

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 $\mu\text{g/l}$ 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 removed in CWs (Matamoros et al., 2009). Although efficiency of pharmaceuticals removal in CWs is rather well known, 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

98 WWTPs (for a few persons) to big WWTP (for example Kolding STRB for 125,000 pe - personal
99 equivalent), but mostly they are useful for medium-size WWTPs (Nielsen, 2003).
100 STRB technology is based on the same processes that occur in natural wetland ecosystems. STRBs are
101 built as concrete constructions or as tight tanks placed in the ground. The whole system is divided into
102 several beds planted with reeds. The long-term experiences indicate that in medium or big WWTPs the
103 number of beds should be at least eight. STRB technology consists of periodical loading of sludge with
104 low content of dry matter (0.5-1.5%) (Kołecka and Obarska-Pempkowiak, 2008; Nielsen, 2003). The
105 time of loading typically takes about 3 - 7 days. After discharging of sludge onto a bed, time for its
106 dewatering (so-called resting time) is needed, therefore sludge should be loaded onto another bed.
107 The resting time is about 21-49 days (Brix, 2017). The sludge is stored in system for about 10-15 years.
108 After this time it is removed from the system and can be used as fertilizer (Kołecka and Obarska-
109 Pempkowiak, 2013; Nielsen, 2011).
110 STRBs are especially useful in rural areas and housing estates where economic considerations limit the
111 use of expensive mechanical equipment. These systems can be established in any area and are simple
112 to build and operate. Their low energy consumption is their main advantage. Additionally, they do not
113 require addition of chemicals for improvement of dewatering capability (Kołecka et al., 2017).
114 Research shows that sludge dewatering efficiency in reed systems is comparable to that of mechanical
115 equipment such as a filter press (content of dry matter can even reach up to 40%). It has also been
116 proven that sludge after long-term treatment in STRBs is stabilized and has a chemical composition
117 similar to that of humus. Additionally, it was proven that the obtained product is safe with regard to
118 its microbiological characteristics (Nielsen, 2007). Unlike most other conventional methods, reject
119 water from STRBs released from the sludge during dewatering is treated as it percolates through the
120 bed (Brix, 2017; Nielsen, 2007).
121 The secondary function of STRBs could be the removal of hazardous pollutants, for example
122 pharmaceuticals, which are classified as new emerging pollutants with a global awareness statute
123 (Gavrilescu et al., 2014). It has been proposed that systems containing plants and soil can participate
124 in elimination of pharmaceuticals and their metabolites.
125 The aim of this study was to recognize the spatial distribution and seasonal changes of selected
126 pharmaceutical in conventional WWTP with STRB technology. The distribution as well as removal
127 potential were analyzed and discussed in the wastewater treatment part of WWTP as well as the
128 sludge processing part in STRB.

129

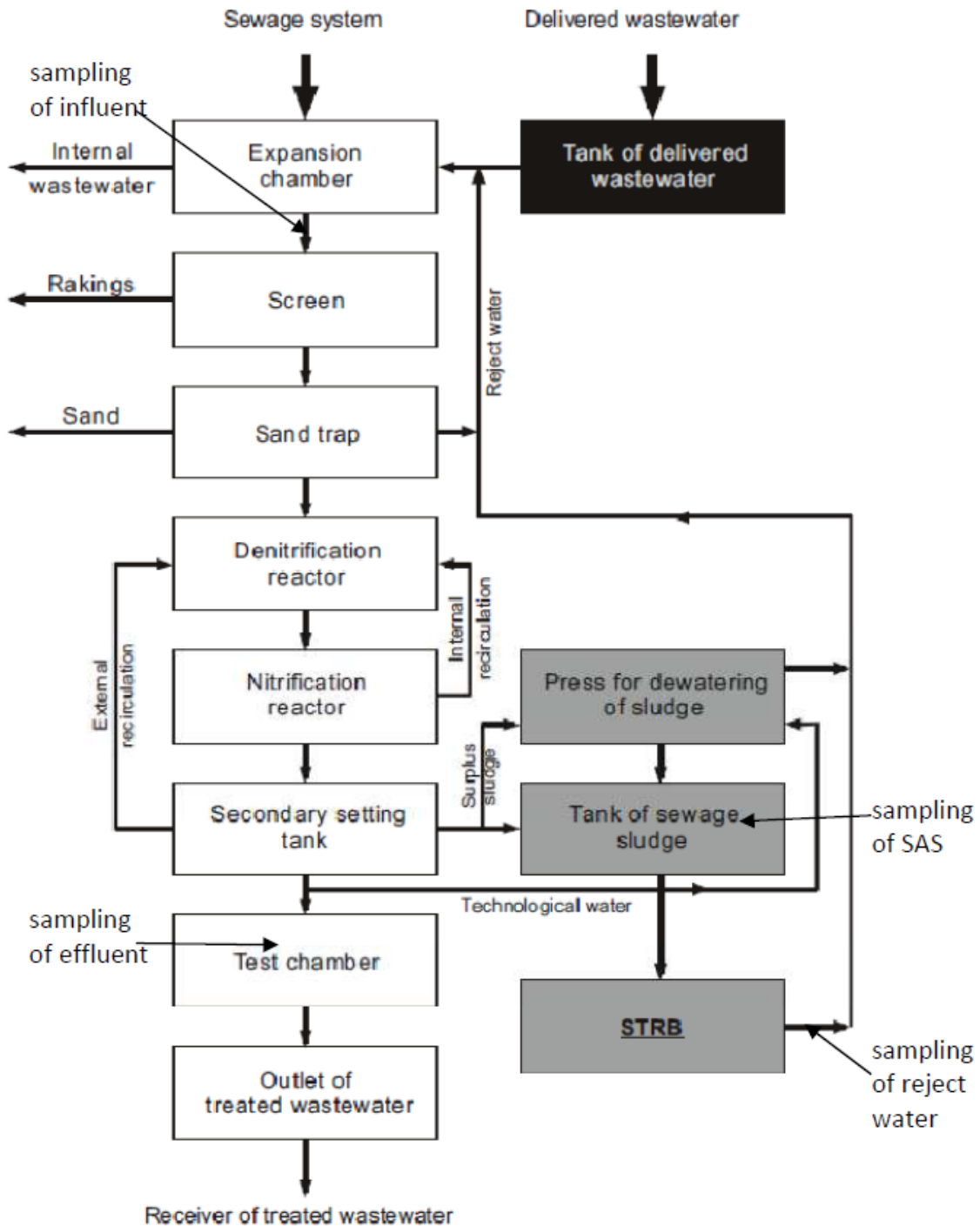
130 2. MATERIALS AND METHODS

131

132 2.1 Object of investigation

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





138
139 **Figure 1. The technological scheme of Gniewino WWTP with sampling points (Kotecka et al., 2017)**
140

141 The STRB was built in 2012. Its total area is 2400 m² and it consists of six beds. The time required to
142 feed sludge to one bed is about 1 day. About 35% of wastewater supplied to the WWTP comes from
143 the dairy and food industry and 17% of wastewater is delivered from septic tanks.
144

145 **2.2 Chemicals**

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

149 hydroxydiclofenac (5OH-diclofenac) (MW: 312.15, CAS:69002-84-2), 4'-hydroxydiclofenac (4OH-
150 diclofenac) (MW: 312.15, CAS: 64118-84-9). Ibuprofen, flurbiprofen, naproxen and diclofenac belong
151 to the non-steroidal anti-inflammatory drugs (NSAIDS). 4'-hydroxydiclofenac and 5-hydroxydiclofenac
152 are primary phase I metabolites of diclofenac (Bort et al., 1999). Two internal standards were used -
153 diclofenac-(acetophenyl ring-¹³C₆) (Internal standard I, MW: 405.16, CAS: 1261393-73-0) and 4'-
154 hydroxydiclofenac-¹³C₆(Internal standard II, MW: 318.01,CAS: 1189656-64-1). All mentioned chemicals
155 were purchased from Sigma-Aldrich. BSTFA+1% TMCS (N,O-Bis(trimethylsilyl)trifluoroacetamide + 1%
156 trimethylchlorosilane) was purchased from Synthese Nord GmbH (Germany). Pyridine was purchased
157 from Sigma-Aldrich. Other organic solvents (HPLC grade purity) were purchased from POCH (Polskie
158 Odczynniki Chemiczne, Poland).

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

164

165 **2.3 Sampling**

166 Measurements were carried out in samples of influent and effluent from the WWTP and in the liquid
167 phase of surplus activated sludge (SAS) as well as reject water from the STRB. The samples were
168 collected every 2 hours during a day (from 7 a.m. until 4 a.m. the next day). Next, the samples were
169 averaged. The samples of influent were collected before the activated sludge reactor and effluent was
170 collected after the secondary settling tank in the test chamber using automatic, specialist equipment.
171 SAS was collected from the tank for sewage sludge and reject water was collected from the well
172 downstream from the STRB using a dredge. The reject water was always collected from the same bed
173 which was fed just before sampling. Between sampling the bed was resting and new sludge was not
174 discharged. Two average samples of reject water were taken. The first sample was taken in the first
175 hour of reject water outflow and the second one during the course of a day in one hour intervals.
176 The samples were taken in 1L plastic bottles and taken immediately to the laboratory without special
177 preservation.

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

179

180 **2.4 Chemical analysis and calculations**

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

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

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

205 The instrumental validation was performed using working calibration standard solutions (0.0001 – 5
 206 µg/mL) and matrix-matched solutions for recovery calculation. The method detection limits (MDL) and
 207 method quantification limits (MQLs) were calculated using equations presented in Migowska et al.
 208 (2012). Recovery of both IS (I and II) was 102 %.

209
 210 **Table 1. Validation parameters of SPE-GC-MS(SIM) analysis of target pharmaceuticals in wastewater**
 211 **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

212 Additionally, the basic parameters, that is suspended solids (SS), COD, BOD₅, total nitrogen (TN),
 213 ammonia nitrogen (N-NH₄⁺), nitrate nitrogen (N-NO₃⁻), nitrite nitrogen (N-NO₂⁻), total phosphorus (TP)
 214 and orthophosphorus (PO₄³⁻) were determined. All determinations were carried out according to Polish
 215 Standards (PN-ISO 15705:2005, PN-EN 1899-1:2002; PN-ISO 5664:2002, PN-EN ISO 10304-1:2009,
 216 +AC:2012, PN-82/C-04576/08, PN-73/C-04576.14, PN-EN ISO 10304-1:2009 +AC:2012, PN-EN ISO
 217 6878:2006 +Ap1:2010 p. 4 +Ap2:2010) and hints from the American Public Health Association (APHA,
 218 2005).

219 The loads of pollutants and pharmaceuticals were calculated taking into account wastewater and
 220 reject water flows as well as the efficiency of the pump feeding the STRB. The quantity of
 221 pharmaceuticals was estimated using the time of bed feeding as well as the outflow of reject water.
 222 Correlations between concentrations of pharmaceuticals and basic parameters were calculated using
 223 Pearson's coefficient (correlation coefficient). Correlation coefficient formulas are used to find how
 224 strong a relationship is between data. The formulas return a value between -1 and 1. 1 indicates a
 225 strong positive relationship, -1 a strong negative relationship and 0 no relationship at all.

227 228 229 **3. RESULTS AND DISCUSSION**

230 231 **3.1 Basic parameters**

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

234
 235 **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-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-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-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-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

236
237

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-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-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-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-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

238

239 In comparison to regular and similar WWTPs in other regions of Poland and Europe, the WWTP in
240 Gniewino received a higher concentration of pollutants in raw wastewater (up to 1200 mg O₂/ L of
241 COD and up to 140 mg TN /L). This is caused by the high share of dairy and fish industry wastewater in
242 the catchment of Gniewino WWTP. For all basic parameters, which include SS, BOD₅, COD, TN and TP,
243 the efficiency of pollutants removal significantly exceeded 90% and final effluent met the requirements
244 of the Polish standards. The previous research confirms the high efficiency of pollutants removal
245 (Kołęcka et al., 2017). In wastewater, the nitrogen occurred in the form of ammonium. In influent,
246 phosphorus was mostly as orthophosphate. In effluent, the share of orthophosphate in total
247 phosphorus was much lower.

248 It is estimated that the liquid phase of SAS accumulated the biggest load of suspended solids (average
249 209.7 ± 28.37 kg/h) (Table 3). Average content of dry matter in SAS was about 1 %, which is why the
250 suspended solid (SS) content in the liquid phase was very high. During further processing, the majority
251 of this load is retained in the STRB as organic matter and the load of SS in reject water was even lower
252 than in effluent.

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

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

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

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

268

269 3.2 Pharmaceutical distribution

270 Tables 4 and 5 present the concentrations (µg/L) and loads (mg/h) of selected pharmaceuticals in
271 wastewater, the liquid phase of SAS as well as reject water from the STRB, respectively. Loads were
272 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

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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

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

294 Paracetamol was detected only in the samples of influent in June and November 2017. This
295 pharmaceutical's presence in wastewater is connected with autumn, the season of flu and cold.
296 Therefore, in September its concentration was high ($28.630 \pm 12.46 \mu\text{g/L}$). Similar to ibuprofen, this
297 pharmaceutical was removed, and it was not detected in effluents in native form.

298 Flurbiprofen was not detected in any analyzed samples.

299 The concentrations of naproxen in influent were from 5.498 ± 0.293 to $22.247 \pm 5.668 \mu\text{g/L}$. Only in
300 November it was detected in effluent, however its concentration was only $0.028 \pm 0.005 \mu\text{g/L}$.
301 Naproxen was found to be removed in anaerobic conditions of activated sludge (Lahti and Oikari,
302 2011).

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

309 There was no straight trend in diclofenac concentration in tested samples between type of matrix and
310 date of samplings. For example, concentrations of diclofenac increased in effluents compared to
311 influents taken in June and September. In these months, in reject water was also found that diclofenac
312 concentration was lower in the first hour of outflow compared to an average sample taken in the next
313 24-h period. In November, concentration of diclofenac was lower in effluent than in influent. In this
314 month, higher concentration in reject water was detected after feeding the STRB, and then it
315 decreased. Certainly, the variation of physico-chemical parameters of influent and changed efficiency
316 of activate sludge are reasons that between the seasons the efficiency of pharmaceuticals removal is
317 difficult to predict. In the case of STRBs the reed and micro-flora living on filters and deposits of sludge
318 have a lower potential to remove / transform / uptake the pharmaceuticals in the cold season.
319 Nevertheless, several processes affect elimination of hazardous substances in STRB systems (biotic
320 processes, e.g. microbiological degradation, biofilm, and plant uptake, and physico-chemical
321 processes, like photodegradation, oxidation, hydrolysis, retention / root sorption).

322 The other factor which we investigated here is the presence of the two most abundant diclofenac
323 metabolites and their concentration variability in the tested WWTP+STRBs system. The two
324 metabolites taken for research, have already been proven to be present in WWTPs samples with high
325 detection frequencies (Stülten et al., 2008). It was noticed that the presence and concentration of
326 diclofenac metabolites in analyzed samples was very changeable. 5OH-diclofenac was not detected in
327 samples in September. In June it was found in wastewater (both influent and effluent) and in reject
328 water taken after one hour of sludge feeding into the bed. Only in November this metabolite was
329 detected in all analyzed samples. The concentration in the liquid phase of SAS and reject water was on
330 a similar level, but the highest concentration was just after the feeding of the STRB. However, the load
331 of 5OH-diclofenac in reject water in comparison to the liquid phase of sewage sludge was much lower.
332 4OH-diclofenac was detected both in influent and in effluent samples, and the lower concentrations
333 were found in effluent samples. Its presence in sewage sludge and reject water was different between
334 seasons of samplings, without a straight trend of distribution. In June it was present in the liquid phase
335 of SAS, but it was not detected in reject water. This season is connected with a high activity of
336 organisms in the beds; therefore, they have potential to support elimination of pharmaceuticals and
337 metabolites. In September its concentration in the liquid phase of SAS was much lower than in reject
338 water and in November its concentration was insignificantly higher in the liquid phase of sewage sludge
339 just after feeding of the STRB (after one hour 4OH-diclofenac was not detected).

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

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

349 The concentrations of target pharmaceuticals were found to be relatively similar to those found in
350 other WWTPs around the world. For example, in Germany the max (medium) concentration of
351 diclofenac, 4OH-diclofenac and 5OH-diclofenac were 5.1 (2.2) µg/L, 1.7 (0.42) µg/L and 0.86 (0.26)
352 µg/L, respectively (Stülten et al., 2008). After 1-year monitoring of pharmaceuticals in Spain, the
353 following ranges of pharmaceuticals were found: diclofenac < LOD - 0.24 µg/L, naproxen 2.02 - 8.50
354 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
355 treated wastewater (Santos et al., 2009). Similar to our study, the several µg/L of diclofenac, ibuprofen
356 and naproxen were found in influents in one of the WWTP in Finland (Lindqvist et al., 2005). In our
357 previous study in another WWTP in Poland ("Wschód", Gdańsk, 2013), we found diclofenac in
358 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
359 in influents and effluents, respectively, while the ibuprofen was found only in influents at a high
360 concentration of 6.722 µg/L (Caban et al., 2014). It must be added that concentration of non-steroidal
361 anti-inflammatory drugs and detection frequencies are one of the highest in wastewater samples (Jelic
362 et al., 2011) because of common use of analgesics and anti-inflammatories throughout the year and
363 their availability without prescription.

364

365 **3.3. Potential of pharmaceutical removal**

366 It was determined that WWTP technology in Gniewino was very effective in removal of ibuprofen,
367 paracetamol and naproxen (Table 6). These pharmaceuticals were removed completely or very
368 efficiently, although their concentrations in influent mostly were very high. The almost total removal
369 of paracetamol and high removal of ibuprofen were presented in the review of Tarpani and Azapagic
370 (2018) and others (Nakada et al. (2006); Bendz et al. (2005); Yu et al. (2006)). Similar high efficiency of
371 naproxen and ibuprofen removal was established in the research from Finland (Lindqvist et al., 2005).
372 In others research naproxen removal varied from 43.3 to 98.6 (Luo et al., 2014).

373 The most problematic to eliminate were diclofenac and its metabolites. The concentration of diclofenac
374 in June and September was higher in effluent than in influent. Only in November diclofenac was
375 removed, but with low efficiency (40.6%) (Table 6). Diclofenac metabolites were removed better than
376 diclofenac (from 58.6 to 78.6 %). The literature data of diclofenac removal in WWTP are variable, from
377 22 % to 93 % (Lonappan et al., 2016; Bendz et al., 2005; Kasprzyk-Hordern et al., 2009), but most of
378 them do not include the metabolites' presence in tested wastewaters. What is more, several reports
379 deal with a problem of higher concentration of diclofenac in treated wastewater (and lower than 0%
380 removal efficiency, reported also for ibuprofen (Tarpani and Azapagic, 2018)). The problem of
381 diclofenac removal was also observed in CWs (Matamoros et al., 2009; Vymazal et al., 2017).

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

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

401

402 **Table 6. The quantity of pharmaceutical removal by Gniewino WWTP and STRB technology applied**
 403 **(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

404 n.d. – not detected

405

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

411

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

	SS mg/L	BOD ₅ mgO ₂ /L	COD mgO ₂ /L	N-NO ₃ ⁻ mg/L	N-NO ₂ ⁻ mg/L	N-NH ₄ ⁺ mg/L	TN mgN/L	P-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

414

415 Based on obtained results and calculation, a strong negative correlation between diclofenac and total
 416 nitrogen and nitrate nitrogen removal was found. Additionally, there is a strong positive correlation
 417 between phosphorus and COD removal with this pharmaceutical removal in the tested WWTP. The
 418 research of Thiebault et al. (2017) showed that diclofenac removal was not strongly correlated to any
 419 of basic parameters. The strongest correlation was found for N-NO₃⁻ (value of coefficient was only 0.53)
 420 and the weakest correlation for total phosphorus (value of coefficient was 0.02). Differences in the
 421 correlation coefficient values may indicate different operating conditions of the wastewater treatment
 422 plants. The similar values of coefficients were obtained for diclofenac and 4-OH-diclofenac, what
 423 suggest that the removal scheme for these two compounds is similar. 5OH-diclofenac has a very strong
 424 positive correlation to BOD. The information about correlation coefficients may be helpful to optimize
 425 the technology in order to remove pharmaceuticals more efficiently. Still, the obtained here values
 426 suggest that the removal schemes of diclofenac and its metabolites are different. The presented
 427 coefficients need a further clarification by the extended research.

428

429 4. CONCLUSIONS

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

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

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

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