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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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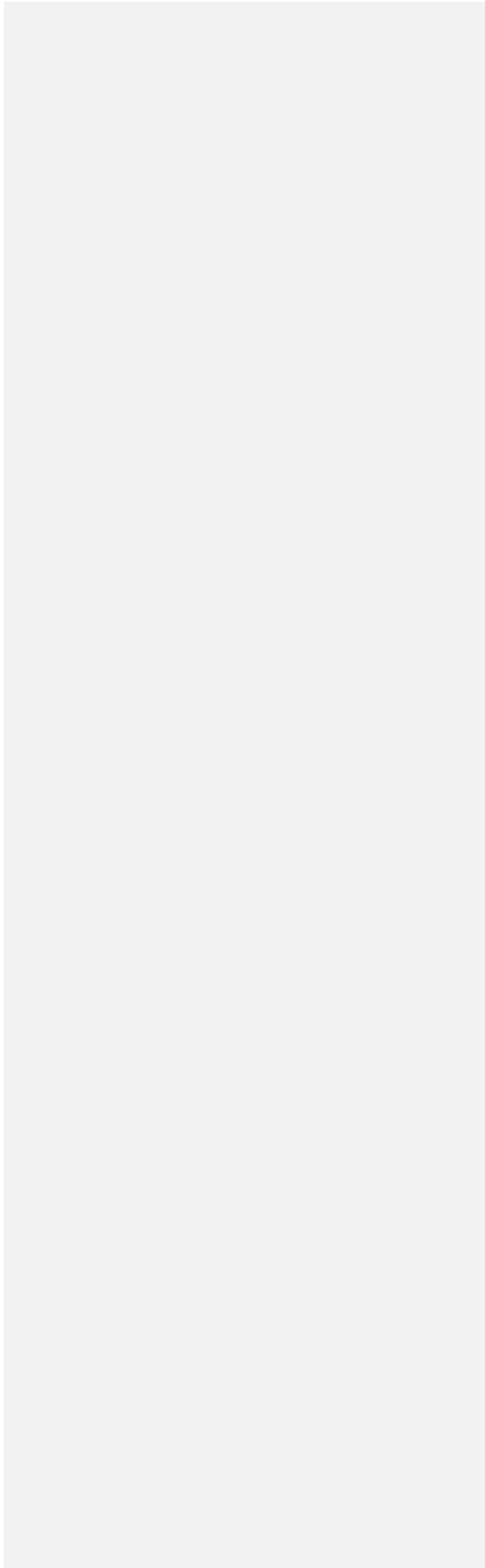
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26 **Abstract**

27 The extend of environment pollution by pharmaceuticals is in a stage that required more
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.
30 The aim of the paper was to check the correlation of the sales data of selected NSAIDs
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
34 part of sold mass can be improperly dispose to sewage system. To support predictions, chemical
35 analysis were conducted in two conventional wastewater treatment plants (WWTPs) located in
36 Poland during 2018 and 2020, thereby before and during pandemic situation. The NSAIDs
37 concentration in the influent was higher than that which would be obtained if all of the
38 administrated mass of the pharmaceutical went through the metabolic pathway of
39 transformation. This mean that substantial mass of sold NSAIDs in improperly dispose to
40 sewage system, and this factor need to be taken into account in future predictions. Furthermore,
41 results indicates that the variance of naproxen and diclofenac concentrations in the influent has
42 no correlation with relatively stable sales throughout whole year. The pandemic situation had
43 yet no direct effect to diclofenac concentrations in influents, despite observed increasing of
44 sales. It was calculated that more than 60 kg of diclofenac was discharged into the Baltic Sea
45 in 2018, and 20 kg in the first half of 2021 from two tested WWTPs. The presence of 4OH-
46 diclofenac in effluents often in higher concentration compared to diclofenac mean that this still
47 biologically active compound need to be taken into account in future risk assessment.

48
49 **Keywords:** wastewater treatment; non-steroidal anti-inflammatory drugs (NSAIDs);
50 diclofenac; metabolites; removal efficiency; prediction of concentration





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,
56 intensification of meat production, an aging population and civilization diseases that require
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
60 al., 2016; Fekadu et al., 2019; Ślósarczyk et al., 2021). What makes problem more complex,
61 the current period of the SARS-CoV-2 epidemic is a factor that is changing the pharmaceuticals
62 sector by yet unknown manner (reduction or increasing of use of selected pharmaceuticals, not
63 necessarily related to the treatment of covid complications). Non-steroidal anti-inflammatory
64 drugs (NSAIDs) are most popularly administrated active compounds, also for flu and cold
65 symptoms, what reflect its high detection frequency and concentrations in the environmental
66 waters (Hong et al., 2018; Jiang et al., 2014; Ślósarczyk et al., 2021). This was confirmed, for
67 example, by research conducted as part of the Morpheus project, where ibuprofen, naproxen
68 and paracetamol were characterised by the highest concentrations compared to the other
69 analysed pharmaceuticals (Morpheus project, 2019). This is a consequence of their popularity
70 of administration, due to the fact that a significant proportion of them are sold without a
71 prescription (OTC, over-the-counter). What essential, NSAIDs are often purchasing in packs
72 containing multiple tablets, which are consumed when needed and stored. That's why, some
73 unpredictable part of stored pharmaceuticals can expired and finally in improper way disposed
74 to toilets and sinks.

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



77 or in the form of easily hydrolysed conjugates. Secondly, unused and expired medicines (pills,
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
81 survey performed in Poland in 2015, 68% of the respondents said they usually disposed of
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
84 Stepnowski, 2021), but exact share of this pathway of pharmaceuticals to domestic wastewater
85 is unrealistic to estimated. Optimistically, the mass of pharmaceuticals returned to pharmacies
86 as waste is increasing in Poland (example of the Gdansk municipality in **Figure S1**,
87 Supplementary Materials). The similar situation was observed in other countries (Basir et al,
88 2020).

89 The technology in the majority of wastewater treatment plants (WWTPs) is not focussed on the
90 removal of pharmaceutically active compounds and their metabolites, and unfortunately a
91 significant load of NSAIDs enter the natural waters by this pathway. Previous works show that
92 NSAIDs can be found in WWTPs in concentrations up to a dozen $\mu\text{g/L}$ in treated wastewater,
93 and there was a low removal rate for diclofenac by standard active sludge-based technologies
94 (Caban et al., 2014; KołECKA et al., 2020, 2019). According to Tiwari et al. (2017) the removal
95 rates of ibuprofen and naproxen are common ranges between 75% and 85% and 50–60%,
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
98 it can be stated that they are persistent in domestic wastewater.

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



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

109 The increasing concern for the natural water quality required the monitoring data be supported
110 by estimation of the loads in the environment. Comparison of sold masses of pharmaceutical
111 with obtained environmental concentration improve determination of pathways and fates, what
112 is essential for problem minimalization. This procedure can be problematic for NSAIDs, as they
113 can be purchase without prescription, and administrated with high dose and delay after
114 purchase, without medical control. Furthermore, most estimation studies of that kind used
115 excretion rate (release of unmetabolized form of pharmaceuticals) as correction factor, but omit
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
118 of the sales data of selected NSAIDs (Poland, 2018 and 2021) and their detected concentrations
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
123 supporting of scenarios reliability. Those two methodology modification distinguish this work
124 from other already published (presented in discussion). In this part of the study, the change of
125 the sales pattern of the investigated NSAIDs due to the SARS-CoV-2 epidemic was taken into
126 account. The second aim was to track the elimination in the WWTP and the actual load into the



127 environment. As Poland have significant share of discharge of pollution to Baltic Sea, two
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
131 highest sales volumes in Poland – ibuprofen, diclofenac and naproxen. The study was supported
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
135 method of calculating loads in a catchment area.

136

137 **2. Materials and methods**

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

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

150 The WWTP located in Gdańsk is called “Wschód”. It serves 807,000 PE. The average flow rate
151 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
156 multiphase active sludge using an A²/O system. Six bioreactors have been installed in the
157 WWTP with a total volume of 158,100 m³. 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
160 internal re-circulations in the biological part. Sewage sludge is treated in a biogas plant. The
161 fermented sludge is discharged to a mechanical dewatering station and dewatered in
162 sedimentation centrifuges. The dewatered sludge is finally burned in a fluidised bed furnace.
163 The influent and effluent were collected in April, June, September and December 2018, and in
164 March and April 2021 as average 24-hour samples using an automatic flow-rate sampler in both
165 tested WWTPs. The samples were taken in 1L plastic (polyethylene) bottles, and brought
166 immediately to the laboratory without special preservation.

167 **2.2. Chemical measurement and statistics**

168 The basic parameters, including the suspended solids (SS), COD, BOD₅, total nitrogen (TN),
169 ammonia nitrogen (N-NH₄⁺), nitrate nitrogen (N-NO₃⁻), nitrite nitrogen (N-NO₂), total
170 phosphorus (TP) and ortho-phosphorus (PO₄³⁻), were determined. All determinations were
171 carried out according to Polish Standards (PN-ISO 15705:2005, PN-EN 1899-1:2002; PN-ISO
172 5664:2002, PN-EN ISO 10304-1:2009, +AC:2012, PN-82/C-04576/08, PN-73/C-04576.14,
173 PN-EN ISO 10304-1:2009 +AC:2012, PN-EN ISO 6878:2006 +Ap1:2010 p. 4 +Ap2:2010)
174 and the advice of the APHA (2005).

175 The chemical analysis of ibuprofen, naproxen, diclofenac and diclofenac metabolite (4OH-
176 diclofenac) was performed with the same validated protocol as in previous studies performed



177 by Caban et al. (2016) and Kolečka et al. (2019, 2020). Briefly, the wastewater samples were
178 adjusted to pH 3, filtered by glass filters, and extracted by solid phase extraction (SPE)
179 techniques (Strata-X columns, elution by methanol). The dry extract was subjected to
180 derivatisation by a silylation reagent, and the obtained sample analysed by a gas chromatograph
181 coupled with a mass spectrometer (GC/MS). Selected ion monitoring (SIM) was used for
182 quantitative analysis. The SPE-GC/MS(SIM) method validation parameters are presented in
183 (Kolečka et al., 2019), whereby the limits of detections were above 2 ng/L.

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

186 **2.3. Assumptions**

187 2.3.1. Metabolism of target pharmaceuticals

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



201 comparable. Information about the biotransformation of diclofenac in the environment can be
202 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
205 is a result of the excretion of the unchanged pharmaceutical, the potential hydrolysis of
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.

209 Furthermore, the changeability of domestic wastewater composition, complex sewage system
210 and seasonal changes of wastewater parameters makes any predictions of these two processes
211 highly imprecise. With mentioned assumptions, three scenarios (S) were defined:

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

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

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

222 Scenarios S1 and S2 are the limit values for the worst-case and the best-case scenarios. Scenario
223 3 was taken into account because of the proven potential of conjugates to be deconjugate in
224 wastewater and basic pH (Gomes et al., 2009; Vieno and Sillanpää, 2014). The scenarios were
225 shown as correction factors (F_S – scenario factor) during the calculation of the loads inflowing



226 to the WWTPs. For example, in the case of ibuprofen, for scenario factor 1, F_S was equal 1,
227 0.13 and 0.22, respectively, for S1, S2 and S3. A limiting factor of these scenarios is the
228 unknown behaviour of the target NSAIDs in the pathway between excretion from the patient to
229 the WWTP. Such processes as adsorption, hydrolysis, and biodegradation can occur. However,
230 the values of the concentrations found in S1, S2 and S3 may be helpful in investigating these
231 processes during the transfer to the WWTPs.

232 **2.4. Input data of sales for the modelling of pharmaceutical concentrations in WWTPs** 233 **influent**

234 NSAIDs have a significant share in the Polish pharmaceutical industry, which is one of the
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
237 (“The Pharmaceutical Industry in Figures, Key Data”, 2018). Compared to the rest of Europe,
238 the Polish pharmaceutical market is in second place in terms of the sales growth rate
239 (Dymaczewski and Jeż-Walkowiak, 2014), with a 4% annual growth rate. In Poland the
240 selected NSAIDs can be purchased outside of a pharmacy setting. Statistical data show that
241 about 90% of Poles had pharmaceuticals in the house during the last 12-month period, and of
242 this, 68% were painkillers and anti-inflammatories (Public Opinion Research Center, 2016).
243 Among NSAIDs, ibuprofen, naproxen and diclofenac are the most commonly used (Patel et al.,
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
248 panel of 6,000 pharmacies. In Poland in 2018, there were 14,300 pharmacies, therefore the
249 obtained data is precise. The data from PEX presents the number of sold packages as the number
250 of defined daily doses (DDD, which exact values can be found in



251 https://www.whocc.no/atc_ddd_index) sold during each month between 2018–2020 and the
252 first six months of 2021. The WHO’s definition of DDD is: “the assumed average maintenance
253 dose per day for a drug used for its main indication in adults”. The DDD numbers for the
254 selected NSAIDs represent sales in the form of ointments / gels, tablets / capsules, and eye
255 drops.

256 **Figure 1** presents the number of DDD of ibuprofen, naproxen and diclofenac sold in
257 pharmacies in Poland during each month of 2018, 2019, 2020 and the first six months of 2021.
258 The yellow squares indicate each January, while the red circle represents the start of the first
259 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
262 arthritis, juvenile rheumatoid arthritis, osteoarthritis and acute musculoskeletal joint pain,
263 among other disorders. In the case of diclofenac, over the final ten months, an increase in sales
264 was observed. Diclofenac is used in the treatment of inflammation and pain of rheumatic and
265 non-rheumatic origin. Diseases of the musculoskeletal system affect 70% of the population over
266 the age of 50 years (Kołodziejaska and Kołodziejczyk, 2018). The increase in the sales of
267 diclofenac could not be only an effect of an increase in musculoskeletal system disorders, but
268 identification of this factor is out of scope of this study. In the case of both naproxen and
269 diclofenac, a clear trend between the sales and the season was not found.

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

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



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

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

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

286

$$287 \quad C \text{ [}\mu\text{g/L]} = \frac{\text{PE} \times \left(\frac{N_{\text{DDD}} \times M_{\text{DDD}} \text{ [}\mu\text{g]}}{N} \right)}{t \text{ [days]} \times f \text{ [}\frac{\text{L}}{\text{day}}\text{]}} \times F_s \text{ (equation 1)}$$

288 Where:

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

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

291 N_{DDD} – 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_{DDD} [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),

297 t [days] – number of days of averaged time in our study per year (2018) or half year (2021),

298 f [L day^{-1}] – average flow in the given WWTP (presented in **Table S1**, Supplementary
299 Materials) averaged over the entire year,

300 F_s – scenario factor, presented above in the text.



301 The upper part of equation can be understood as the μg of the specific NSAID for a single
302 person in Poland per one day, and the lower part as the number of litres of wastewater per
303 inhabitant per day in the target WWTP.

304

305 **3. Results and discussion**

306 **3.1. Concentration of target pharmaceuticals in influent and effluent**

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

311 Ibuprofen was found in each of the tested samples of influent within the range of 4.03–91.43
312 $\mu\text{g/L}$ (median 12.85 $\mu\text{g/L}$ for WWTP Gdansk and median 25.07 $\mu\text{g/L}$ for WWTP Gniewino)
313 with high variance. In the effluents, its concentration was between $<\text{MDL}$ (Method Detection
314 Limit) and 0.48 $\mu\text{g/L}$. A similar pattern was found for naproxen, but the median concentration
315 in the influent was lower (median 3.17 $\mu\text{g/L}$ for WWTP Gdansk and median 5.41 $\mu\text{g/L}$ for
316 WWTP Gniewino).

317 Diclofenac was found in each of the tested samples of influent and effluent. For three sampling
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
321 (Kolecka et al., 2019), and in studies performed in other countries (Oosterhuis et al., 2013;
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).



326 Metabolite 4OH-diclofenac was found in 9 of 12 tested samples of influent and 11 of 12 tested
327 samples of effluent. For this compound, a high variance of the concentration was noted. In 5
328 sampling dates, a higher concentration of 4OH-diclofenac was noted in the effluent compared
329 to the influent (both WWTPs). The cause may be the release of 4OH-diclofenac from
330 conjugates. Another cause could be temporal storage in the sludge with release. Research
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
334 biotransformation by the bacteria community of the activated sludge. Such a degradation
335 pattern has been observed in biodegradation studies (Kosjek et al., 2009; Murshid and
336 Dhakshinamoorthy, 2019). On the other hand, very low biodegradation of diclofenac in the
337 inoculum of sewage sludge was observed (Lee et al., 2012). According to Cherek et al. (2015),
338 the removal of diclofenac takes place mostly by adsorption from the surface of activated sludge,
339 and diclofenac can be toxic for this bacteria community. This may be why the efficiency of
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
344 metabolites in the effluent has been noted in the literature (Osorio et al., 2014; Sathishkumar et
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
347 poses a risk to the environment. In our study, we did not study of diclofenac glucuronide
348 metabolites. Its presence in wastewater is probable, but it decomposes in $\text{pH} > 6$ (Vree et al.,
349 1993); the pH of influent is approximately 8 thereby the diclofenac can be released by
350 hydrolysis even after excretion and passage to WWTP. The other transformation products of



351 diclofenac can also be found in wastewater (Jewell et al., 2016), but because a lack of analytical
352 standards, they are unable to quantify.

353 The variance in the concentration the pharmaceuticals in the influent has no correlation with
354 the sales data presented in **Figure 1**. For example, the sales of diclofenac are relatively stable
355 throughout the whole year, but its concentration in the wastewater differs substantially (**Table**
356 **S2**, Supplementary Materials). This is most probably an issue of changeable matrix of
357 wastewater and heterogeneous consumption of the diclofenac contained medicines.

358

359 **3.2. Concentrations by prediction scenario**

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

364 The calculated concentrations of pharmaceuticals in scenario S1 for Gdańsk WWTP and
365 Gniewino WWTP were the highest for each of the target NSAIDs, which is logical. The highest
366 values were noted for ibuprofen, followed by naproxen and diclofenac for WWTP Gniewino in
367 2018.

368 A clear pattern in the relationship between the C_m and the predicted concentrations for the three
369 scenarios is observed – the measured concentrations are between two extreme scenarios: S1 –
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.



376 This was also observed for the predicted concentrations. The averaged time was 12 (in case of
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
380 often used months after being purchased. Furthermore, the pills often end in the sink or toilet
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**).

384 The concentration in S3 is close to the C_m in the case of ibuprofen, but much higher in the case
385 of naproxen. S3 assumed that all of the released conjugates are hydrolysed in the wastewater.
386 This process most probably occurs, but to an unknown extent. The fact that the value of C_m is
387 between S1 and S3 is the result of multiple factors, and the accurate determination of C_m by
388 any model is unrealistic. What is certain is that knowing the sales data and the basic operational
389 data from the WWTP, we can determine the boundaries of the pharmaceutical concentrations
390 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 $\mu\text{g/L}$ and 52.74 $\mu\text{g/L}$, respectively, in one
394 WWTP in Switzerland (Lienert et al., 2007). The annual consumption of ibuprofen in
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
398 consumed about 2.5 times more ibuprofen than currently in Poland. This is reflected in the
399 predicted concentrations, which were about three times higher in the referred study than those
400 presented in this study by scenario S2.



401 In 2013 in the Netherlands, two WWTPs were tested (Oosterhuis et al., 2013). In the first one,
402 the predicted concentration of diclofenac in the effluents was 0.47 µg/L, while the observed
403 value was 0.34 (n=6). In the second WWTP, the predicted C was 0.43 µg/L, and the observed
404 one – 0.25 µg/L. This means that C_{pred} was 138% and 172% of C_{real} , respectively, for the first
405 and second WWTP. In our study, we were unable to predict the concentration in the effluents,
406 because of the high variability in the removal efficiency.

407 In the report from Japan (He et al., 2020), C_{pred} and C_{real} were 0.188 µg/L and 0.135 µg/L, and
408 0.077 µg/L and 0.520 µg/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
410 an acceptable prediction was when $0.1 < C_{pred} / C_{real} < 10$. We applied this concept in our study.

411 The calculated ratio of C_{pred} to C_{real} is presented in **Figure 4**. In the cases of ibuprofen and
412 naproxen, most of the prediction scenarios give accurate values (ratios between 0.10 and 8.66).

413 In the case of diclofenac, scenario 1 gives the highest overestimation, while scenario 2 gives
414 the lower ratios. In the case of this pharmaceutical, the sorption into sludge, mentioned above,
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.

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

424 Our study present relatively short chemical monitoring, and in the future will be continued.

425 However, it can be considered as a “seed” investigation for creating a database. A large database



426 of many studies would allow the use of a machine learning in order to identify the greatest
427 threats related to the presence of pharmaceuticals in the environment (Ahmad et al., 2021,
428 Mahmood and Wang, 2021). Furthermore, after prediction of concentration in WWTP, the
429 predicted environmental concentration (PEC) can be estimated, as it was performed previously
430 in several studies for river waters, knowing dilution factors (Cardini et al., 2021; Meyer et al.,
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,
434 2013). HELCOM propose an annual average Environmental Quality Standard (AA-EQS) in
435 marine waters of 0.005 µg/l (5 ng/L) for diclofenac (Helcom, 2018).

436

437 **3.3. Potential of removal**

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

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

451 The removal efficiency of the target NSAIDs by the two tested WWTPs is presented in **Figure**
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
454 whole year in both WWTPs. The removal of diclofenac was between -242% and 98%. The
455 negative value means that a higher concentration was found in the effluent compared to the
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
458 diclofenac and its metabolite was not connected with the sampling period and the technical
459 systems of WWTPs. To present this more clearly, the RE (Removal Efficiency, %) of the basic
460 parameters was higher than 83% (except for N-NO₂ and N-NO₃). In most of analysed samples,
461 the RE was higher than 95% (**Table S3**, Supplementary Materials). The removal of N-NO₂ and
462 N-NO₃ was very variable and ranged from -1511% (caused by an increase of the value in the
463 effluent compared to the influent) to 96%. This may indicate periodic problems with the
464 denitrification process.

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



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

478

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

480 The calculations of loads of pharmaceuticals from single point source (such as WWTPs) can
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
484 et al., 2020). Based on the Helcom report (HELCOM, 2018), it can be stated that the average
485 concentration of diclofenac in treated wastewater which ends up in the Baltic Sea is 1.41 ± 1.10
486 $\mu\text{g/L}$. The calculation based on a meta-analysis by Sathishkumar et al. (2020), who reviewed
487 more than 50 publications from the last 15 years, estimates that the global average diclofenac
488 concentration is 2.28 ± 3.43 $\mu\text{g/L}$ in treated wastewater. In our study the median concentration
489 of diclofenac in effluents was 1.27 $\mu\text{g/L}$ and 1.11 $\mu\text{g/L}$ in Gdańsk and Gniewino wastewater,
490 respectively. This values are close the HELCOM predictions, and values for both tested
491 WWTPs are similar. We are unable to compare median concentration before and after pandemic
492 period, because we have not sufficient number of monitoring data in 2021. It can be just say
493 that levels of concentration were similar. The increasing pattern of diclofenac sale (**Figure 1**)
494 mean that median concentration both in influents and effluents have potential to increase. In
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.



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

502 It was calculated that 60 kg of diclofenac was discharged into the Baltic Sea during the whole
503 of 2018, and 20 kg in the first half of 2021 from the WWTP Gdansk. In the first half of the year
504 2018, a similar amount was discharged as in the first half of 2021. For WWTP Gniewino, the
505 equivalent values were much smaller and did not exceed 1 kg. What scare, that more metabolite
506 of diclofenac compared to the native pharmaceutical was released from the WWTP Gdańsk.
507 From the data presented in **Table 2**, it is easy to calculate the number of kg removed by a single
508 WWTP.

509 For comparison, in the city of St. Petersburg in Russia (5,230,000 inhabitants), about 400 kg of
510 diclofenac is introduced into the Baltic Sea annually (Helcom, 2018). The annual load of
511 diclofenac originating from the three largest cities of Estonia (Tallinn, Narva and Pärnu with a
512 total 5,440,000 inhabitants) is only about 30 kg per year (Lember et al., 2016) despite similar
513 number of people as in St. Petersburg. For WWTP Gdańsk person equivalent is estimated
514 806,815 and it release 60 kg of diclofenac per year, what mean that single “person” are
515 responsible for release of 74 mg of diclofenac per year, in St. Petersburg adequate number is
516 76 mg, while in Estonia region only 5 mg per year. Unfortunately, for most countries, such
517 numbers are unavailable. Nevertheless, there is potential for calculation of annual loads of
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
521 with rivers ended in Baltic Sea and with underground and surface runoff. In our study we were
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.



524 Comparison of the total introduced mass of ibuprofen (**Table 2**) with the whole sold mass of
525 this NSAID in Poland in 2018 shows that the citizens of Gdańsk consume 1.46% of the total
526 sales mass of the ibuprofen purchased in Poland and 1.45% of diclofenac. The percentage of
527 the Gdańsk population is 1.53% of the total Polish population (2018). These values are
528 comparable. On the other hand, the percentage of the use of naproxen is 0.40%. This may result
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
532 could not be explained by the consumption pattern.

533

534 **4. Conclusion**

535 Strategy to perform chemical analysis in influents and effluent of WWTPs in parallel with
536 observation of sale pattern during tested periods allow to obtain several findings. Our research
537 indicates that the changes of naproxen and diclofenac concentrations in the influent of both of
538 tested WWTPs has no correlation with relatively stable sales throughout whole tested period.
539 It was noted that pandemic have effect into sale of ibuprofen. However, this had no effect on
540 its concentration in the wastewater. This was probably connected with fact that NSAIDs are
541 often stored and used whenever needed, not directly after being purchased. The developed
542 scenarios of discharging of pharmaceuticals indicated that the quantity of pharmaceuticals sold
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
546 the influent was higher than that which would be obtained if all of the administrated mass of
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



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

552 Our research as well as literature data shows high variance of the removal of the selected
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
555 diclofenac (60 kg) was released from Gdańsk WWTP into the Baltic Sea during 2018 and the
556 first half of 2021 (19 kg). It is remarkable that during the same time, a higher mass of 4-hydroxy
557 metabolite of diclofenac was released, and part of this mass was released during treatment by
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
562 and changes in found concentrations in WWTPs not overlap, what was probably effect of
563 administration of NSAIDs in delay after purchase, variation of matrix composition (difference
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
566 implementation of passive sampling techniques of extraction in wastewaters, thanks to which
567 average in time concentration can be obtained (Caban et al., 2021). What scared, that sales data
568 show increase in diclofenac purchasing, what at least in first half of 2021 have no reflect into
569 found concentration in two tested WWTPs, but as mentioned previously can be an problem in
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
572 the activity of diclofenac. This study apply simple calculation with three release scenarios. In
573 the future the database of found real concentrations and sale data should be created and a



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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784



1 **Figures' captions**

2

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

7 **Figure 2.** Box and whisker plot presenting the distributions of the concentrations [$\mu\text{g/L}$] of
8 ibuprofen, naproxen, diclofenac and 4-hydroxy diclofenac (4OH-diclofenac) in six samples of
9 influent and effluent of the two tested WWTPs in Poland (location: Gdańsk and Gniewino,
10 2018 and 2021).

11 **Figure 3.** Concentrations [$\mu\text{g/L}$] of ibuprofen, naproxen and diclofenac in influent by three
12 prediction scenarios (S1, S2, S3) and real median concentration (C_m) found in wastewater
13 treatment plants (WWTP) in Gdańsk and Gniewino (Poland, 2018 and 2021).

14 **Figure 4.** Ratio of values of predicted concentrations by the three prediction scenarios (S1, S2,
15 S3) to real median concentrations of selected NSAIDs (C_m) for the two investigated wastewater
16 treatment plants (Gdańsk and Gniewino, both in Poland) between sampling years 2018 and
17 2021.

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

20

21

22 Figure 1.

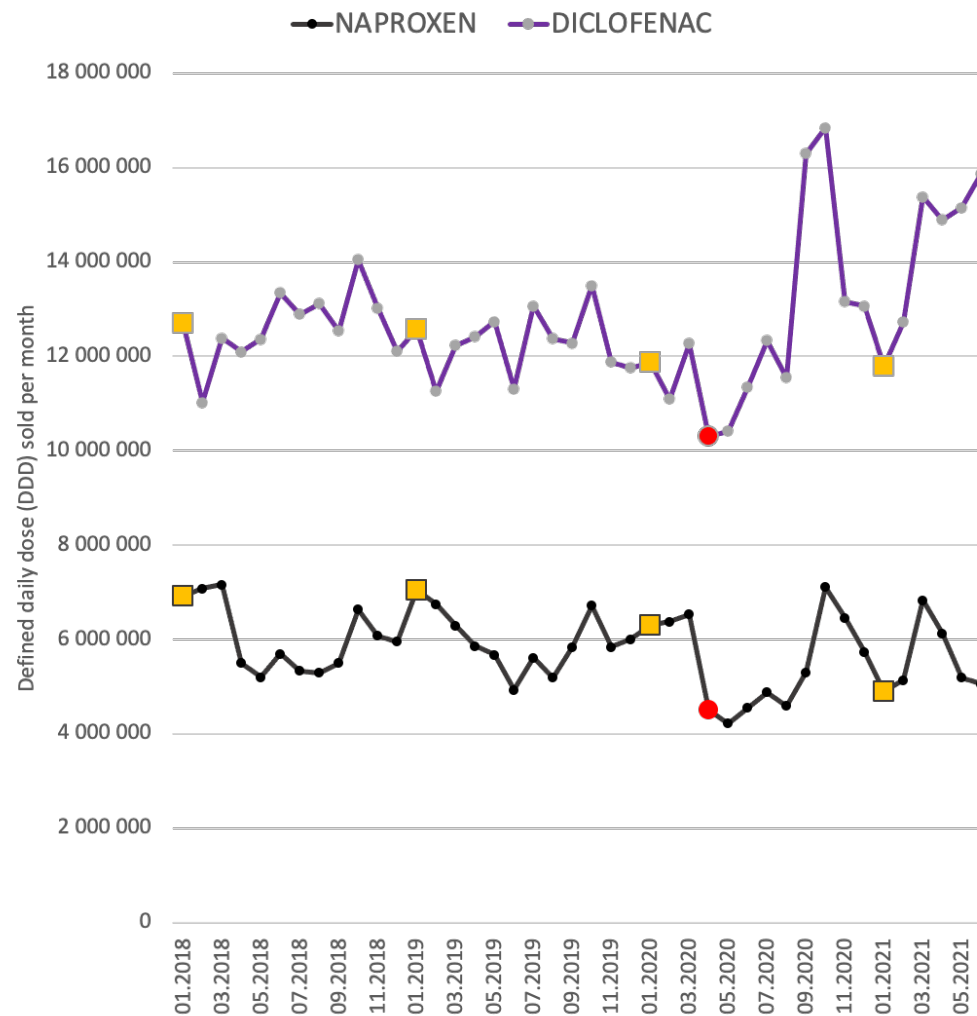
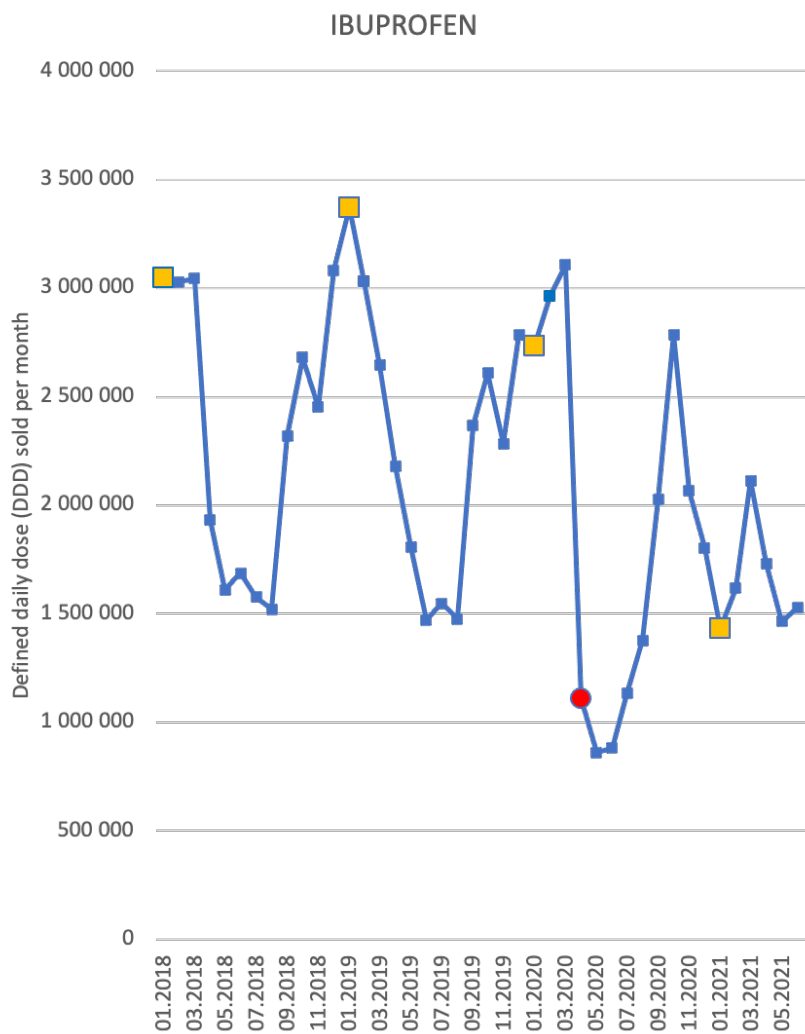
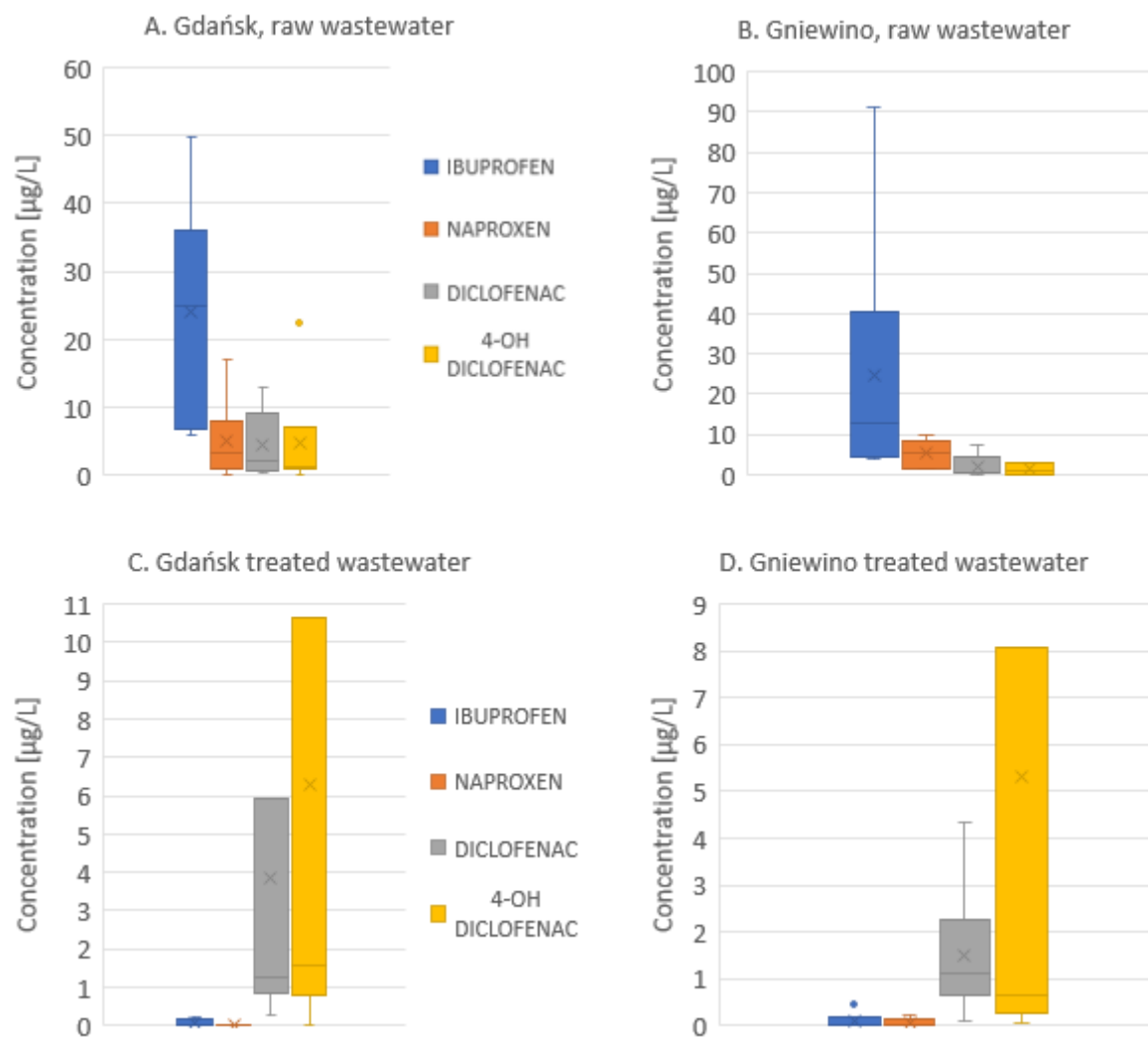
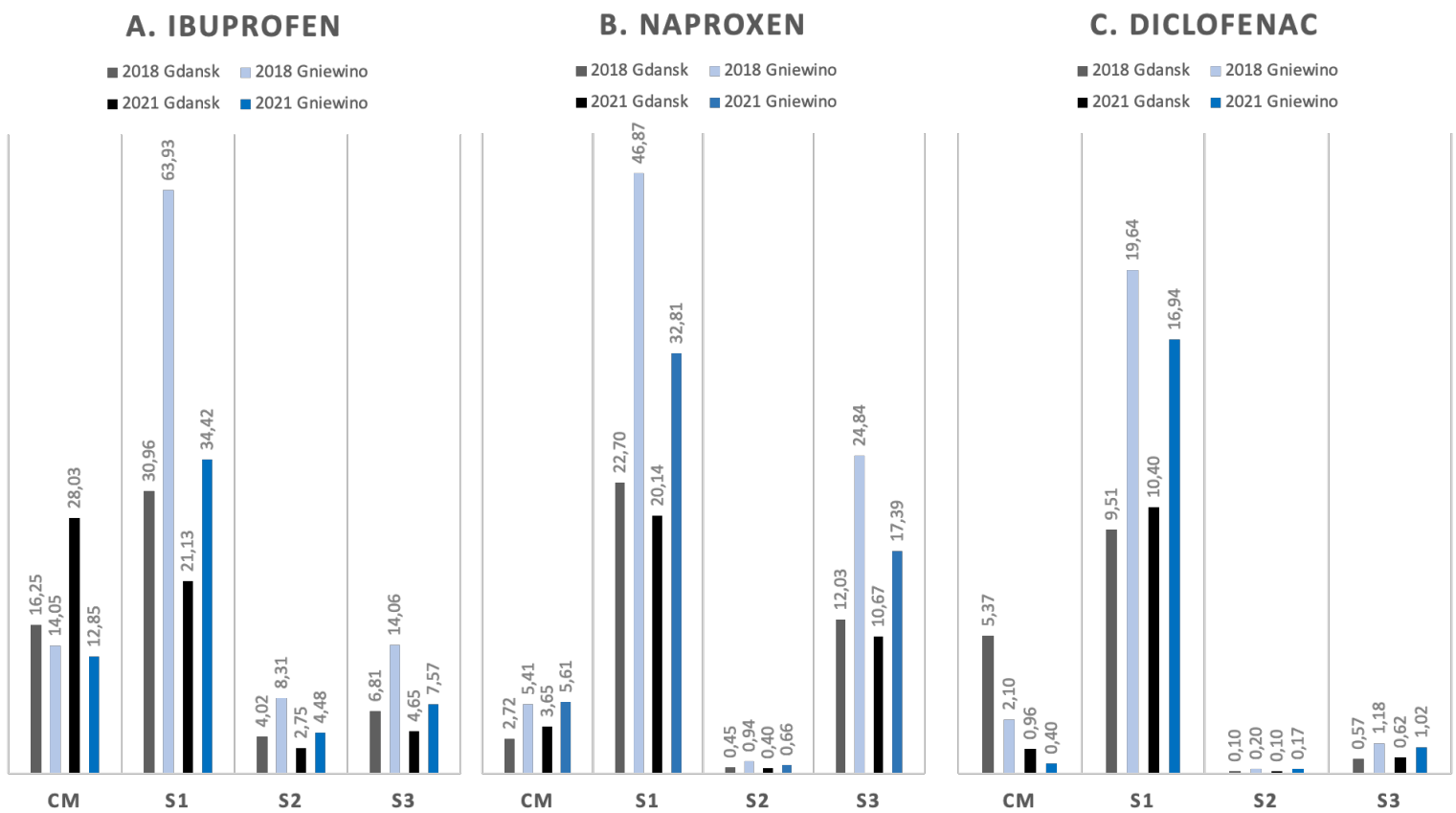


Figure 2

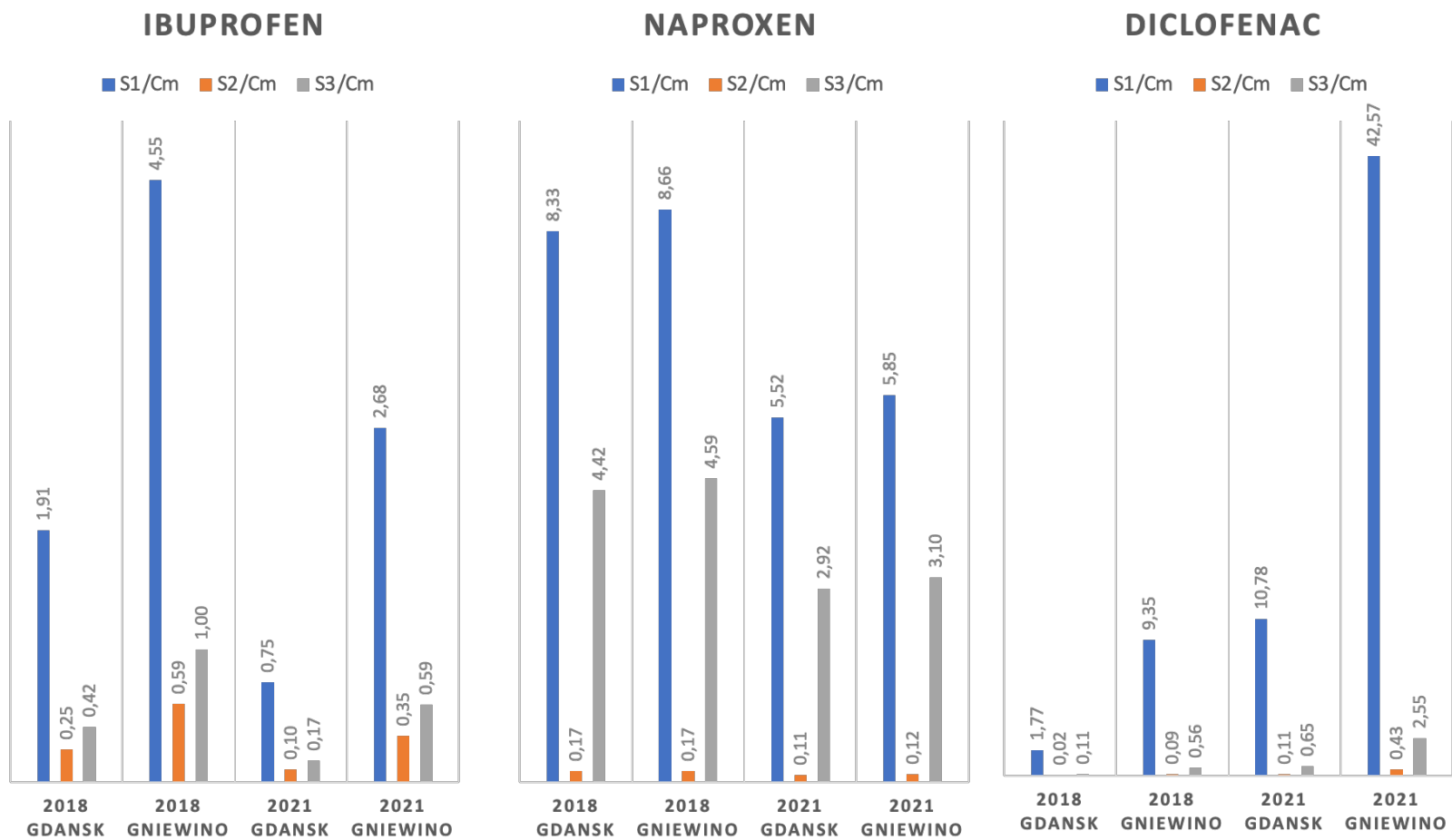


1 Figure 3



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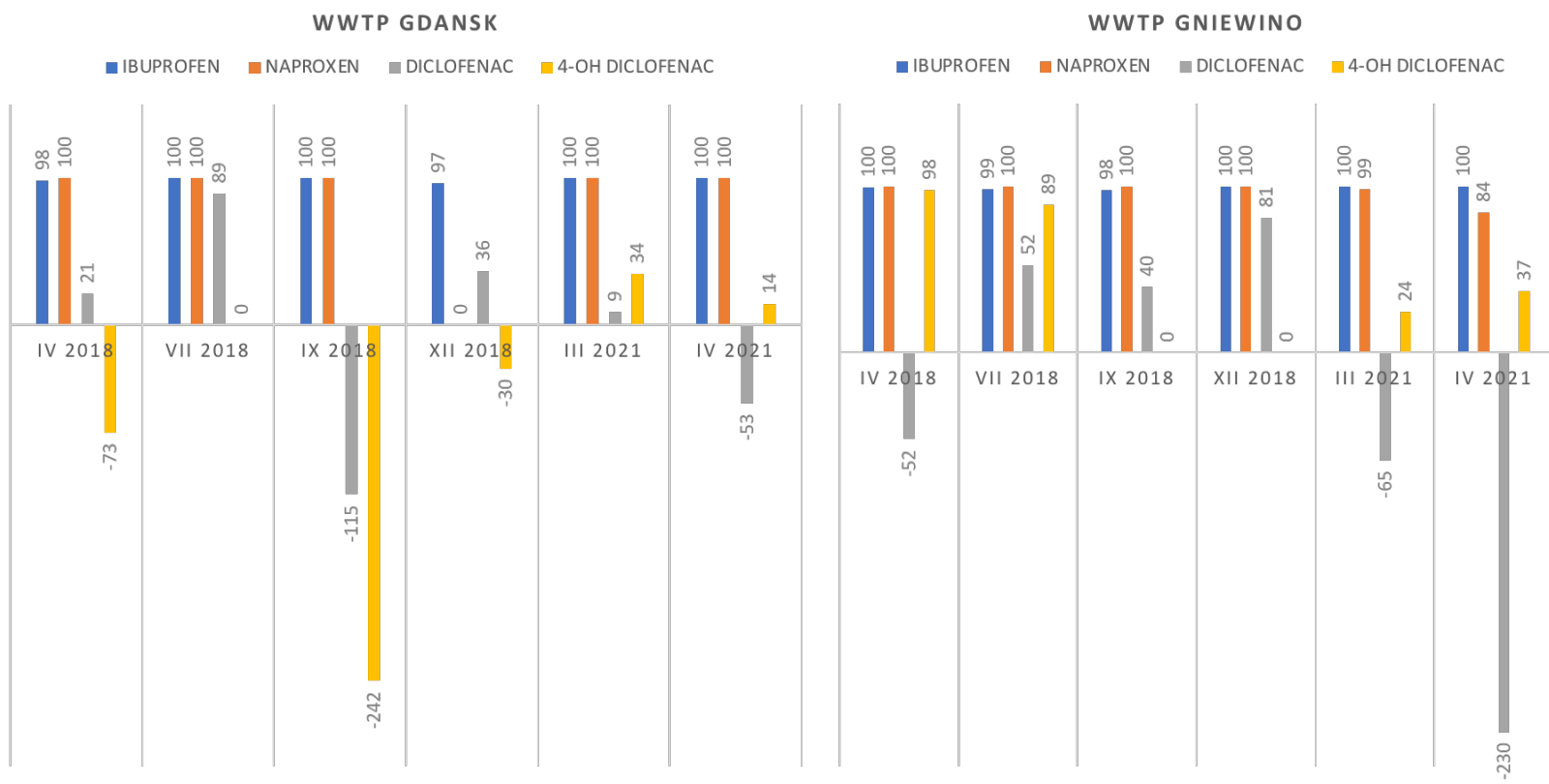
3 Figure 4



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 (4OH-diclofenac) 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 excreted [%] | II. Share of conjugates of 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 |