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5	From the pills to environment – prediction and tracking of non-steroidal anti-
6	inflammatory drug concentrations in wastewater
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#### 26 Abstract

The extend of environment pollution by pharmaceuticals is in a stage that required more 27 28 automatic and integrated solutions. The non-steroidal anti-inflammatory drugs (NSAIDs) are 29 one of the most popular pharmaceutical in the world and emerging pollutants of natural waters. The aim of the paper was to check the correlation of the sales data of selected NSAIDs 30 31 (ibuprofen, naproxen, diclofenac) and their concentration in the WWTP in order to enable 32 predicting their loads, having only the sales data. For calculations, we apply three discharge 33 scenarios (the fates between purchased to the presence in influents), having in mind that some part of sold mass can be improperly dispose to sewage system. To support predictions, chemical 34 analysis were conducted in two conventional wastewater treatment plants (WWTPs) located in 35 36 Poland during 2018 and 2020, thereby before and during pandemic situation. The NSAIDs concentration in the influent was higher than that which would be obtained if all of the 37 administrated mass of the pharmaceutical went through the metabolic pathway of 38 transformation. This mean that substantial mass of sold NSAIDs in improperly dispose to 39 sewage system, and this factor need to be taken into account in future predictions. Furthermore, 40 41 results indicates that the variance of naproxen and diclofenac concentrations in the influent has no correlation with relatively stable sales throughout whole year. The pandemic situation had 42 43 yet no direct effect to diclofenac concentrations in influents, despite observed increasing of sales. It was calculated that more than 60 kg of diclofenac was discharged into the Baltic Sea 44 45 in 2018, and 20 kg in the first half of 2021 from two tested WWTPs. The presence of 4OHdiclofenac in effluents often in higher concentration compared to diclofenac mean that this still 46 biologically active compound need to be taken into account in future risk assessment. 47

**Keywords:** wastewater treatment; non-steroidal anti-inflammatory drugs (NSAIDs); diclofenac; metabolites; removal efficiency; prediction of concentration

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#### 52 1. Introduction

53 The pollution of the environment by pharmaceuticals and its metabolites is still emerging 54 problem, irrespective to the development of society (Caban and Stepnowski, 2021). Generally, 55 the trend of pharmaceuticals production and use is increasing, and greater access to medicines, intensification of meat production, an aging population and civilization diseases that require 56 57 daily treatment are some of the reasons. This situation has a direct effect on the quality of the 58 environment, while selected pharmaceuticals can be found in natural water in concentrations 59 higher than assessed risk level, especially in hot spots as wastewater discharge areas (Beek et al., 2016; Fekadu et al., 2019; Ślósarczyk et al., 2021). What makes problem more complex, 60 the current period of the SARS-CoV-2 epidemic is a factor that is changing the pharmaceuticals 61 62 sector by yet unknown manner (reduction or increasing of use of selected pharmaceuticals, not necessarily related to the treatment of covid complications). Non-steroidal anti-inflammatory 63 drugs (NSAIDs) are most popularly administrated active compounds, also for flu and cold 64 symptoms, what reflect its high detection frequency and concentrations in the environmental 65 waters (Hong et al., 2018; Jiang et al., 2014; Ślósarczyk et al., 2021). This was confirmed, for 66 example, by research conducted as part of the Morpheus project, where ibuprofen, naproxen 67 and paracetamol were characterised by the highest concentrations compared to the other 68 69 analysed pharmaceuticals (Morpheus project, 2019). This is a consequence of their popularity of administration, due to the fact that a significant proportion of them are sold without a 70 71 prescription (OTC, over-the-counter). What essential, NSAIDs are often purchasing in packs containing multiple tablets, which are consumed when needed and stored. That's why, some 72 unpredictable part of stored pharmaceuticals can expired and finally in improper way disposed 73 to toilets and sinks. 74

There are two main reasons for NSAIDs being present in domestic wastewater. Firstly, after administration, they are metabolised and some parts can be released in either their native form

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or in the form of easily hydrolysed conjugates. Secondly, unused and expired medicines (pills, 77 78 gels, syrups) often improperly end up going down the drain. According to national and EU law, 79 pharmaceuticals are dangerous wastes and should be collected separately. Unfortunately, there 80 is low societal awareness and systematic problems with returning programs. In the statistical survey performed in Poland in 2015, 68% of the respondents said they usually disposed of 81 82 expired pharmaceuticals in their household waste or sink/toilet (Rogowska et al., 2019). Similar 83 studies around the world confirm that unfortunately this problem is common (Caban and Stepnowski, 2021), but exact share of this pathway of pharmaceuticals to domestic wastewater 84 is unrealistic to estimated. Optimistically, the mass of pharmaceuticals returned to pharmacies 85 as waste is increasing in Poland (example of the Gdansk municipality in Figure S1, 86 87 Supplementary Materials). The similar situation was observed in other countries (Basir et al, 2020). 88

The technology in the majority of wastewater treatment plants (WWTPs) is not focussed on the 89 removal of pharmaceutically active compounds and their metabolites, and unfortunately a 90 significant load of NSAIDs enter the natural waters by this pathway. Previous works show that 91 92 NSAIDs can be found in WWTPs in concentrations up to a dozen µg/L in treated wastewater, and there was a low removal rate for diclofenac by standard active sludge-based technologies 93 94 (Caban et al., 2014; Kołecka et al., 2020, 2019). According to Tiwari et al. (2017) the removal rates of ibuprofen and naproxen are common ranges between 75% and 85% and 50-60%, 95 96 respectively. Diclofenac revealed low and varied removal rate ranging from 10 to 50%. 97 Furthermore, the tested NSAIDs have a 100% frequency of detection in the raw wastewater, so it can be stated that they are persistent in domestic wastewater. 98

The high quantity of sold pharmaceuticals then have a direct affect on the loads into the environment. The concentrations of pharmaceuticals released with the treated wastewater are small (de Oliveira et al., 2020), compared to nutrients, for example. However, the continuity of

the discharging contributes to the pseudo-persistence of the NSAIDs in the aquatic environment (Szymonik et al., 2017). The chronic impact of diclofenac on water organisms has also unfortunately been proven (Duarte et al., 2020). The problem of pharmaceuticals released into the environment was also noticed by HELCOM, which shows that there are large knowledge gaps in this field. Another issue is that more pharmaceuticals are to be included in the regional assessment of the state of the Baltic Sea, and respective indicators developed or advanced (BSR Water, 2021).

109 The increasing concern for the natural water quality required the monitoring data be supported by estimation of the loads in the environment. Comparison of sold masses of pharmaceutical 110 with obtained environmental concentration improve determination of pathways and fates, what 111 is essential for problem minimalization. This procedure can be problematic for NSAIDs, as they 112 can be purchase without prescription, and administrated with high dose and delay after 113 purchase, without medical control. Furthermore, most estimation studies of that kind used 114 excretion rate (release of unmetabolized form of pharmaceuticals) as correction factor, but omit 115 116 the fact that some part of purchased medicine is disposed to sinks and toilets, and seems that 117 with NSAIDs it is important factor. Thereby, this research aimed firstly to check the correlation of the sales data of selected NSAIDs (Poland, 2018 and 2021) and their detected concentrations 118 119 in the WWTP to enable further prediction of the loads having only the sales data with three 120 scenarios of excretion rates. The use of three scenarios aimed to check how share of sold mass 121 of pharmaceuticals improperly dispose to toilets affect obtained concentrations in raw 122 wastewaters. Furthermore, monitoring of diclofenac main metabolite was applied for supporting of scenarios reliability. Those two methodology modification distinguish this work 123 from other already published (presented in discussion). In this part of the study, the change of 124 the sales pattern of the investigated NSAIDs due to the SARS-CoV-2 epidemic was taken into 125 126 account. The second aim was to track the elimination in the WWTP and the actual load into the

environment. As Poland have significant share of discharge of pollution to Baltic Sea, two 127 128 WWTPs located in northern Poland were selected. Target WWTPs are differentiated in the 129 catchment and volumes of discharging wastewater to cross-check estimations. Both release the 130 effluent directly into the Baltic Sea. The NSAIDs selected for the research are those with the highest sales volumes in Poland - ibuprofen, diclofenac and naproxen. The study was supported 131 132 by sales data from PEX PharmaSequence, a consulting and research company that has focussed 133 on the pharmaceutical market and the healthcare sector in Poland for almost twenty years. The 134 obtained results could be helpful in managing the problem of pharmaceuticals by providing a method of calculating loads in a catchment area. 135

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#### 137 2.Materials and methods

#### 138 2.1. Characteristic of tested WWTPs and sampling of wastewater

The WWTP in Gniewino operates 15,000 PE (Population Equivalent). The average flow rate 139 of the wastewater was about 830,000 L/day in 2018 and 1,108,000 L/day in 2021 (presented in 140 Table S1). About 35% of the wastewater supplied to the WWTP comes from the dairy and fish 141 142 industries and 17% is delivered from septic tanks. The wastewater is treated by means of mechanical, biological and chemical removal processes of the organic matter and nutrients. The 143 144 mechanical part of the WWTP consists of a bar screen, grit chamber and skimming tank. The biological part is based on multiphase active sludge. It consists of two chambers: denitrification 145 146 and nitrification. Internal recirculation of activated sludge is carried out between the chambers. 147 A coagulant is dosed into the nitrification chamber to chemically precipitate phosphorus compounds with use of PIX coagulant. Sewage sludge is treated by an STRB (sludge treatment 148 reed bed) system, which is planted with Phragmites australis. 149

The WWTP located in Gdańsk is called "Wschód". It serves 807,000 PE. The average flow rate of the wastewater was about 92,000,000 L/day in 2018 and 97,106,350 L/day in 2020 (**Table** 

152 S1). It mainly treats municipal wastewater. Only 6.3 % of the wastewater is from the food, 153 shipbuilding and chemical industries. The Gdańsk WWTP uses mechanical and biological 154 processes to treat the wastewater. The mechanical part of the WWTP consists of four bar 155 screens, aerated grit chambers and three primary settling tanks. The biological part is based on multiphase active sludge using an A<sup>2</sup>/O system. Six bioreactors have been installed in the 156 157 WWTP with a total volume of 158,100 m<sup>3</sup>. Biological processes of organic matter 158 decomposition take place, and nitrogen and phosphorus compounds are removed in these 159 bioreactors. Each bioreactor consists of an anaerobic, anoxic and aerobic zone. There are two internal re-circulations in the biological part. Sewage sludge is treated in a biogas plant. The 160 fermented sludge is discharged to a mechanical dewatering station and dewatered in 161 162 sedimentation centrifuges. The dewatered sludge is finally burned in a fluidised bed furnace.

The influent and effluent were collected in April, June, September and December 2018, and in March and April 2021 as average 24-hour samples using an automatic flow-rate sampler in both tested WWTPs. The samples were taken in 1L plastic (polyethylene) bottles, and brought immediately to the laboratory without special preservation.

#### 167 2.2. Chemical measurement and statistics

The basic parameters, including the suspended solids (SS), COD, BOD<sub>5</sub>, total nitrogen (TN), ammonia nitrogen (N-NH<sub>4</sub><sup>+</sup>), nitrate nitrogen (N-NO<sub>3</sub><sup>-</sup>), nitrite nitrogen (N-NO<sub>2</sub>), total phosphorus (TP) and ortho-phosphorus (PO<sub>4</sub><sup>3-</sup>), 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 the advice of the APHA (2005).

The chemical analysis of ibuprofen, naproxen, diclofenac and diclofenac metabolite (4OHdiclofenac) was performed with the same validated protocol as in previous studies performed by Caban et al. (2016) and Kołecka et al. (2019, 2020). Briefly, the wastewater samples were adjusted to pH 3, filtered by glass filters, and extracted by solid phase extraction (SPE) techniques (Strata-X columns, elution by methanol). The dry extract was subjected to derivatisation by a silylation reagent, and the obtained sample analysed by a gas chromatograph coupled with a mass spectrometer (GC/MS). Selected ion monitoring (SIM) was used for quantitative analysis. The SPE-GC/MS(SIM) method validation parameters are presented in (Kołecka et al., 2019), whereby the limits of detections were above 2 ng/L.

The statistical analysis (box and whisker plot, correlation study by determination of Pearsoncoefficients) was performed in the Microsoft Excel software.

186 **2.3. Assumptions** 

187 2.3.1. Metabolism of target pharmaceuticals

Generally, NSAIDs are almost totally absorbed in the gastric system, and metabolised by dual 188 phases of metabolism (phases I and II) (Mulkiewicz et al., 2021). In phase I, the NSAIDs are 189 transformed to hydroxy or carboxy metabolites. Then in phase II, the conjugation (mostly with 190 glucuronide) is obtained. The elimination proceeds mostly by urine (to some extent by bile), in 191 192 which a mixture of the native pharmaceutical and its phase I, and phase II metabolites are obtained. Table 1 presents the share of the unchanged form of selected NSAIDs in urine, which 193 194 is not more than 12%. The conjugated form of the target pharmaceuticals is 9% and 51% for ibuprofen and naproxen, respectively. In the case of diclofenac, the databases report a 5% share 195 196 of conjugate. 4OH-diclofenac has been indicated as the main metabolite of diclofenac. For this 197 reason, it has been used as an indicator in the studies. More specific information of the profile of the metabolites can be found below. It needs to be highlighted that the presented data of the 198 metabolism of pharmaceuticals should be treated as average values, because the metabolism 199 varies between people and their condition, and the data presented in the literature are often not 200

201 comparable. Information about the biotransformation of diclofenac in the environment can be

found in the literature (Guzik et al., 2013; Mulkiewicz et al., 2021).

203 2.3.2. Scenarios of discharging of pharmaceuticals into wastewater

204 For scenarios we assumed that the concentration that can be found in the influent of a WWTP is a result of the excretion of the unchanged pharmaceutical, the potential hydrolysis of 205 206 conjugates, and disposal of unwanted medicines in the toilet and sink. What was omitted is the 207 fact that degradation by microbiome can be obtained in domestic wastewater, as well as the 208 sorption to solids during passage to the WWTP. For this factors we have no inputs data. Furthermore, the changeability of domestic wastewater composition, complex sewage system 209 and seasonal changes of wastewater parameters makes any predictions of these two processes 210 highly imprecise. With mentioned assumptions, three scenarios (S) were defined: 211

S1 – all of the sold mass of pharmaceuticals is released into the municipal wastewater without
being metabolised due to the improper disposal of expired / unwanted drugs and the washing
from the skin after the application of shower gels (an unrealistic, worst-case scenario).

S2 – only the excreted unchanged fraction of the administrated pharmaceuticals reach the
WWTP in influents (13%, 2% and 1% of the sold mass, respectively, for ibuprofen, naproxen
and diclofenac, as presented in Table 1, column I).

S3 – the concentration in influents is the result of the sum of the unchanged fraction and
deconjugation of conjugates during transport to the WWTP (22%, 53% and 6% of the sold
mass, respectively, for ibuprofen, naproxen and diclofenac, as presented in Table 1, column I
+ column II).

Scenarios S1 and S2 are the limit values for the worst-case and the best-case scenarios. Scenario 3 was taken into account because of the proven potential of conjugates to be deconjugate in wastewater and basic pH (Gomes et al., 2009; Vieno and Sillanpää, 2014). The scenarios were shown as correction factors ( $F_s$  – scenario factor) during the calculation of the loads inflowing to the WWTPs. For example, in the case of ibuprofen, for scenario factor 1, F<sub>S</sub> was equal 1, 0.13 and 0.22, respectively, for S1, S2 and S3. A limitating factor of these scenarios is the unknown behaviour of the target NSAIDs in the pathway between excretion from the patient to the WWTP. Such processes as adsorption, hydrolysis, and biodegradation can occur. However, the values of the concentrations found in S1, S2 and S3 may be helpful in investigating these processes during the transfer to the WWTPs.

# 232 2.4. Input data of sales for the modelling of pharmaceutical concentrations in WWTPs233 influent

NSAIDs have a significant share in the Polish pharmaceutical industry, which is one of the 234 235 fastest growing sectors in the country. In terms of market value, Poland is ranked in eighth 236 place in Europe, while in terms of pharmaceutical production, Poland ranks fifteenth place ("The Pharmaceutical Industry in Figures, Key Data", 2018). Compared to the rest of Europe, 237 the Polish pharmaceutical market is in second place in terms of the sales growth rate 238 (Dymaczewski and Jeż-Walkowiak, 2014), with a 4% annual growth rate. In Poland the 239 240 selected NSAIDs can be purchased outside of a pharmacy setting. Statistical data show that about 90% of Poles had pharmaceuticals in the house during the last 12-month period, and of 241 242 this, 68% were painkillers and anti-inflammatories (Public Opinion Research Center, 2016). Among NSAIDs, ibuprofen, naproxen and diclofenac are the most commonly used (Patel et al., 243 244 2019). Although data on drugs are taken from Poland, they point to a relatively big problem 245 that affects the whole world (Peña et al., 2021, Pharmaceutical Strategy for Europe, 2020). The 246 sales data of the selected NSAIDs was provided by the PEX PharmaSequence sp. z o.o. 247 company. They were developed on the basis of information from a nationwide representative panel of 6,000 pharmacies. In Poland in 2018, there were 14,300 pharmacies, therefore the 248 obtained data is precise. The data from PEX presents the number of sold packages as the number 249 250 defined daily doses (DDD, which exact values of can be found in

https://www.whocc.no/atc\_ddd\_index) sold during each month between 2018–2020 and the first six months of 2021. The WHO's definition of DDD is: "the assumed average maintenance dose per day for a drug used for its main indication in adults". The DDD numbers for the selected NSAIDs represent sales in the form of ointments / gels, tablets / capsules, and eye drops.

Figure 1 presents the number of DDD of ibuprofen, naproxen and diclofenac sold in pharmacies in Poland during each month of 2018, 2019, 2020 and the first six months of 2021. The yellow squares indicate each January, while the red circle represents the start of the first lockdown in Poland (March 2020) caused by the SARS-CoV-2 pandemic.

260 Based on these data, a few trends can be observed. Firstly, the sales of naproxen were relatively 261 stable over the last three years. This medicament is used to treat symptoms of rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis and acute musculoskeletal joint pain, 262 among other disorders. In the case of diclofenac, over the final ten months, an increase in sales 263 was observed. Diclofenac is used in the treatment of inflammation and pain of rheumatic and 264 non-rheumatic origin. Diseases of the musculoskeletal system affect 70% of the population over 265 266 the age of 50 years (Kołodziejska and Kołodziejczyk, 2018). The increase in the sales of diclofenac could not be only an effect of an increase in musculoskeletal system disorders, but 267 268 identification of this factor is out of scope of this study. In the case of both naproxen and diclofenac, a clear trend between the sales and the season was not found. 269

The sales of ibuprofen in pharmacies shows an increasing trend of sold DDD in the cold seasons before the March 2020, when the epidemic in Poland started. Unofficial information indicated that using ibuprofen may contribute to a worse course of the Covid-19 disease probably resulted in the observed very significant decrease in sales.

The data presented in **Figure 1** represent sales only in pharmacies. Public stores are responsible for 45% of painkiller sales in Poland (Association - Drugs only from pharmacies, 2018). In

relation to the most popular products in this category, based on the two most commonly used active substances – ibuprofen and paracetamol – the value of sales in the non-pharmacy channel accounts for over 47% on the Polish market. For the purpose of the further calculations, the number of DDD for ibuprofen was increased by the out-of-pharmacy sales. The most up-todate report (Gardocka-Jałowiec et al., 2020) shows that sales in the OTC sector also follow the trends shown in **Figure 1** – a significant decrease in March, followed by an increase in the following months.

#### 283 2.5. Mathematical model for prediction of NSAID concentrations in raw wastewater

To estimate the concentration of NSAIDs in the domestic wastewater in the two tested WWTPs,the following equation was used:

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$$C [\mu g/L] = \frac{PE \times (\frac{N_{DDD} \times M_{DDD} [\mu g]}{N}}{t [days] \times f[\frac{L}{day}]} \times F_S (equation 1)$$

288 Where:

289 C – estimated concentration of specific NSAIDs in raw wastewater [ $\mu g L^{-1}$ ],

290 PE – person equivalent for the target WWTP (for Gdańsk 806,815, for Gniewino 15,000),

291 N<sub>DDD</sub> – number of DDD sold per year in Poland (Figure 1, in the case of 2021, the number of

- 292 DDD sold in the first six months),
- 293 M<sub>DDD</sub> [µg] mass of DDD for specific NSAIDs (1,200 mg, 500 mg and 100 mg for ibuprofen,
- 294 naproxen and diclofenac, respectively, recalculated to µg),
- 295 N number of people in Poland (38,410,000 in 2018, 37,578,000 in 2021, data from the Central
- 296 Statistical Office in Poland),
- t [days] number of days of averaged time in our study per year (2018) or half year (2021),
  - f [L day<sup>-1</sup>] average flow in the given WWTP (presented in Table S1, Supplementary

Z komentarzem [KK1]: previous change

- 299 Materials) averaged over the entire year,
  - $F_s$  scenario factor, presented above in the text.

The upper part of equation can be understood as the  $\mu$ g of the specific NSAID for a single person in Poland per one day, and the lower part as the number of litres of wastewater per inhabitant per day in the target WWTP.

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305 3. Results and discussion

306 3.1. Concentration of target pharmaceuticals in influent and effluent

The concentrations of the target NSAIDs found in the influent (raw wastewater) and effluent (treated wastewater) during screening in the two tested WWTPs are presented as box-andwhisker plots in **Figure 2** and by the actual concentration in each sampling campaign in **Table S2** (Supplementary Materials).

Ibuprofen was found in each of the tested samples of influent within the range of 4.03–91.43  $\mu g/L$  (median 12.85  $\mu g/L$  for WWTP Gdansk and median 25.07  $\mu g/L$  for WWTP Gniewino) with high variance. In the effluents, its concentration was between <MDL (Method Detection Limit) and 0.48  $\mu g/L$ . A similar pattern was found for naproxen, but the median concentration in the influent was lower (median 3.17  $\mu g/L$  for WWTP Gdansk and median 5.41  $\mu g/L$  for WWTP Gniewino).

Diclofenac was found in each of the tested samples of influent and effluent. For three sampling 317 318 campaigns, the concentrations of diclofenac in the effluent were higher than those noted in the 319 influent. This fact may suggest that during treatment, diclofenac can be released from 320 conjugates. A similar situation was noted in our previous research in other WWTPs in Poland (Kołecka et al., 2019), and in studies performed in other countries (Oosterhuis et al., 2013; 321 322 Pedrouzo et al., 2011). Diclofenac was detected previously also in wastewater from residential 323 area, stormwater, sewage water from industrial areas, small and medium-sized enterprises 324 effluents, influent sewage water, effluent sewage water, and sewage sludge (Gercken et al., 325 2018).

Metabolite 4OH-diclofenac was found in 9 of 12 tested samples of influent and 11 of 12 tested 326 samples of effluent. For this compound, a high variance of the concentration was noted. In 5 327 sampling dates, a higher concentration of 4OH-diclofenac was noted in the effluent compared 328 to the influent (both WWTPs). The cause may be the release of 4OH-diclofenac from 329 conjugates. Another cause could be temporal storage in the sludge with release. Research 330 331 conducted by Szymonik et al. (2017) indicated that about 80% of diclofenac is absorbed in 332 sewage sludge, while for hydroxy metabolite there is no data. Another process that could be 333 responsible for the increase of the hydroxy metabolite of diclofenac in the effluent is its biotransformation by the bacteria community of the activated sludge. Such a degradation 334 pattern has been observed in biodegradation studies (Kosjek et al., 2009; Murshid and 335 336 Dhakshinamoorthy, 2019). On the other hand, very low biodegradation of diclofenac in the inoculum of sewage sludge was observed (Lee et al., 2012). According to Cherik et al. (2015), 337 the removal of diclofenac takes place mostly by adsorption from the surface of activated sludge, 338 and diclofenac can be toxic for this bacteria community. This may be why the efficiency of 339 340 diclofenac removal in activated sludge processes usually does not exceed 50% (Clara et al., 341 2005; Petrie et al., 2013; Tiwari et al., 2017). The studies indicate that the most effective process 342 in the removal of diclofenac is the adsorption process on activated carbon (Lee et al., 2012). 343 However, this method is not commonly used in WWTPs. Also the presence of diclofenac metabolites in the effluent has been noted in the literature (Osorio et al., 2014; Sathishkumar et 344 345 al., 2020; Stülten et al., 2008). 4OH-diclofenac produces a thirtieth of the activity of diclofenac 346 in the human body, therefore it seems logical that its presence in high concentrations in effluent poses a risk to the environment. In our study, we did not study of diclofenac glucuronide 347 metabolites. Its presence in wastewater is probable, but it decomposes in pH>6 (Vree et al., 348 1993); the pH of influent is approximately 8 thereby the diclofenac can be released by 349 350 hydrolysis even after excretion and passage to WWTP. The other transformation products of standards, whey are unable to quantify.
The variance in the concentration the pharmaceuticals in the influent has no correlation with
the sales data presented in Figure 1. For example, the sales of diclofenac are relatively stable
throughout the whole year, but its concentration in the wastewater differs substantially (Table
S2, Supplementary Materials). This is most probably an issue of changeable matrix of

diclofenac can also be found in wastewater (Jewell et al., 2016), but because a lack of analytical

357 wastewater and heterogeneous consumption of the diclofenac contained medicines.

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#### 359 3.2. Concentrations by prediction scenario

Based on the sales data, the estimated concentrations of ibuprofen, naproxen and diclofenac were calculated using equation (1). In **Figure 3**, the concentrations by scenarios S1, S2, S3 and the median concentrations (Cm) found in the samples of raw wastewater are presented, separately for 2018 and 2021.

The calculated concentrations of pharmaceuticals in scenario S1 for Gdańsk WWTP and Gniewino WWTP were the highest for each of the target NSAIDs, which is logical. The highest values were noted for ibuprofen, followed by naproxen and diclofenac for WWTP Gniewino in 2018.

368 A clear pattern in the relationship between the Cm and the predicted concentrations for the three scenarios is observed - the measured concentrations are between two extreme scenarios: S1 -369 370 with assumption that all purchased pharmaceuticals are released to domestic wastewater vs. S2 371 - only a small fraction of unmetabolised pharmaceuticals are released to domestic wastewater. 372 Of course, there is an order of difference between S1 and S2 values, while keeping in mind the 373 very low fraction released as unchanged molecules (Table 1). There were also large differences 374 between the median concentration of the targets NSAIDs between the years and the tested 375 WWTP, which means that the sufficient sampling frequency produces representative results.

This was also observed for the predicted concentrations. The averaged time was 12 (in case of 376 377 2018) or 6 month (is a case of 2021). In our opinion, the lower time of the averaged time can 378 be used, but only for pharmaceuticals that are known to be used soon after being purchased. In 379 the case of NSAIDs, houses frequently contain a stock of these medicines, and the pills are often used months after being purchased. Furthermore, the pills often end in the sink or toilet 380 381 after expiration, which could be roughly estimated as happening a year after being purchased. 382 With this in mind, the lack of a clear dependency of the lower ibuprofen concentration in 2021 383 compared to 2018 is noted, despite much lower sales DDD in the 2018 (Figure 1).

The concentration in S3 is close to the Cm in the case of ibuprofen, but much higher in the case of naproxen. S3 assumed that all of the released conjugates are hydrolysed in the wastewater. This process most probably occurs, but to an unknown extent. The fact that the value of Cm is between S1 and S3 is the result of multiple factors, and the accurate determination of Cm by any model is unrealistic. What is certain is that knowing the sales data and the basic operational data from the WWTP, we can determine the boundaries of the pharmaceutical concentrations in the raw wastewater.

391 Predicting the concentrations of NSAIDs using their sales data has been performed before in 392 some countries. For example, using a similar model to our S2 scenario, the predicted 393 concentrations of diclofenac and ibuprofen were 9.86 µg/L and 52.74 µg/L, respectively, in one WWTP in Switzerland (Lienert et al., 2007). The annual consumption of ibuprofen in 394 395 Switzerland in 2004 was 23.150 kg, while in 2018 in Poland it was 46.108 kg. At the same 396 time, the number of people in Switzerland in 2004 was 7,415,100, while in Poland in 2018 it 397 was about five times higher. This means, that sixteen year ago, the people in Switzerland consumed about 2.5 times more ibuprofen than currently in Poland. This is reflected in the 398 399 predicted concentrations, which were about three times higher in the referred study than those 400 presented in this study by scenario S2.

In 2013 in the Netherlands, two WWTPs were tested (Oosterhuis et al., 2013). In the first one, the predicted concentration of diclofenac in the effluents was 0.47  $\mu$ g/L, while the observed value was 0.34 (n=6). In the second WWTP, the predicted C was 0.43  $\mu$ g/L, and the observed one – 0.25  $\mu$ g/L. This means that C<sub>pred</sub> was 138% and 172% of C<sub>real</sub>, respectively, for the first and second WWTP. In our study, we were unable to predict the concentration in the effluents, because of the high variability in the removal efficiency.

407 In the report from Japan (He et al., 2020), Cpred and Creal were 0.188 µg/L and 0.135 µg/L, and 408  $0.077 \ \mu g/L$  and  $0.520 \ \mu g/L$  in the influents for diclofenac and ibuprofen, respectively. In the 409 mentioned work, it was stated that an accurate prediction was when  $0.5 < C_{pred} / C_{real} < 2$ , and an acceptable prediction was when  $0.1 < C_{pred} / C_{real} < 10$ . We applied this concept in our study. 410 The calculated ratio of C<sub>pred</sub> to C<sub>real</sub> is presented in Figure 4. In the cases of ibuprofen and 411 412 naproxen, most of the prediction scenarios give accurate values (ratios between 0.10 and 8.66). In the case of diclofenac, scenario 1 gives the highest overestimation, while scenario 2 gives 413 the lower ratios. In the case of this pharmaceutical, the sorption into sludge, mentioned above, 414 415 can lower the medium concentration and hinder the application of selected models. This 416 sorption could be applied in the prediction model, but the variability of the matrix in raw 417 wastewater makes this concept difficult.

Finally, it was confirmed that for diclofenac and other tested NSAIDs, the concentrations in the influent are higher than that which would be obtained if all of the administrated mass of pharmaceuticals goes through the metabolic pathway of transformation (scenario S1). In the case of diclofenac and naproxen, the accelerated load to the WWTPs is mostly connected with fact that these substances are used in gels and creams, from which a low topical administration rate was reported (Pradal et al., 2019).

Our study present relatively short chemical monitoring, and in the future will be continued. However, it can be considered as a "seed" investigation for creating a database. A large database

of many studies would allow the use of a machine learning in order to identify the greatest 426 threats related to the presence of pharmaceuticals in the environment (Ahmad et al., 2021, 427 428 Mahmood and Wang, 2021). Furthermore, after prediction of concentration in WWTP, the predicted environmental concentration (PEC) can be estimated, as it was performed previously 429 in several studies for river waters, knowing dilution factors (Cardini et al., 2021; Meyer et al., 430 431 2016). Comparison of PEC with predicted-no-effect-concentrations (PNECs, determined 432 experimentally using test organisms) is a basis of the simple risk assessment (Beek et al., 2016). 433 PNEC for diclofenac was determined to be 0.1 µg/L in surface water (European Commission, 2013). HELCOM propose an annual average Environmental Quality Standard (AA-EQS) in 434 marine waters of 0.005 µg/l (5 ng/L) for diclofenac (Helcom, 2018). 435

436

#### 437 **3.3. Potential of removal**

After entering into the WWTP, micropollutants are removed by processes of adsorption on 438 particulate matter, biodegradation and bio-transformation. The other physicochemical 439 440 processes, such as hydrolysis and UV-degradation, may be less important. The WWTPs are not designed for pharmaceutical removal, but co-elimination can be obtained. The potential for 441 442 removal (even between quite similar pharmaceuticals in the same group) can be diverse. NSAIDs are a good example. Diclofenac is resistant to bioremediation (Vieno and Sillanpää, 443 444 2014). Research conducted by Lonappan et al. (2016) indicated that the most effective method of diclofenac removal is adsorption on activated carbon followed by ozonation. Reverse 445 446 osmosis could be also used to remove diclofenac. However, this method is only used for small 447 amounts of wastewaters, e.g. from hospitals (Ali-Taleshi and Nejadkoorki, 2016).

Ibuprofen is biodegradable. For this reason, this pharmaceutical is removed very efficiently in wastewater treatment processes (Zembrzuska et al., 2019). The same is true of naproxen, which is removed under anaerobic conditions during activated sludge processes (Kołecka et al., 2019).

The removal efficiency of the target NSAIDs by the two tested WWTPs is presented in Figure 451 452 5 for each sampling campaign. Its value varies for each of the tested pharmaceuticals, but 453 generally the native forms of ibuprofen and naproxen were eliminated almost totally during the whole year in both WWTPs. The removal of diclofenac was between -242% and 98%. The 454 negative value means that a higher concentration was found in the effluent compared to the 455 456 influent. The reduction of 4OH-diclofenac was highly variable and differed between the 457 research facilities and the individual samples, similarly to diclofenac. The removal of diclofenac and its metabolite was not connected with the sampling period and the technical 458 systems of WWTPs. To present this more clearly, the RE (Removal Efficiency, %) of the basic 459 parameters was higher than 83% (except for N-NO<sub>2</sub> and N-NO<sub>3</sub>). In most of analysed samples, 460 the RE was higher than 95% (Table S3, Supplementary Materials). The removal of N-NO2 and 461 N-NO3 was very variable and ranged from -1511% (caused by an increase of the value in the 462 effluent compared to the influent) to 96%. This may indicate periodic problems with the 463 464 denitrification process.

There was no direct correlation (determined by Pearson coefficient) between the removal of the 465 nutrients, suspended solids and target pharmaceuticals. This correction was also different in the 466 analysed WWTPs (Table S4, Supplementary Materials). The correlation between the trends of 467 468 concentrations of the target NSAIDs and changes in the basic parameters is ambiguous. Research by Thiebault et al. (2017) showed that diclofenac removal was not strongly correlated 469 470 with any of the basic parameters. The lack of a direct correlation between the removal of carbon 471 and nitrogen, and the removal of NSAIDs was also found by Kołecka et al. (2020). On the other 472 hand, our previous study proved a strong negative correlation between diclofenac and total nitrogen and nitrate nitrogen removal, as well as a strong positive correlation between 473 phosphorus and COD removal with the removal of this pharmaceutical (Kołecka et al., 2019). 474 475 Similarly, a strong correlation of diclofenac removal to nitrogen removal was proven in one

WWTP in Turkey (Sari et al., 2014). Clearly, the presence of NSAIDs removal with basicparameters need further investigation, and its plant-dependent.

478

#### 479 3.4. Loads of NSAIDs to Baltic Sea

The calculations of loads of pharmaceuticals from single point source (such as WWTPs) can 480 481 gives meaningful argument to take an action of reduction by decision makers. This is crucial 482 for diclofenac, as this is one of the emerging pharmaceuticals in environmental monitoring with 483 proved presence in natural water in higher than risk level (Ślósarczyk et al., 2021; Szymczycha et al., 2020). Based on the Helcom report (HELCOM, 2018), it can be stated that the average 484 concentration of diclofenac in treated wastewater which ends up in the Baltic Sea is 1.41±1.10 485 486 µg/L. The calculation based on a meta-analysis by Sathishkumar et al. (2020), who reviewed more than 50 publications from the last 15 years, estimates that the global average diclofenac 487 concentration is  $2.28\pm3.43 \ \mu g/L$  in treated wastewater. In our study the median concentration 488 of diclofenac in effluents was 1.27 µg/L and 1.11 µg/L in Gdańsk and Gniewino wastewater, 489 respectively. This values are close the HELCOM predictions, and values for both tested 490 491 WWTPs are similar. We are unable to compare median concentration before and after pandemic period, because we have not sufficient number of monitoring data in 2021. It can be just say 492 493 that levels of concentration were similar. The increasing pattern of diclofenac sale (Figure 1) mean that median concentration both in influents and effluents have potential to increase. In 494 495 consequence, the treat to environment from a side of diclofenac is predicted to be increased. 496 This need investigation in future. Furthermore, the presence of diclofenac metabolite with 497 proven biological activity in concentrations close to the native pharmaceuticals need to be taken 498 into account in future risks assessments.

Based on the median concentrations of the target NSAIDs, the mass that was introduced with
urban wastewater and potentially discharged to the Baltic Sea during the whole of 2018 and
first six months of 2021 was calculated (**Table 2**).

It was calculated that 60 kg of diclofenac was discharged into the Baltic Sea during the whole of 2018, and 20 kg in the first half of 2021 from the WWTP Gdansk. In the first half of the year 2018, a similar amount was discharged as in the first half of 2021. For WWTP Gniewino, the equivalent values were much smaller and did not exceed 1 kg. What scare, that more metabolite of diclofenac compared to the native pharmaceutical was released from the WWTP Gdańsk. From the data presented in **Table 2**, it is easy to calculate the number of kg removed by a single WWTP.

509 For comparison, in the city of St. Petersburg in Russia (5,230,000 inhabitants), about 400 kg of diclofenac is introduced into the Baltic Sea annually (Helcom, 2018). The annual load of 510 diclofenac originating from the three largest cities of Estonia (Tallinn, Narva and Pärnu with a 511 total 5,440,000 inhabitants) is only about 30 kg per year (Lember et al., 2016) despite similar 512 number of people as in St. Petersburg. For WWTP Gdańsk person equivalent is estimated 513 514 806,815 and it release 60 kg of diclofenac per year, what mean that single "person" are responsible for release of 74 mg of diclofenac per year, in St. Petersburg adequate number is 515 516 76 mg, while in Estonia region only 5 mg per year. Unfortunately, for most countries, such numbers are unavailable. Nevertheless, there is potential for calculation of annual loads of 517 518 NSAIDs with WWTP effluents produced in urban area near coast of Baltic Sea, or even for 519 whole Baltic Sea catchment. The bottleneck is the availability of the data on NSAIDs sales. 520 Furthermore, the total release of NSAIDs to Baltic Sea need calculations of loads of NSAIDs with rivers ended in Baltic Sea and with underground and surface runoff. In our study we were 521 522 focused in liquid fraction of wastewater released to natural water, while solid faction in utilized 523 in different ways, depending of tested WWTPs, and not taken to calculations.

Comparison of the total introduced mass of ibuprofen (Table 2) with the whole sold mass of 524 525 this NSAID in Poland in 2018 shows that the citizens of Gdańsk consume 1.46% of the total sales mass of the ibuprofen purchased in Poland and 1.45% of diclofenac. The percentage of 526 527 the Gdańsk population is 1.53% of the total Polish population (2018). These values are comparable. On the other hand, the percentage of the use of naproxen is 0.40%. This may result 528 529 from regional differences in sales patterns. This estimation is also imperfect due to the fact that 530 Gdańsk is a tourist destination, and during the summer, the number of people significantly 531 increases. Diclofenac shows substantial variability of its concentration in wastewater, which could not be explained by the consumption pattern. 532

533

#### 534 4. Conclusion

Strategy to perform chemical analysis in influents and effluent of WWTPs in parallel with 535 observation of sale pattern during tested periods allow to obtain several findings. Our research 536 indicates that the changes of naproxen and diclofenac concentrations in the influent of both of 537 538 tested WWTPs has no correlation with relatively stable sales throughout whole tested period. It was noted that pandemic have effect into sale of ibuprofen. However, this had no effect on 539 its concentration in the wastewater. This was probably connected with fact that NSAIDs are 540 541 often stored and used whenever needed, not directly after being purchased. The developed scenarios of discharging of pharmaceuticals indicated that the quantity of pharmaceuticals sold 542 543 can be used to predict the concentrations of NSAIDs in the WWTPs' influents. Each of three 544 scenarios produces an acceptable prediction. The S1 and S2 scenarios give boundaries for 545 maximum and minimum observed concentrations. For the tested NSAIDs, the concentration in the influent was higher than that which would be obtained if all of the administrated mass of 546 547 pharmaceutical went through the metabolic pathway of transformation. This mean that part of 548 sold pharmaceuticals mass is directly introduced to sewage system, what is in agreement with

previous questionnaire of inhabitants. Furthermore, release from easily hydrolysed conjugates
is also probable (scenario S3). This also show that this factor need to be taken into account to
improve future predictions of concentrations knowing sales data.

Our research as well as literature data shows high variance of the removal of the selected 552 553 NSAIDs in WWTPs. In tested WWTPs the removal efficiency was not directly connected with 554 a reduction of the basic parameters. Additionally, it was calculated that a substantial mass of diclofenac (60 kg) was released from Gdańsk WWTP into the Baltic Sea during 2018 and the 555 556 first half of 2021 (19 kg). It is remarkable that during the same time, a higher mass of 4-hydroxy metabolite of diclofenac was released, and part of this mass was released during treatment by 557 558 activated sludge. Such observations make it reasonable to introduce this metabolite into the risk 559 assessments of pharmaceuticals.

560 Despite agreement of calculated concentration with reals median concentrations, the chemical 561 monitoring is still necessary. In a case of NSAIDs targeted in our study the patterns of sales and changes in found concentrations in WWTPs not overlap, what was probably effect of 562 administration of NSAIDs in delay after purchase, variation of matrix composition (difference 563 564 in accumulation on wastewater solid fraction and biodegradation - hypothetically), impossible 565 to estimate dose of medication discharged down the toilet. The improvement of study could be implementation of passive sampling techniques of extraction in wastewaters, thanks to which 566 average in time concentration can be obtained (Caban et al., 2021). What scared, that sales data 567 show increase in diclofenac purchasing, what at least in first half of 2021 have no reflect into 568 found concentration in two tested WWTPs, but as mentioned previously can be an problem in 569 570 future, because of delay in NSAIDs administration. The noted high concentration of 4OH-571 diclofenac mean that in future monitoring it need to be added as target, as it have thirtieth of the activity of diclofenac. This study apply simple calculation with three release scenarios. In 572 the future the database of found real concentrations and sale data should be created and a 13

574 machine learning can be an tool to makes such prediction more reliable and automatic, and

- 575 finally improve risk assessment.
- 576

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#### **1** Figures' captions

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Figure 1. Number of daily defined doses (DDD) of ibuprofen, naproxen, and diclofenac sold
in Poland during 2018, 2019, 2020 and the first six months of 2021. The yellow squares indicate
each January, while the red circle represents the start of the lockdown in Poland (March 2020)
caused by the SARS-CoV-2 pandemic.

Figure 2. Box and whisker plot presenting the distributions of the concentrations [μg/L] of
ibuprofen, naproxen, diclofenac and 4-hydroxy diclofenac (4OH-diclofenac) in six samples of
influent and effluent of the two tested WWTPs in Poland (location: Gdańsk and Gniewino,
2018 and 2021).

Figure 3. Concentrations [µg/L] of ibuprofen, naproxen and diclofenac in influent by three
prediction scenarios (S1, S2, S3) and real median concentration (C<sub>m</sub>) found in wastewater
treatment plants (WWTP) in Gdańsk and Gniewino (Poland, 2018 and 2021).

Figure 4. Ratio of values of predicted concentrations by the three prediction scenarios (S1, S2,
S3) to real median concentrations of selected NSAIDs (C<sub>m</sub>) for the two investigated wastewater
treatment plants (Gdańsk and Gniewino, both in Poland) between sampling years 2018 and
2021.

Figure 5. Removal efficiency of target NSAIDs in two tested WWTPs located in Gdansk and
Gniewino during each sampling performed for the purpose of this study between 2018 and 2021

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## Figure 2







### 3 Figure 4





## Figure 5

#### **Tables' titles**

**Table 1.** Approximated share of the eliminated fraction of ibuprofen, naproxen, diclofenac as a free and conjugated fraction in relation to an administered dose for diclofenac (Davies and Anderson, 1997a; Lienert et al., 2007; Verbeeck et al., 1983), and ibuprofen (Davies, 1998; Mazaleuskaya et al., 2015). For naproxen, public databases were used as other sources of data: pubchem.ncbi.nlm.nih.gov, drugbank.ca, Davies and Anderson (1997b), Vree et al. (1993).

**Table 2**. Calculated mass [kg] of ibuprofen, naproxen, diclofenac and 4-hydroxydiclofenac (4OHdiclofenac) found in influents and released with effluents from treatment plants (WWTP) located in Gdańsk and Gniewino (Poland) during the whole of 2018 and the first six months of 2021.

## Table 1

Compound	I. Share of unchanged compound	II. Share of conjugates of			
	excreted [%]	compound excreted [%]			
Ibuprofen	13 = 12 (urine) + 1 (feces)	9 (ibuprofen glucuronide)			
Naproxen	2 (feces)	51 (naproxen acyl glucuronide)			
Diclofenac	1 (urine)	approx. 5			

Table 2

Localization	WWTP Gdansk				WWTP Gniewino			
Wastewater	Influent		Effluent		Influent		Effluent	
Year	2018	2021	2018	2021	2018	2021	2018	2021
Ibuprofen	544.18	496.79	1.91	0.00	4.23	2.60	0.01	0.00
Naproxen	91.19	64.70	0.00	0.00	1.63	1.13	0.00	0.08
Diclofenac	179.86	17.09	59.97	19.34	0.63	0.08	0.44	0.35
4-OH diclofenac	40.73	27.62	107.92	20.37	0.42	0.19	0.21	0.27