, reject water from STRBs released from the sludge during dewatering is treated as it percolates through the bed (Brix, 2017; Nielsen, 2007).

The secondary function of STRBs could be the removal of hazardous pollutants, for example pharmaceuticals, which are classified as new emerging pollutants with a global awareness statute (Gavrilescu et al., 2014). It has been proposed that systems containing plants and soil can participate in elimination of pharmaceuticals and their metabolites.

The aim of this study was to recognize the spatial distribution and seasonal changes of selected pharmaceutical in conventional WWTP with STRB technology. The distribution as well as removal potential were analyzed and discussed in the wastewater treatment part of WWTP as well as the sludge processing part in STRB.

2. MATERIALS AND METHODS

2.1 Object of investigation

The research was conducted in a conventional WWTP for 15,000 pe (personal equivalent) located in Gniewino (Poland, Pomerania Province). Wastewater is treated using mechanical, biological and chemical removal of the organic matter and nutrients and sewage sludge is treated by an STRB system, which is planted with *Phragmites australis*. The technological scheme with sampling points is presented in Figure 1.

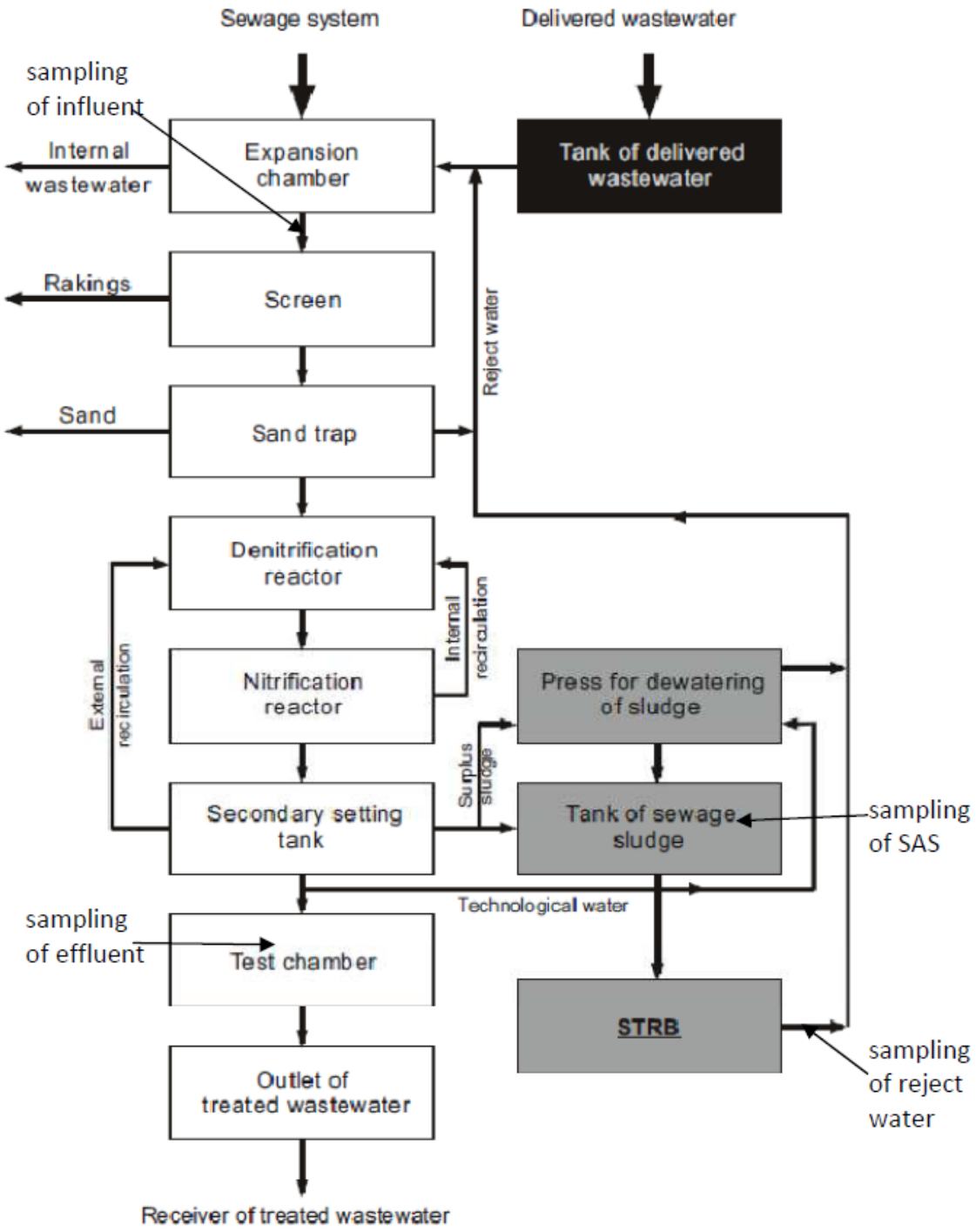

Figure 1. The technological scheme of Gniewino WWTP with sampling points (Kołecka et al., 2017)

The STRB was built in 2012. Its total area is 2400 m^2 and it consists of six beds. The time required to feed sludge to one bed is about 1 day. About 35% of wastewater supplied to the WWTP comes from the dairy and food industry and 17% of wastewater is delivered from septic tanks.

2.2 Chemicals

The following pharmaceuticals were taken as targets: ibuprofen (MW: 206.28, CAS: 15687-27-1), paracetamol (acetaminophen, MW: 151.16, CAS: 103-90-2), flurbiprofen (MW: 244.26, CAS: 5104-49-4), naproxen (MW: 230.26, CAS: 22204-53-1), diclofenac (MW: 318.13, CAS: 15307-79-6), 5-

hydroxydiclofenac (5OH-diclofenac) (MW: 312.15, CAS:69002-84-2), 4'-hydroxydiclofenac (4OH-diclofenac) (MW: 312.15, CAS: 64118-84-9). Ibuprofen, flurbiprofen, naproxen and diclofenac belong to the non-steroidal anti-inflammatory drugs (NSAIDS). 4'-hydroxydiclofenac and 5-hydroxydiclofenac are primary phase I metabolites of diclofenac (Bort et al., 1999). Two internal standards were used - diclofenac-(acetophenyl ring-$^{13}C_6$) (Internal standard I, MW: 405.16, CAS: 1261393-73-0) and 4'-hydroxydiclofenac-$^{13}C_6$(Internal standard II, MW: 318.01,CAS: 1189656-64-1). All mentioned chemicals were purchased from Sigma-Aldrich. BSTFA+1% TMCS (N,O-Bis(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane) was purchased from Synthese Nord GmbH (Germany). Pyridine was purchased from Sigma-Aldrich. Other organic solvents (HPLC grade purity) were purchased from POCH (Polskie Odczynniki Chemiczne, Poland).

The stock solutions (1 mg/mL) of each analyte (targets and internal standards IS) were prepared in methanol. The working solutions were prepared in methanol as well. The solutions of the two internal standards were prepared in methanol and had concentrations of 0.5 µg/mL of each IS. These solutions were used in further experiments and were added to the samples before extraction or into the standards samples before the validation procedure.

2.3 Sampling

Measurements were carried out in samples of influent and effluent from the WWTP and in the liquid phase of surplus activated sludge (SAS) as well as reject water from the STRB. The samples were collected every 2 hours during a day (from 7 a.m. until 4 a.m. the next day). Next, the samples were averaged. The samples of influent were collected before the activated sludge reactor and effluent was collected after the secondary settling tank in the test chamber using automatic, specialist equipment. SAS was collected from the tank for sewage sludge and reject water was collected from the well downstream from the STRB using a dredge. The reject water was always collected from the same bed which was fed just before sampling. Between sampling the bed was resting and new sludge was not discharged. Two average samples of reject water were taken. The first sample was taken in the first hour of reject water outflow and the second one during the course of a day in one hour intervals.

The samples were taken in 1L plastic bottles and taken immediately to the laboratory without special preservation.

In 2017 the samples were collected 3 times: 12th of June, 05th of September and 16th of November.

2.4 Chemical analysis and calculations

The wastewater samples were analyzed immediately or frozen at -20 °C. 100 mL of sample was taken for solid-phase extraction (SPE). The 50 µL of working ISs solution was added before extraction. The pH of the sample was adjusted to 3 (±0.1) using an aqueous HCl solution. Then the sample was filtered first by using a paper filter, then glass-fiber filters. An additional study was taken and samples were filtered, then pH was subsequently adjusted. The results showed slight differences between the pre-treatment protocols, therefore the first protocol mentioned was utilized for subsequent analysis. The Strata-X columns (200 mg, 3 mL, Phenomenex) were taken for SPE. The columns where conditioned using methanol (3 mL) and deionized water (3 mL). Samples were passed through the column under pressure (water pump). Two-step washing was used, first with 5 % aq. methanol, then hexane, each 3 mL with subsequent sorbent drying by air flow. The elution was performed by 2x3 mL methanol. The extract was transferred to chromatographic vials, and the solvent was evaporated. The dry residues were subjected to derivatisation by BSTFA+1% TMCS:pyridine mixture (1:1, v/v), 100 µL per sample. Reaction time and temperature were 30 min and 60 °C, respectively. After cooling down the samples were transferred to glass inserts in chromatographic vials. The determinations were performed by GC/MS(SIM) method.

The GC separations were performed on a Zb-5 fused silica capillary column (30 m×0.25 mm×0.25 µm, Zebron, Phenomenex) using the following temperature program: 100°C for 1 min, from 100°C to 300°C at 10 °C/min, and finally 10 min at 300 ºC (total time: 31 min).The pressure of the helium carrier gas was set at 100 kPa. The gas chromatograph (GC-2010 Plus-Shimadzu (Kyoto, Japan)) was coupled to a mass spectrometer (GCMS-QP 2010 SE). The transfer line was heated to 300 ºC. Mass spectra (EI, 70

eV, 200 °C) were recorded by scanning the mass in the 50–850 m/z range at a rate of 3 scans per second and in SIM mode. TMS-derivatives of analytes were identified by their characteristic retention times and m/z values (min. 3 m/z values) in specific time windows, and ratios between ions taken for quantification and ions taken for confirmation (Table 1).

The instrumental validation was performed using working calibration standard solutions (0.0001 – 5 µg/mL) and matrix-matched solutions for recovery calculation. The method detection limits (MDL) and method quantification limits (MQLs) were calculated using equations presented in Migowska et al. (2012). Recovery of both IS (I and II) was 102 %.

Table 1. Validation parameters of SPE-GC-MS(SIM) analysis of target pharmaceuticals in wastewater samples (Bold - m/z value for quantification, IS - internal standard used)

Analyte	SIM ion [m/z]	IS	MDL [ng/L]	MQL [ng/L]	Recovery %
Ibuprofen	**160**, 278, 263, 234	I	4	12	85
Paracetamol	**206**,280, 295	I	4	11	95
Flurbiprofen	**180**, 301, 316, 165	I	2	6	87
Naproxen	**185**, 243, 302, 287	I	2	6	88
Diclofenac	**214**, 242, 367, 276	I	2	5	101
5OH-diclofenac	**302**, 365, 455, 457	II	5	14	74
4OH-diclofenac	**302**, 330, 455	II	3	10	100

Additionally, the basic parameters, that is suspended solids (SS), COD, BOD_5, total nitrogen (TN), ammonia nitrogen ($N\text{-}NH_4^+$), nitrate nitrogen ($N\text{-}NO_3^-$), nitrite nitrogen ($N\text{-}NO_2^-$), total phosphorus (TP) and orthophosphorus (PO_4^{3-}) were determined. All determinations were carried out according to Polish Standards (PN-ISO 15705:2005, PN-EN 1899-1:2002; PN-ISO 5664:2002, PN-EN ISO 10304-1:2009, +AC:2012, PN-82/C-04576/08, PN-73/C-04576.14, PN-EN ISO 10304-1:2009 +AC:2012, PN-EN ISO 6878:2006 +Ap1:2010 p. 4 +Ap2:2010) and hints from the American Public Health Association (APHA, 2005).

The loads of pollutants and pharmaceuticals were calculated taking into account wastewater and reject water flows as well as the efficiency of the pump feeding the STRB. The quantity of pharmaceuticals was estimated using the time of bed feeding as well as the outflow of reject water.

Correlations between concentrations of pharmaceuticals and basic parameters were calculated using Pearson's coefficient (correlation coefficient). Correlation coefficient formulas are used to find how strong a relationship is between data. The formulas return a value between -1 and 1. 1 indicates a strong positive relationship, -1 a strong negative relationship and 0 no relationship at all.

3. RESULTS AND DISCUSSION

3.1 Basic parameters

Tables 2 and 3 present the characteristics of basic parameters (concentrations and loads) in wastewater as well as in the liquid phase of SAS and reject water from the STRB in Gniewino.

Table 2. The average values of basic parameters concentrations in Gniewino WWTP

Parameter	Influent	Effluent	Liquid phase of SAS	Reject water from STRB (1st hour)	Reject water from STRB (after 1 hour)
SS, mg/L	277 ± 54.4	6.33 ± 3.86	8389 ± 1005	10.0 ± 7.07	4.67 ± 0.47
BOD, mgO_2/L	894 ± 82.1	4.33 ± 1.25	920 ± 140	83.3 ± 14.34	80.0 ± 16.33
COD, mgO_2/L	1224 ± 89.3	38.0 ± 6.32	1228 ± 253	185.3 ± 35.24	160.0 ± 47.21
$N\text{-}NO_3^-$, mg/L	1.60 ± 0.31	2.74 ± 1.73	13.84 ± 5.07	17.77 ± 7.89	27.53 ± 1.31
$N\text{-}NO_2^-$, mg/L	0.60 ± 0.13	0.050 ± 0.012	1.74 ± 0.26	5.62 ± 3.78	4.33 ± 2.83
$N\text{-}NH_4^+$, mg/L	100.8 ± 4.42	0.41 ± 0.14	3.80 ± 0.83	160.3 ± 25.53	177.4 ± 10.01

TN, mgN/L	132.1 ± 8.66	9.64 ± 2.85	103.4 ± 13.9	230.4 ± 27.04	237.5 ± 11.18
$P\text{-}PO_4^{3-}$, mg/L	14.03 ± 2.43	0.10 ± 0.045	106.9 ± 13.1	16.50 ± 1.27	16.87 ± 2.22
TP, mgP/L	17.93 ± 5.37	0.45 ± 0.29	116.2 ± 15.9	41.37 ± 0.73	40.40 ± 2.36

Table 3. The average loads of basic parameters in Gniewino WWTP

Parameter	Influent	Effluent	Liquid phase of SAS	Reject water from STRB (1st hour)	Reject water from STRB (after 1 hour)
SS, kg/h	10.75 ± 2.25	0.24 ± 0.14	209.7 ± 28.37	0.0015 ± 0.0011	0.00042 ± 0.000042
BOD, kg/h	34.93 ± 5.29	0.17 ± 0.057	23.00 ± 3.49	0.0126 ± 0.0021	0.0072 ± 0.0015
COD, kg/h	47.79 ± 6.47	1.50 ± 0.36	30.71 ± 6.34	0.0280 ± 0.0051	0.0145 ± 0.0043
$N\text{-}NO_3^-$, kg/h	0.062 ± 0.014	0.104 ± 0.065	0.344 ± 0.127	0.0027 ± 0.0012	0.0025 ± 0.00012
$N\text{-}NO_2^-$, kg/h	0.022 ± 0.004	0.0021 ± 0.0005	0.043 ± 0.010	0.00080 ± 0.00006	0.00039 ± 0.00005
$N\text{-}NH_4^+$, kg/h	3.92 ± 0.27	0.016 ± 0.0042	0.095 ± 0.020	0.0240 ± 0.0037	0.0160 ± 0.00082
TN, kg/h	5.13 ± 0.35	0.37 ± 0.12	2.60 ± 0.33	0.034 ± 0.0039	0.021 ± 0.0012
$P\text{-}PO_4^{3-}$, kg/h	0.52 ± 0.076	0.0038 ± 0.0019	2.67 ± 0.33	0.0024 ± 0.00019	0.0015 ± 0.00019
TP, kg/h	0.69 ± 0.16	0.018 ± 0.012	2.90 ± 0.40	0.0062 ± 0.00014	0.0036 ± 0.00022

In comparison to regular and similar WWTPs in other regions of Poland and Europe, the WWTP in Gniewino received a higher concentration of pollutants in raw wastewater (up to 1200 mg O_2/ L of COD and up to140 mg TN /L). This is caused by the high share of dairy and fish industry wastewater in the catchment of Gniewino WWTP. For all basic parameters, which include SS, BOD_5, COD, TN and TP, the efficiency of pollutants removal significantly exceeded 90% and final effluent met the requirements of the Polish standards. The previous research confirms the high efficiency of pollutants removal (Kołecka et al., 2017). In wastewater, the nitrogen occurred in the form of ammonium. In influent, phosphorus was mostly as orthophosphate. In effluent, the share of orthophosphate in total phosphorus was much lower.

It is estimated that the liquid phase of SAS accumulated the biggest load of suspended solids (average 209.7 ± 28.37 kg/h) (Table 3). Average content of dry matter in SAS was about 1 %, which is why the suspended solid (SS) content in the liquid phase was very high. During further processing, the majority of this load is retained in the STRB as organic matter and the load of SS in reject water was even lower than in effluent.

COD and BOD in the liquid phase of SAS were at the same level as in influent to WWTP, and the load of COD and BOD in reject water decreased significantly (average up to 99%). In the liquid phase of SAS nitrogen occurred mostly in organic form while in reject water primarily as ammonium.

The concentration of nitrogen and its form in the liquid phase of SAS was rather low. In sewage sludge the nitrogen is mostly restricted to the solid phase. Its concentration in SAS was on average 5.05% of dry matter. The nitrogen was kept in the STRB where denitrification and nitrification processes occur (Kołecka et al., 2017). In reject water from STRB the highest loads had nutrient compounds (Table 2), which were probably released from storage sludge.

Average concentration of phosphorus in SAS was 3.7% of dry matter. In the liquid phase of SAS phosphorus was mostly as orthophosphate. In reject water the concentration of phosphorus was much lower than in the liquid phase of SAS. The phosphorus was probably partly taken by reeds and partly bound in the bed.

Although concentrations of some parameters in reject water was rather high, their loading was lower than in effluent. They can be safely recirculated and discharged at the beginning of the technological line of wastewater treatment (like it is in this case) and can even be released to the environment.

3.2 Pharmaceutical distribution

Tables 4 and 5 present the concentrations (µg/L) and loads (mg/h) of selected pharmaceuticals in wastewater, the liquid phase of SAS as well as reject water from the STRB, respectively. Loads were calculated knowing the flow of wastewater in each of the tested parts of the studied technology.

Table 4. The concentrations of selected pharmaceuticals in Gniewino WWTP, µg/L

Analyte	Influent	Effluent	Liquid phase of SAS	Reject water from STRB (1st hour)	Reject water from STRB (after 1 hour)
			12.06.2017		
Ibuprofen	16.624 ± 0.495	<MDL	<MDL	<MDL	2.554 ± 0.318
Paracetamol	0.837 ± 0.077	<MDL	<MDL	<MDL	<MDL
Flurbiprofen	<MDL	<MDL	<MDL	<MDL	<MDL
Naproxen	6.175 ± 0.057	<MDL	<MDL	<MDL	<MDL
Diclofenac	2.251 ± 0.104	5.630 ± 0.264	2.433 ± 0.496	0.705 ± 0.027	2.050 ± 0.342
5OH-diclofenac	4.686 ± 0.626	0.321 ± 0.025	<MDL	<MDL	4.245 ± 0.357
4OH-diclofenac	15.217 ± 2.399	8.560 ± 0.591	4.533 ± 1.112	<MDL	<MDL
			05.09.2017		
Ibuprofen	34.508 ± 5.644	<MDL	<MDL	<MDL	<MDL
Paracetamol	<MDL	<MDL	<MDL	<MDL	<MDL
Flurbiprofen	<MDL	<MDL	<MDL	<MDL	<MDL
Naproxen	22.247 ± 5.668	<MDL	<MDL	<MDL	<MDL
Diclofenac	4.477 ± 0.655	5.189 ± 1.507	0.841 ± 0.133	1.832 ± 0.195	3.926 ± 1.132
5OH-diclofenac	<MDL	<MDL	<MDL	<MDL	<MDL
4OH-diclofenac	18.153 ± 5.899	5.915 ± 1.284	0.889 ± 0.134	5.588 ± 1.530	5.680 ± 0.563
			16.11.2017		
Ibuprofen	27.965 ± 1.494	<MDL	<MDL	1.002 ± 0.377	2.235 ± 1.270
Paracetamol	28.630 ± 12.46	<MDL	<MDL	<MDL	<MDL
Flurbiprofen	<MDL	<MDL	<MDL	<MDL	<MDL
Naproxen	5.498 ± 0.293	0.028 ± 0.005	<MDL	<MDL	<MDL
Diclofenac	2.688 ± 0.599	1.597 ± 0.046	1.421 ± 0.032	0.986 ± 0.404	0.824 ± 0.249
5OH-diclofenac	5.033 ± 0.726	1.805 ± 0.027	1.939 ± 0.164	2.044 ± 0.654	1.860 ± 0.099
4OH-diclofenac	5.042 ± 0.720	1.782 ± 0.040	1.900 ± 0.099	1.742 ± 0.483	<MDL
			The average values		
Ibuprofen	26.366 ± 7.388	<MDL	<MDL	0.334 ± 0.172	1.596 ± 0.936
Paracetamol	9.822 ± 3.303	<MDL	<MDL	<MDL	<MDL
Flurbiprofen	<MDL	<MDL	<MDL	<MDL	<MDL
Naproxen	11.307 ± 5.741	0.009 ± 0.003	<MDL	<MDL	<MDL
Diclofenac	3.139 ± 0.963	4.139 ± 1.806	1.565 ± 0.658	1.174 ± 0.479	2.267 ± 1.276
5OH-diclofenac	3.240 ± 1.295	0.709 ± 0.086	0.646 ± 0.914	0.681 ± 0.364	2.035 ± 1.137
4OH-diclofenac	12.804 ± 5.618	5.419 ± 2.789	2.441 ± 1.536	2.443 ± 1.335	1.893 ± 0.978

<MDL- below the method detection limit

Table 5. The loads of selected pharmaceuticals in Gniewino WWTP, mg/h

Analyte	Influent	Effluent	Liquid phase of SAS	Reject water from STRB (1st hour)	Reject water from STRB (after 1 hour)
			12.06.2017		

Ibuprofen	661.55	<MDL	<MDL	<MDL	0.23
Paracetamol	33.31	<MDL	<MDL	<MDL	<MDL
Flurbiprofen	<MDL	<MDL	<MDL	<MDL	<MDL
Naproxen	245.73	<MDL	<MDL	<MDL	<MDL
Diclofenac	89.58	224.05	60.83	0.11	0.18
5OH-diclofenac	186.48	12.77	<MDL	<MDL	0.38
4OH-diclofenac	605.56	340.65	113.33	<MDL	<MDL
			05.09.2017		
Ibuprofen	1462.38	<MDL	<MDL	<MDL	<MDL
Paracetamol	<MDL	<MDL	<MDL	<MDL	<MDL
Flurbiprofen	<MDL	<MDL	<MDL	<MDL	<MDL
Naproxen	942.78	<MDL	<MDL	<MDL	<MDL
Diclofenac	189.73	219.90	21.03	0.27	0.35
5OH-diclofenac	<MDL	<MDL	<MDL	<MDL	<MDL
4OH-diclofenac	769.29	250.67	22.23	0.84	0.51
			16.11.2017		
Ibuprofen	969.52	<MDL	<MDL	0.15	0.20
Paracetamol	992.57	<MDL	<MDL	<MDL	<MDL
Flurbiprofen	<MDL	<MDL	<MDL	<MDL	<MDL
Naproxen	190.61	0.97	<MDL	<MDL	<MDL
Diclofenac	93.19	55.37	35.53	0.15	0.07
5OH-diclofenac	174.49	62.58	48.48	0.31	0.17
4OH-diclofenac	174.80	61.78	47.50	0.26	<MDL
			The average values		
Ibuprofen	1031.2 ±329.9	<MDL	<MDL	0.15 ± 0.08	0.22 ± 0.14
Paracetamol	512.9 ± 279.6	<MDL	<MDL	<MDL	<MDL
Flurbiprofen	<MDL	<MDL	<MDL	<MDL	<MDL
Naproxen	459.7 ± 242.3	0.97 ± 0.01	<MDL	<MDL	<MDL
Diclofenac	124.2 ± 46.4	166.4 ± 78.6	39.1 ± 16.4	0.18 ±0.07	0.20 ± 0.11
5OH-diclofenac	174.5 ± 85.2	37.7 ± 24.9	48.5 ± 24.1	0.10 ± 0.09	0.18 ± 0.11
4OH-diclofenac	516.6 ± 250.7	217.7 ± 116.2	61.0 ± 38.4	0.37 ± 0.25	0.17 ±0.10

<MDL- below the method detection limit

Very high concentrations and loads were found in the case of ibuprofen (up to 35 µg/L and 1400 mg/h, respectively). However, it was detected only in influents. Technological processes of the WWTP completely removed this pharmaceutical from wastewater. In the samples from June and November ibuprofen was found in reject water from the STRB and its concentrations were 2.554 ± 0.318 and 2.235 ± 1.270 µg/L. The presence of the ibuprofen in reject water can be an effect of a memory of beds (the elution of ibuprofen from the previous loadings of sewage sludge), the release the ibuprofen from the solid part of sludge (the solid part of the sludge was not analyzed during this study for content of pharmaceuticals), or the release of this pharmaceutical from its conjugates during passage through the beds structures. The created *de novo* of ibuprofen from its metabolites in CWs was already amplified (Hijosa-Valsero et al., 2016). However, none of these hypotheses can be confirmed by this study. Nevertheless, the load of ibuprofen in reject water was only about 0.02% in comparison to its load in influent. In the case of CWs, the ibuprofen was found in native form after the 400 h of wastewater

loadings (Matamoros and Garci, 2005), which suggests high stability of this pharmaceutical in soil-plants systems.

Paracetamol was detected only in the samples of influent in June and November 2017. This pharmaceutical's presence in wastewater is connected with autumn, the season of flu and cold. Therefore, in September its concentration was high (28.630 ± 12.46 μg/L). Similar to ibuprofen, this pharmaceutical was removed, and it was not detected in effluents in native form.

Flurbiprofen was not detected in any analyzed samples.

The concentrations of naproxen in influent were from 5.498 ± 0.293 to 22.247 ± 5.668 μg/L. Only in November it was detected in effluent, however its concentration was only 0.028 ± 0.005 μg/L. Naproxen was found to be removed in anaerobic conditions of activated sludge (Lahti and Oikari, 2011).

Diclofenac and its two metabolites were the analytes with the lowest removal potential in tested WWTP. Their presences were found in wastewater before and after treatment, in the liquid phase of SAS as well as in reject water. The poor removal of diclofenac in both aerobic and anaerobic conditions was already shown (Lahti and Oikari, 2011). In the case of the tested STRB system (similarity to CWs, (Imfeld et al., 2009)) both aerobic and anaerobic sectors are present depending on the depth of the bed.

There was no straight trend in diclofenac concentration in tested samples between type of matrix and date of samplings. For example, concentrations of diclofenac increased in effluents compared to influents taken in June and September. In these months, in reject water was also found that diclofenac concentration was lower in the first hour of outflow compared to an average sample taken in the next 24-h period. In November, concentration of diclofenac was lower in effluent than in influent. In this month, higher concentration in reject water was detected after feeding the STRB, and then it decreased. Certainly, the variation of physico-chemical parameters of influent and changed efficiency of activate sludge are reasons that between the seasons the efficiency of pharmaceuticals removal is difficult to predict. In the case of STRBs the reed and micro-flora living on filters and deposits of sludge have a lower potential to remove / transform / uptake the pharmaceuticals in the cold season. Nevertheless, several processes affect elimination of hazardous substances in STRB systems (biotic processes, e.g. microbiological degradation, biofilm, and plant uptake, and physico-chemical processes, like photodegradation, oxidation, hydrolysis, retention / root sorption).

The other factor which we investigated here is the presence of the two most abundant diclofenac metabolites and their concentration variability in the tested WWTP+STRBs system. The two metabolites taken for research, have already been proven to be present in WWTPs samples with high detection frequencies (Stülten et al., 2008). It was noticed that the presence and concentration of diclofenac metabolites in analyzed samples was very changeable. 5OH-diclofenac was not detected in samples in September. In June it was found in wastewater (both influent and effluent) and in reject water taken after one hour of sludge feeding into the bed. Only in November this metabolite was detected in all analyzed samples. The concentration in the liquid phase of SAS and reject water was on a similar level, but the highest concentration was just after the feeding of the STRB. However, the load of 5OH-diclofenac in reject water in comparison to the liquid phase of sewage sludge was much lower. 4OH-diclofenac was detected both in influent and in effluent samples, and the lower concentrations were found in effluent samples. Its presence in sewage sludge and reject water was different between seasons of samplings, without a straight trend of distribution. In June it was present in the liquid phase of SAS, but it was not detected in reject water. This season is connected with a high activity of organisms in the beds; therefore, they have potential to support elimination of pharmaceuticals and metabolites. In September its concentration in the liquid phase of SAS was much lower than in reject water and in November its concentration was insignificantly higher in the liquid phase of sewage sludge just after feeding of the STRB (after one hour 4OH-diclofenac was not detected).

The variations of pharmaceuticals' and metabolites' presence and concentrations in the tested system are not easy to explain currently. The distribution of diclofenac and its metabolites was already tested in conventional WWTPs (Stülten et al., 2008). In natural water, seven metabolites of diclofenac were already identified (Lonappan et al., 2016). There are examples that the concentrations of native

compounds in raw wastewater are lower compared to treated wastewater (Lindqvist et al., 2005) because of the process of pharmaceutical release from conjugates by bacteria enzymes. What is more, the several new compound / degradation products can occur in the water from photodegradation and biodegradation in aerobic and anaerobic conditions; in the case of diclofenac seven products were determined (Poirier-Larabie et al., 2016).

The concentrations of target pharmaceuticals were found to be relatively similar to those found in other WWTPs around the world. For example, in Germany the max (medium) concentration of diclofenac, 4OH-diclofenac and 5OH-diclofenac were 5.1 (2.2) µg/L, 1.7 (0.42) µg/L and 0.86 (0.26) µg/L, respectively (Stülten et al., 2008). After 1-year monitoring of pharmaceuticals in Spain, the following ranges of pharmaceuticals were found: diclofenac < LOD - 0.24 µg/L, naproxen 2.02 - 8.50 µg/L and 0.54 - 5.09 µg/L, ibuprofen 3.73 - 353 µg/L and <LOD - 26.5 µg/L, respectively for raw and treated wastewater (Santos et al., 2009). Similar to our study, the several µg/L of diclofenac, ibuprofen and naproxen were found in influents in one of the WWTP in Finland (Lindqvist et al., 2005). In our previous study in another WWTP in Poland ("Wschód", Gdańsk, 2013), we found diclofenac in concentrations of 2.061-2.092 µg/L and 0.155-0.635 µg/L, naproxen 3.489-7.040 and 0.152-2.512 µg/L in influents and effluents, respectively, while the ibuprofen was found only in influents at a high concentration of 6.722 µg/L (Caban et al., 2014). It must be added that concentration of non-steroidal anti-inflammatory drugs and detection frequencies are one of the highest in wastewater samples (Jelic et al., 2011) because of common use of analgesics and anti-inflammatories throughout the year and their availability without prescription.

3.3. Potential of pharmaceutical removal

It was determined that WWTP technology in Gniewino was very effective in removal of ibuprofen, paracetamol and naproxen (Table 6). These pharmaceuticals were removed completely or very efficiently, although their concentrations in influent mostly were very high. The almost total removal of paracetamol and high removal of ibuprofen were presented in the review of Tarpani and Azapagic (2018) and others (Nakada et al. (2006); Bendz et al. (2005); Yu et al. (2006)). Similar high efficiency of naproxen and ibuprofen removal was established in the research from Finland (Lindqvist et al., 2005). In others research naproxen removal varied from 43.3 to 98.6 (Luo et al., 2014).

The most problematic to eliminate were diclofenac and it metabolites. The concentration of diclofenac in June and September was higher in effluent than in influent. Only in November diclofenac was removed, but with low efficiency (40.6%) (Table 6). Diclofenac metabolites were removed better than diclofenac (from 58.6 to 78.6 %). The literature data of diclofenac removal in WWTP are variable, from 22 % to 93 % (Lonappan et al., 2016; Bendz et.al., 2005, Kasprzyk-Hordern et al., 2009), but most of them do not include the metabolites' presence in tested wastewaters. What is more, several reports deal with a problem of higher concentration of diclofenac in treated wastewater (and lower than 0% removal efficiency, reported also for ibuprofen (Tarpani and Azapagic, 2018)). The problem of diclofenac removal was also observed in CWs (Matamoros et al., 2009; Vymazal et al., 2017).

Table 6 also presents estimated removal efficiency of target chemicals by STRBs. In a few cases there was no possibility to calculate the removal potential, because the compound was not found in inflow water. In other cases, the removal potential was higher than 94 % (mostly higher than 99 %), which looked very successful compared to the CWs technology. For example, in a review of pharmaceuticals removal potential of CWs, the diclofenac was removed in the amount of 0-78 % depending on the CWs' types (Li et al., 2014). The mentioned removal did not mean that pharmaceuticals were totally eliminated. These compounds could be "trapped and stored" in beds (by sorption process) or transformed into other, not tested or not currently known products. The transformation / degradation of diclofenac in natural conditions is already known. There is evidence of transformation of diclofenac in soil within a few days (Dodgen et al., 2014).The sorption of diclofenac in the organic phase of the beds is highly probable because this chemical has a logP = 4.51. What is more, the high affinity of diclofenac for the organic phase of soil in CWs has been proven (Matamoros and Bayona, 2006). The uptake of pharmaceuticals by reeds and subsequent biotransformation is also a pathway for the removal of diclofenac in STRB because the reeds have already proven to have potential for such

remediation (Podlipná et al., 2013) and diclofenac is able to be absorbed by plants (Wu et al., 2015). Nevertheless, during the cold season without reed vegetation, the adsorption is the main mechanism for the elimination of diclofenac by the STRB. To know exactly what happens to pharmaceuticals in sludge stored in reed beds further research is needed. Determination of pharmaceuticals should be done in the solid phase of sludge as well as in different parts of the reed.

Table 6. The quantity of pharmaceutical removal by Gniewino WWTP and STRB technology applied (NA – not applicable)

Analyte	Removal potential of WWTP [%]				Removal potential of STBR [%]			
	12.06.2017	05.09.2017	16.11.2017	Medium	12.06.2017	05.09.2017	16.11.2017	Medium
Ibuprofen	100.0	100.0	100.0	100.0	n.d.	n.d.	n.d.	n.d.
Paracetamol	100.0	100.0	100.0	100.0	n.d.	n.d.	n.d.	n.d.
Flurbiprofen	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Naproxen	100.0	100.0	99.5	99.8	n.d.	n.d.	n.d.	n.d.
Diclofenac	-150.1	-15.9	40.6	NA	99.3	95.9	99.5	98.2
5OH-diclofenac	93.2	n.d.	64.1	78.6	n.d.	n.d.	99.1	99.1
4OH-diclofenac	43.7	67.4	64.7	58.6	99.5	94.1	99.9	97.8

n.d. – not detected

As it was mentioned ibuprofen and paracetamol was completely removed from wastewater. Naproxen in effluent was found only in one sample and flurbiprofen was not detected in any sample. For this reason, it is not possible to calculate the correlation coefficient between these pharmaceuticals and the basic parameters removal rate. Table 7 presents the correlation coefficient for diclofenac and its metabolites.

Table 7. The correlation coefficient between pharmaceuticals and basic parameters removal in wastewater from Gniewino WWTP

	SS mg/L	BOD_5 mgO_2/L	COD mgO_2/L	$N\text{-}NO_3^-$ mg/L	$N\text{-}NO_2^-$ mg/L	$N\text{-}NH_4^+$ mg/L	TN mgN/L	$P\text{-}PO_4^{3-}$ mg/L	TP mgP/L
Diclofenac	-0.24	-0.55	0.87	-1.00	0.27	-0.10	-0.87	1.00	0.96
5OH-diclofenac	-0.71	1.00	-0.03	0.56	0.69	-0.80	0.87	-0.49	-0.73
4OH-diclofenac	0.15	-0.83	0.62	-0.94	-0.12	0.29	-0.99	0.99	0.99

Based on obtained results and calculation, a strong negative correlation between diclofenac and total nitrogen and nitrate nitrogen removal was found. Additionally, there is a strong positive correlation between phosphorus and COD removal with this pharmaceutical removal in the tested WWTP. The research of Thiebault et al. (2017) showed that diclofenac removal was not strongly correlated to any of basic parameters. The strongest correlation was found for $N\text{-}NO_3^-$ (value of coefficient was only 0.53) and the weakest correlation for total phosphorus (value of coefficient was 0.02). Differences in the correlation coefficient values may indicate different operating conditions of the wastewater treatment plants. The similar values of coefficients were obtained for diclofenac and 4-OH-diclofenac, what suggest that the removal scheme for these two compounds is similar. 5OH-diclofenac has a very strong positive correlation to BOD. The information about correlation coefficients may be helpful to optimize the technology in order to remove pharmaceuticals more efficiently. Still, the obtained here values suggest that the removal schemes of diclofenac and its metabolites are different. The presented coefficients need a further clarification by the extended research.

4. CONCLUSIONS

Basing on the performed research the following main results can be formulated:

- Basic pollutants in wastewater in Gniewino WWTP were removed very efficiently and the effluent met requirements of the Polish standards.
- There was no scheme of spatial and seasonal distribution of target analyte in the tested WWTP.
- Ibuprofen was found in the highest concentration among analyzed pharmaceuticals; however technological processes of WWTP completely removed the native form of this pharmaceutical from wastewater.
- Flurbiprofen was not detected in any analyzed samples.
- The presence of naproxen in wastewater was highly connected with the time of the year associated with flu season. Similarly to ibuprofen, naproxen was absent in effluents.
- Diclofenac and it metabolites were the pharmaceuticals with the lowest removal potential in WWTP. It was also found in the liquid phase of SAS as well as in reject water. However, removal potential of STRB from liquid phase of SAS was higher than 94 % (in most cases even higher than 99 %), independent of the sampling period.
- Removal of diclofenac from liquid phase of SAS in STRB did not mean that the pharmaceuticals were totally eliminated. These compounds could be "trapped and stored" in beds (by sorption process) or transformed into another form - not recognized so far.

There is a very strong potential that the pharmaceuticals are stored in the sludge. For better understanding and recognition of processes of pharmaceutical removal in STRB further research on the solid phase of sludge as well as different parts of the reed is needed. It is essential to analyze the distribution of pharmaceutical metabolites in the WWTP because it is often observed that the concentration of the native form of pharmaceuticals is higher in the treated wastewater than in raw wastewater (this and previous mentioned studies). The spatial distribution of target chemicals in the tested WWTP+STRB system varies according to the seasons. Further research will be ongoing to determine the mechanism of removal of diclofenac and its metabolites in STRB because this technology possesses valuable properties.

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