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From the pills to environment – prediction and tracking of non-steroidal anti-inflammatory drug concentrations in wastewater

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Abstract

The extend of environment pollution by pharmaceuticals is in a stage that required more automatic and integrated solutions. The non-steroidal anti-inflammatory drugs (NSAIDs) are 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 (ibuprofen, naproxen, diclofenac) and their concentration in the WWTP in order to enable predicting their loads, having only the sales data. For calculations, we apply three discharge 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 analysis were conducted in two conventional wastewater treatment plants (WWTPs) located in 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 administrated mass of the pharmaceutical went through the metabolic pathway of transformation. This mean that substantial mass of sold NSAIDs in improperly dispose to sewage system, and this factor need to be taken into account in future predictions. Furthermore, 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 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 in 2018, and 20 kg in the first half of 2021 from two tested WWTPs. The presence of 4OH-diclofenac in effluents often in higher concentration compared to diclofenac mean that this still biologically active compound need to be taken into account in future risk assessment.

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

1. Introduction

The pollution of the environment by pharmaceuticals and its metabolites is still emerging problem, irrespective to the development of society (Caban and Stepnowski, 2021). Generally, 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 daily treatment are some of the reasons. This situation has a direct effect on the quality of the environment, while selected pharmaceuticals can be found in natural water in concentrations 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, the current period of the SARS-CoV-2 epidemic is a factor that is changing the pharmaceuticals 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 drugs (NSAIDs) are most popularly administrated active compounds, also for flu and cold symptoms, what reflect its high detection frequency and concentrations in the environmental waters (Hong et al., 2018; Jiang et al., 2014; Ślósarczyk et al., 2021). This was confirmed, for example, by research conducted as part of the Morpheus project, where ibuprofen, naproxen and paracetamol were characterised by the highest concentrations compared to the other 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 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 unpredictable part of stored pharmaceuticals can expired and finally in improper way disposed to toilets and sinks.

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 or in the form of easily hydrolysed conjugates. Secondly, unused and expired medicines (pills, gels, syrups) often improperly end up going down the drain. According to national and EU law, pharmaceuticals are dangerous wastes and should be collected separately. Unfortunately, there 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 expired pharmaceuticals in their household waste or sink/toilet (Rogowska et al., 2019). Similar 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 is unrealistic to estimated. Optimistically, the mass of pharmaceuticals returned to pharmacies as waste is increasing in Poland (example of the Gdansk municipality in **Figure S1**, Supplementary Materials). The similar situation was observed in other countries (Basir et al, 2020).

The technology in the majority of wastewater treatment plants (WWTPs) is not focussed on the removal of pharmaceutically active compounds and their metabolites, and unfortunately a significant load of NSAIDs enter the natural waters by this pathway. Previous works show that 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 (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%, respectively. Diclofenac revealed low and varied removal rate ranging from 10 to 50%. 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.

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).

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 with obtained environmental concentration improve determination of pathways and fates, what is essential for problem minimalization. This procedure can be problematic for NSAIDs, as they can be purchase without prescription, and administrated with high dose and delay after purchase, without medical control. Furthermore, most estimation studies of that kind used excretion rate (release of unmetabolized form of pharmaceuticals) as correction factor, but omit the fact that some part of purchased medicine is disposed to sinks and toilets, and seems that 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 in the WWTP to enable further prediction of the loads having only the sales data with three scenarios of excretion rates. The use of three scenarios aimed to check how share of sold mass of pharmaceuticals improperly dispose to toilets affect obtained concentrations in raw wastewaters. Furthermore, monitoring of diclofenac main metabolite was applied for supporting of scenarios reliability. Those two methodology modification distinguish this work from other already published (presented in discussion). In this part of the study, the change of the sales pattern of the investigated NSAIDs due to the SARS-CoV-2 epidemic was taken into 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 WWTPs located in northern Poland were selected. Target WWTPs are differentiated in the catchment and volumes of discharging wastewater to cross-check estimations. Both release the 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 by sales data from PEX PharmaSequence, a consulting and research company that has focussed on the pharmaceutical market and the healthcare sector in Poland for almost twenty years. The obtained results could be helpful in managing the problem of pharmaceuticals by providing a method of calculating loads in a catchment area.

2.Materials and methods

2.1. Characteristic of tested WWTPs and sampling of wastewater

The WWTP in Gniewino operates 15,000 PE (Population Equivalent). The average flow rate of the wastewater was about 830,000 L/day in 2018 and 1,108,000 L/day in 2021 (presented in **Table S1**). About 35% of the wastewater supplied to the WWTP comes from the dairy and fish 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 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 and nitrification. Internal recirculation of activated sludge is carried out between the chambers. 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 reed bed) system, which is planted with *Phragmites australis*.

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**

S1). It mainly treats municipal wastewater. Only 6.3 % of the wastewater is from the food, shipbuilding and chemical industries. The Gdańsk WWTP uses mechanical and biological processes to treat the wastewater. The mechanical part of the WWTP consists of four bar screens, aerated grit chambers and three primary settling tanks. The biological part is based on multiphase active sludge using an A^2/O system. Six bioreactors have been installed in the WWTP with a total volume of 158,100 m^3. Biological processes of organic matter decomposition take place, and nitrogen and phosphorus compounds are removed in these 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 fermented sludge is discharged to a mechanical dewatering station and dewatered in 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.

2.2. Chemical measurement and statistics

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

The chemical analysis of ibuprofen, naproxen, diclofenac and diclofenac metabolite (4OH-diclofenac) 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 Pearson coefficients) was performed in the Microsoft Excel software.

2.3. Assumptions

2.3.1. Metabolism of target pharmaceuticals

Generally, NSAIDs are almost totally absorbed in the gastric system, and metabolised by dual phases of metabolism (phases I and II) (Mulkiewicz et al., 2021). In phase I, the NSAIDs are transformed to hydroxy or carboxy metabolites. Then in phase II, the conjugation (mostly with glucuronide) is obtained. The elimination proceeds mostly by urine (to some extent by bile), in 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 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 of conjugate. 4OH-diclofenac has been indicated as the main metabolite of diclofenac. For this 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 metabolism of pharmaceuticals should be treated as average values, because the metabolism varies between people and their condition, and the data presented in the literature are often not comparable. Information about the biotransformation of diclofenac in the environment can be found in the literature (Guzik et al., 2013; Mulkiewicz et al., 2021).

2.3.2. Scenarios of discharging of pharmaceuticals into wastewater

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 conjugates, and disposal of unwanted medicines in the toilet and sink. What was omitted is the fact that degradation by microbiome can be obtained in domestic wastewater, as well as the 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 and seasonal changes of wastewater parameters makes any predictions of these two processes highly imprecise. With mentioned assumptions, three scenarios (S) were defined:

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_S 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.

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

NSAIDs have a significant share in the Polish pharmaceutical industry, which is one of the fastest growing sectors in the country. In terms of market value, Poland is ranked in eighth 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, the Polish pharmaceutical market is in second place in terms of the sales growth rate (Dymaczewski and Jeż-Walkowiak, 2014), with a 4% annual growth rate. In Poland the 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 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., 2019). Although data on drugs are taken from Poland, they point to a relatively big problem that affects the whole world (Peña et al., 2021, Pharmaceutical Strategy for Europe, 2020). The sales data of the selected NSAIDs was provided by the PEX PharmaSequence sp. z o.o. 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 obtained data is precise. The data from PEX presents the number of sold packages as the number of defined daily doses (DDD, which exact values 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.

Based on these data, a few trends can be observed. Firstly, the sales of naproxen were relatively 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, among other disorders. In the case of diclofenac, over the final ten months, an increase in sales was observed. Diclofenac is used in the treatment of inflammation and pain of rheumatic and non-rheumatic origin. Diseases of the musculoskeletal system affect 70% of the population over 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 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.

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-to-date 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.

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:

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

Where:

C – estimated concentration of specific NSAIDs in raw wastewater [µg L^{-1}],

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

N_{DDD} – number of DDD sold per year in Poland (**Figure 1**, in the case of 2021, the number of DDD sold in the first six months),

M_{DDD} [µg] – mass of DDD for specific NSAIDs (1,200 mg, 500 mg and 100 mg for ibuprofen, naproxen and diclofenac, respectively, recalculated to µg),

N – number of people in Poland (38,410,000 in 2018, 37,578,000 in 2021, data from the Central 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^{-1}] – average flow in the given WWTP (presented in **Table S1**, Supplementary Materials) averaged over the entire year,

F_s – scenario factor, presented above in the text.

Z komentarzem [KK1]: previous change

The upper part of equation can be understood as the µg 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.

3. Results and discussion

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-and-whisker 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 µg/L (median 12.85 µg/L for WWTP Gdansk and median 25.07 µg/L for WWTP Gniewino) with high variance. In the effluents, its concentration was between <MDL (Method Detection Limit) and 0.48 µg/L. A similar pattern was found for naproxen, but the median concentration in the influent was lower (median 3.17 µg/L for WWTP Gdansk and median 5.41 µg/L for WWTP Gniewino).

Diclofenac was found in each of the tested samples of influent and effluent. For three sampling campaigns, the concentrations of diclofenac in the effluent were higher than those noted in the influent. This fact may suggest that during treatment, diclofenac can be released from 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; Pedrouzo et al., 2011). Diclofenac was detected previously also in wastewater from residential area, stormwater, sewage water from industrial areas, small and medium-sized enterprises effluents, influent sewage water, effluent sewage water, and sewage sludge (Gercken et al., 2018).

Metabolite 4OH-diclofenac was found in 9 of 12 tested samples of influent and 11 of 12 tested samples of effluent. For this compound, a high variance of the concentration was noted. In 5 sampling dates, a higher concentration of 4OH-diclofenac was noted in the effluent compared to the influent (both WWTPs). The cause may be the release of 4OH-diclofenac from conjugates. Another cause could be temporal storage in the sludge with release. Research conducted by Szymonik et al. (2017) indicated that about 80% of diclofenac is absorbed in sewage sludge, while for hydroxy metabolite there is no data. Another process that could be 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 pattern has been observed in biodegradation studies (Kosjek et al., 2009; Murshid and 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), the removal of diclofenac takes place mostly by adsorption from the surface of activated sludge, and diclofenac can be toxic for this bacteria community. This may be why the efficiency of diclofenac removal in activated sludge processes usually does not exceed 50% (Clara et al., 2005; Petrie et al., 2013; Tiwari et al., 2017). The studies indicate that the most effective process in the removal of diclofenac is the adsorption process on activated carbon (Lee et al., 2012). 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 al., 2020; Stülten et al., 2008). 4OH-diclofenac produces a thirtieth of the activity of diclofenac 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 metabolites. Its presence in wastewater is probable, but it decomposes in pH>6 (Vree et al., 1993); the pH of influent is approximately 8 thereby the diclofenac can be released by hydrolysis even after excretion and passage to WWTP. The other transformation products of diclofenac can also be found in wastewater (Jewell et al., 2016), but because a lack of analytical 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 wastewater and heterogeneous consumption of the diclofenac contained medicines.

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.

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 – with assumption that all purchased pharmaceuticals are released to domestic wastewater vs. S2 – only a small fraction of unmetabolised pharmaceuticals are released to domestic wastewater. Of course, there is an order of difference between S1 and S2 values, while keeping in mind the very low fraction released as unchanged molecules (**Table 1**). There were also large differences between the median concentration of the targets NSAIDs between the years and the tested 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 2018) or 6 month (is a case of 2021). In our opinion, the lower time of the averaged time can be used, but only for pharmaceuticals that are known to be used soon after being purchased. In 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 after expiration, which could be roughly estimated as happening a year after being purchased. With this in mind, the lack of a clear dependency of the lower ibuprofen concentration in 2021 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.

Predicting the concentrations of NSAIDs using their sales data has been performed before in some countries. For example, using a similar model to our S2 scenario, the predicted 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 Switzerland in 2004 was 23.150 kg, while in 2018 in Poland it was 46.108 kg. At the same time, the number of people in Switzerland in 2004 was 7,415,100, while in Poland in 2018 it 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 predicted concentrations, which were about three times higher in the referred study than those 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 μg/L, while the observed value was 0.34 (n=6). In the second WWTP, the predicted C was 0.43 μg/L, and the observed one – 0.25 μg/L. This means that C_{pred} was 138% and 172% of C_{real}, 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.

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 0.077 μg/L and 0.520 μg/L in the influents for diclofenac and ibuprofen, respectively. In the 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. The calculated ratio of C_{pred} to C_{real} is presented in **Figure 4**. In the cases of ibuprofen and 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 the lower ratios. In the case of this pharmaceutical, the sorption into sludge, mentioned above, can lower the medium concentration and hinder the application of selected models. This sorption could be applied in the prediction model, but the variability of the matrix in raw 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 threats related to the presence of pharmaceuticals in the environment (Ahmad et al., 2021, Mahmood and Wang, 2021). Furthermore, after prediction of concentration in WWTP, the predicted environmental concentration (PEC) can be estimated, as it was performed previously in several studies for river waters, knowing dilution factors (Cardini et al., 2021; Meyer et al., 2016). Comparison of PEC with predicted-no-effect-concentrations (PNECs, determined experimentally using test organisms) is a basis of the simple risk assessment (Beek et al., 2016). 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 marine waters of 0.005 μg/l (5 ng/L) for diclofenac (Helcom, 2018).

3.3. Potential of removal

After entering into the WWTP, micropollutants are removed by processes of adsorption on particulate matter, biodegradation and bio-transformation. The other physicochemical 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 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, 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 osmosis could be also used to remove diclofenac. However, this method is only used for small 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 5** for each sampling campaign. Its value varies for each of the tested pharmaceuticals, but 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 negative value means that a higher concentration was found in the effluent compared to the influent. The reduction of 4OH-diclofenac was highly variable and differed between the 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 systems of WWTPs. To present this more clearly, the RE (Removal Efficiency, %) of the basic parameters was higher than 83% (except for N-NO_2 and N-NO_3). In most of analysed samples, the RE was higher than 95% (**Table S3**, Supplementary Materials). The removal of N-NO_2 and N-NO_3 was very variable and ranged from -1511% (caused by an increase of the value in the effluent compared to the influent) to 96%. This may indicate periodic problems with the denitrification process.

There was no direct correlation (determined by Pearson coefficient) between the removal of the nutrients, suspended solids and target pharmaceuticals. This correction was also different in the analysed WWTPs (**Table S4**, Supplementary Materials). The correlation between the trends of 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 with any of the basic parameters. The lack of a direct correlation between the removal of carbon and nitrogen, and the removal of NSAIDs was also found by Kołecka et al. (2020). On the other 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 phosphorus and COD removal with the removal of this pharmaceutical (Kołecka et al., 2019). 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 basic parameters need further investigation, and its plant-dependent.

3.4. Loads of NSAIDs to Baltic Sea

The calculations of loads of pharmaceuticals from single point source (such as WWTPs) can gives meaningful argument to take an action of reduction by decision makers. This is crucial for diclofenac, as this is one of the emerging pharmaceuticals in environmental monitoring with 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 concentration of diclofenac in treated wastewater which ends up in the Baltic Sea is 1.41±1.10 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 concentration is 2.28±3.43 μg/L in treated wastewater. In our study the median concentration of diclofenac in effluents was 1.27 μg/L and 1.11 μg/L in Gdańsk and Gniewino wastewater, respectively. This values are close the HELCOM predictions, and values for both tested 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 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 consequence, the treat to environment from a side of diclofenac is predicted to be increased. This need investigation in future. Furthermore, the presence of diclofenac metabolite with proven biological activity in concentrations close to the native pharmaceuticals need to be taken 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.

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 diclofenac originating from the three largest cities of Estonia (Tallinn, Narva and Pärnu with a total 5,440,000 inhabitants) is only about 30 kg per year (Lember et al., 2016) despite similar number of people as in St. Petersburg. For WWTP Gdańsk person equivalent is estimated 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 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 NSAIDs with WWTP effluents produced in urban area near coast of Baltic Sea, or even for whole Baltic Sea catchment. The bottleneck is the availability of the data on NSAIDs sales. 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 focused in liquid fraction of wastewater released to natural water, while solid faction in utilized 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 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 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 from regional differences in sales patterns. This estimation is also imperfect due to the fact that Gdańsk is a tourist destination, and during the summer, the number of people significantly increases. Diclofenac shows substantial variability of its concentration in wastewater, which could not be explained by the consumption pattern.

4. Conclusion

Strategy to perform chemical analysis in influents and effluent of WWTPs in parallel with observation of sale pattern during tested periods allow to obtain several findings. Our research indicates that the changes of naproxen and diclofenac concentrations in the influent of both of 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 its concentration in the wastewater. This was probably connected with fact that NSAIDs are 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 can be used to predict the concentrations of NSAIDs in the WWTPs' influents. Each of three scenarios produces an acceptable prediction. The S1 and S2 scenarios give boundaries for 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 pharmaceutical went through the metabolic pathway of transformation. This mean that part of 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 NSAIDs in WWTPs. In tested WWTPs the removal efficiency was not directly connected with 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 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 activated sludge. Such observations make it reasonable to introduce this metabolite into the risk assessments of pharmaceuticals.

Despite agreement of calculated concentration with reals median concentrations, the chemical 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 administration of NSAIDs in delay after purchase, variation of matrix composition (difference in accumulation on wastewater solid fraction and biodegradation – hypothetically), impossible 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 average in time concentration can be obtained (Caban et al., 2021). What scared, that sales data show increase in diclofenac purchasing, what at least in first half of 2021 have no reflect into found concentration in two tested WWTPs, but as mentioned previously can be an problem in future, because of delay in NSAIDs administration. The noted high concentration of 4OH-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 the future the database of found real concentrations and sale data should be created and a machine learning can be an tool to makes such prediction more reliable and automatic, and finally improve risk assessment.

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Figures' captions

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_m) 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_m) 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

22 Figure 1.

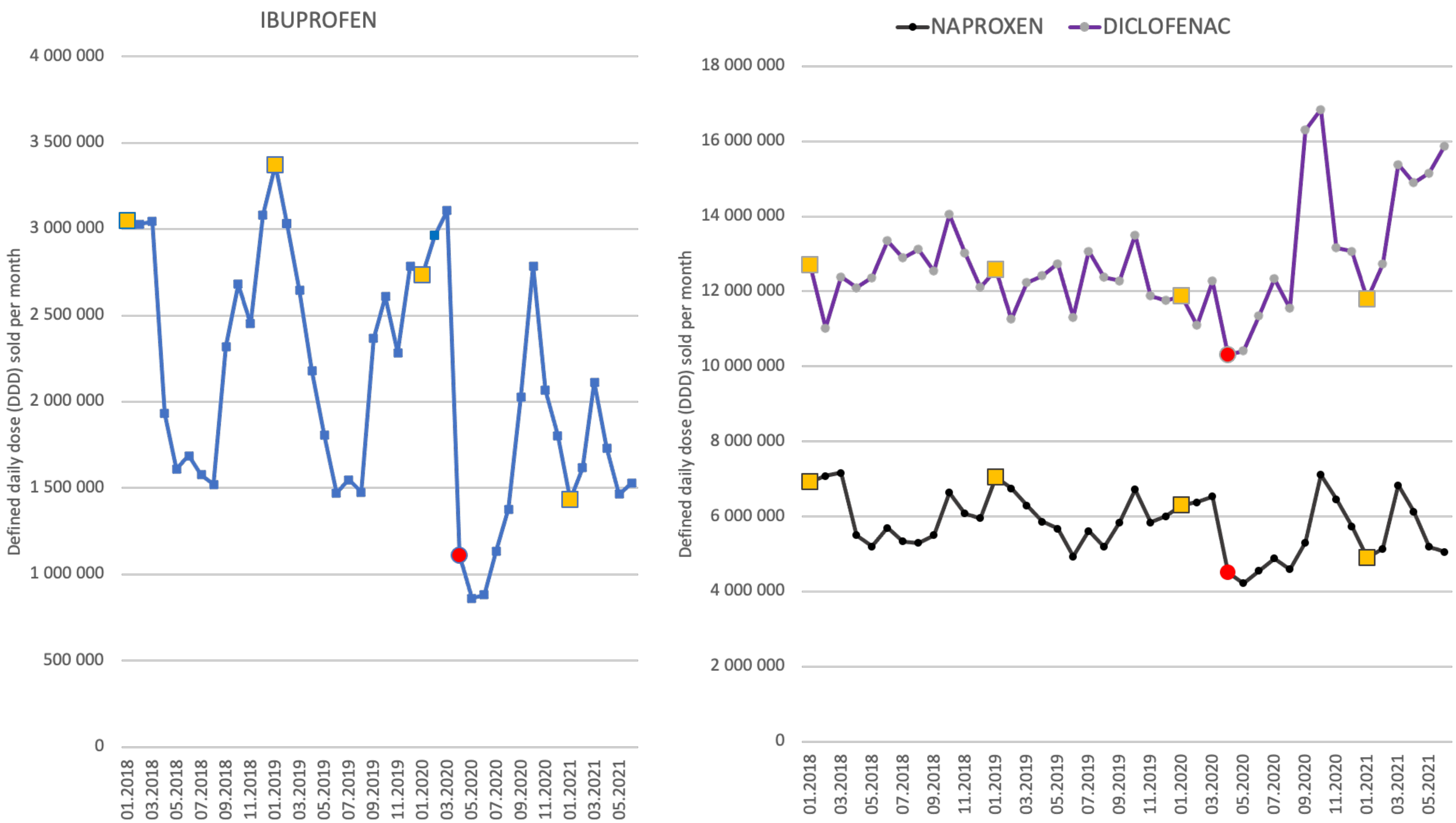

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Figure 2

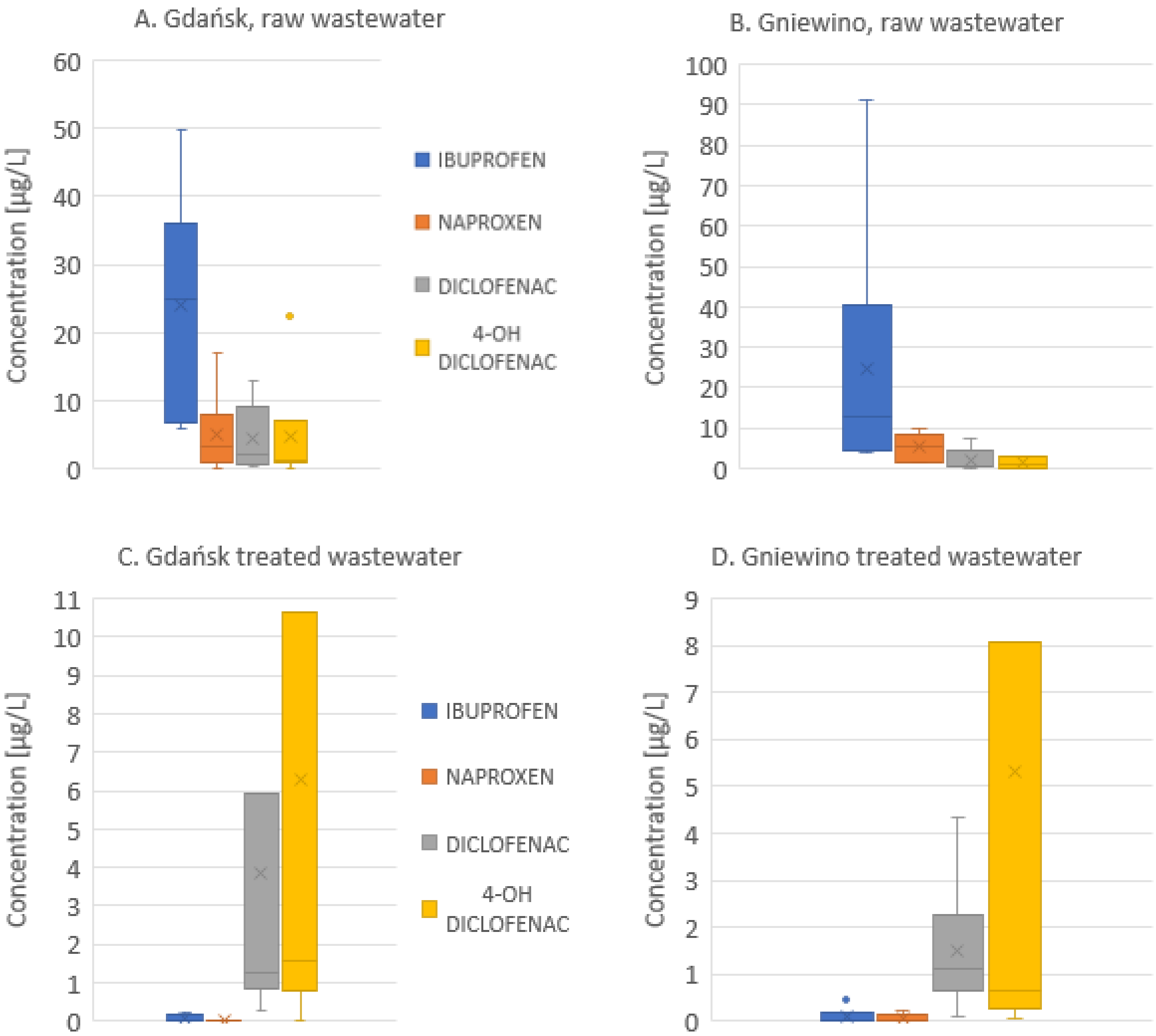
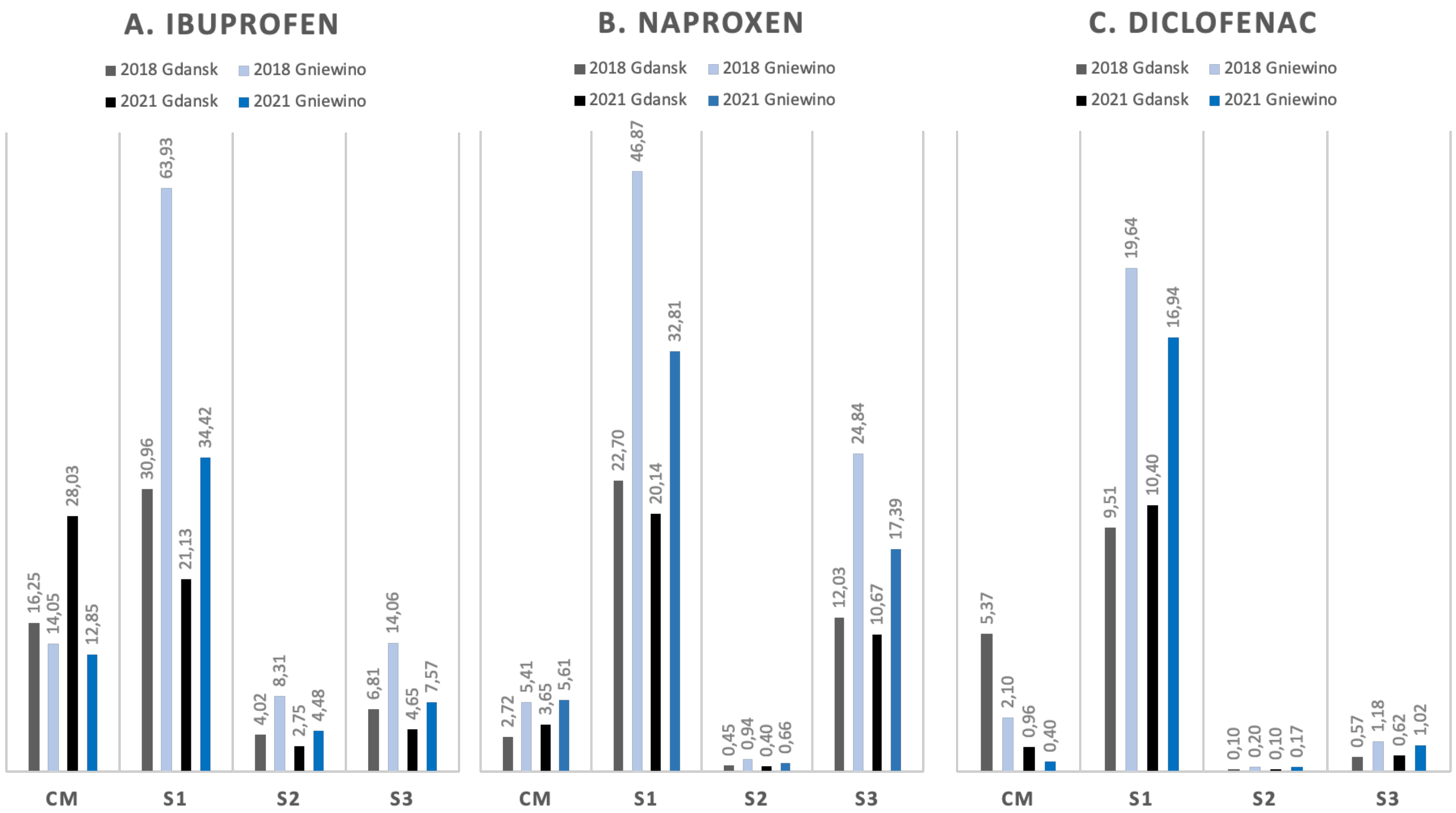
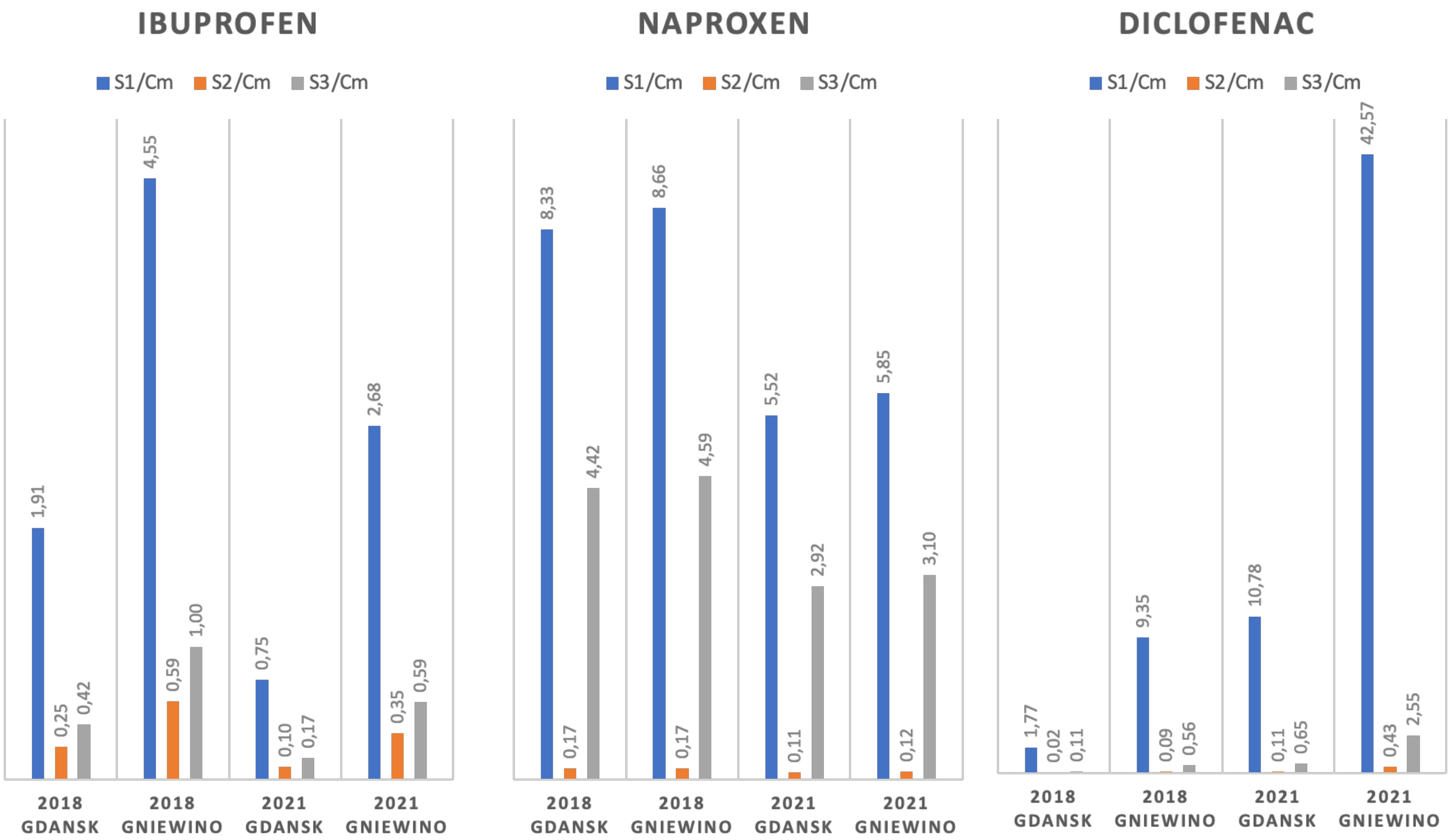

A. Gdańsk, raw wastewater
Concentration [µg/L]
IBUPROFEN
NAPROXEN
DICLOFENAC
4-OH DICLOFENAC
B. Gniewino, raw wastewater
Concentration [µg/L]
C. Gdańsk treated wastewater
Concentration [µg/L]
IBUPROFEN
NAPROXEN
DICLOFENAC
4-OH DICLOFENAC
D. Gniewino treated wastewater
Concentration [µg/L]

Figure 3

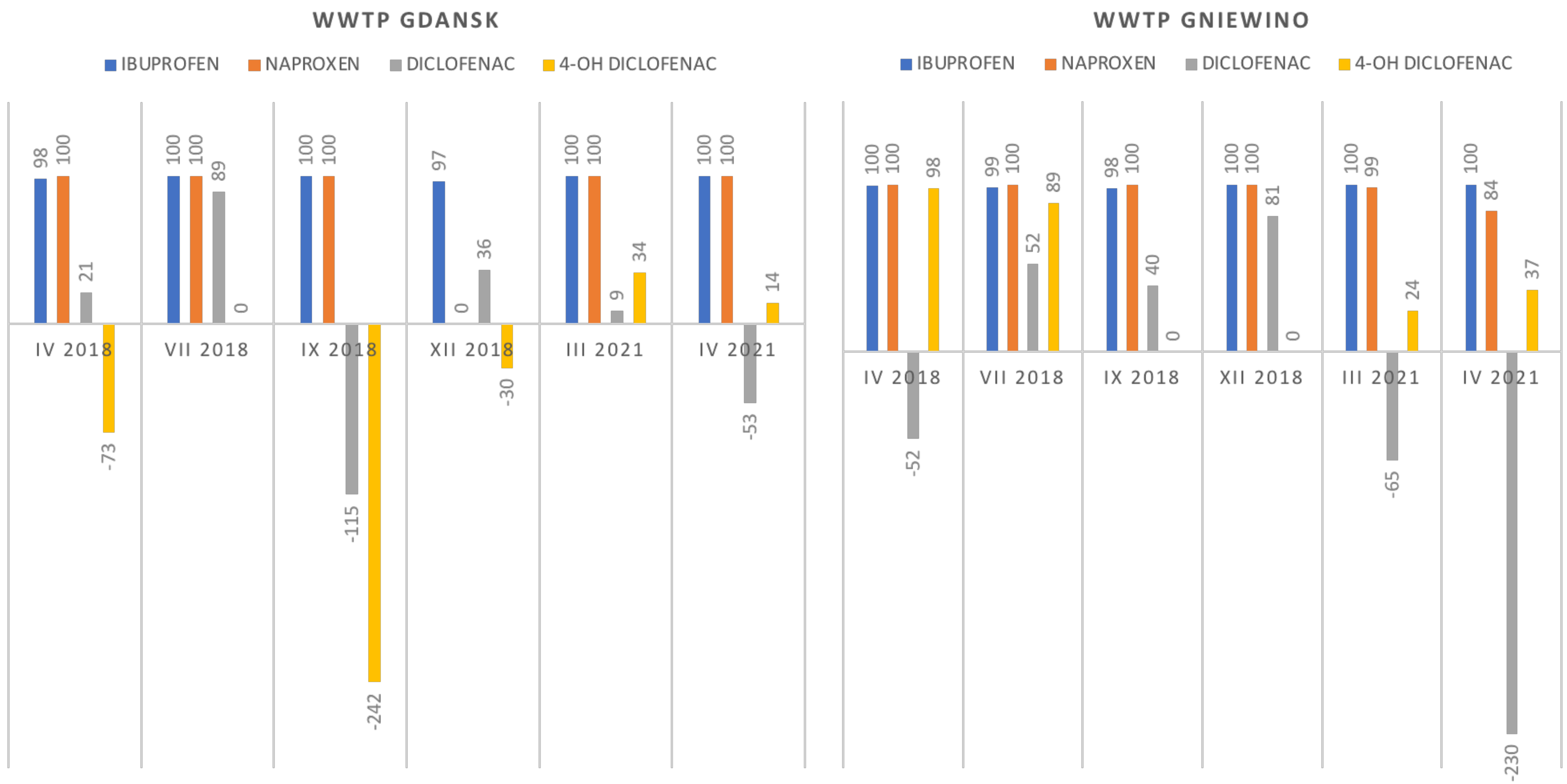

A. IBUPROFEN
2018 Gdansk
2018 Gniewino
2021 Gdansk
2021 Gniewino
16,25
14,05
28,03
12,85
30,96
63,93
21,13
34,42
4,02
8,31
2,75
4,48
6,81
14,06
4,65
7,57
CM
S1
S2
S3
B. NAPROXEN
2018 Gdansk
2018 Gniewino
2021 Gdansk
2021 Gniewino
2,72
5,41
3,65
5,61
22,70
46,87
20,14
32,81
0,45
0,94
0,40
0,66
12,03
24,84
10,67
17,39
CM
S1
S2
S3
C. DICLOFENAC
2018 Gdansk
2018 Gniewino
2021 Gdansk
2021 Gniewino
5,37
2,10
0,96
0,40
9,51
19,64
10,40
16,94
0,10
0,20
0,10
0,17
0,57
1,18
0,62
1,02
CM
S1
S2
S3

3 Figure 4


IBUPROFEN
S1/Cm S2/Cm S3/Cm
1,91 0,25 0,42
4,55 0,59 1,00
0,75 0,10 0,17
2,68 0,35 0,59
2018 GDANSK
2018 GNIEWINO
2021 GDANSK
2021 GNIEWINO
NAPROXEN
S1/Cm S2/Cm S3/Cm
8,33 0,17 4,42
8,66 0,17 4,59
5,52 0,11 2,92
5,85 0,12 3,10
2018 GDANSK
2018 GNIEWINO
2021 GDANSK
2021 GNIEWINO
DICLOFENAC
S1/Cm S2/Cm S3/Cm
1,77 0,02 0,11
9,35 0,09 0,56
10,78 0,11 0,65
42,57 0,43 2,55
2018 GDANSK
2018 GNIEWINO
2021 GDANSK
2021 GNIEWINO

4

Figure 5


WWTP GDANSK
IBUPROFEN
NAPROXEN
DICLOFENAC
4-OH DICLOFENAC
IV 2018
VII 2018
IX 2018
XII 2018
III 2021
IV 2021
98
100
21
-73
100
100
89
0
100
100
-115
-242
97
0
36
-30
100
100
9
34
100
100
-53
14
WWTP GNIEWINO
IBUPROFEN
NAPROXEN
DICLOFENAC
4-OH DICLOFENAC
IV 2018
VII 2018
IX 2018
XII 2018
III 2021
IV 2021
100
100
-52
98
99
100
52
89
98
100
40
0
100
100
81
0
100
99
-65
24
100
84
-230
37

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