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The impact of ultrasonic field on the pharmacological residues biodegradation

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ABSTRACT

The most commonly detected pharmaceuticals in surface waters are non-steroidal anti-inflammatory drugs, oestrogens (included in hormonal agents), and preparations used in veterinary medicine. The conducted research aimed to determine the possibility of removing from wastewater selected non-steroidal anti-inflammatory pharmaceuticals (ketoprofen and diclofenac), which pose a threat to the water and wastewater environment. From among the available methods supporting the removal of pharmaceuticals from wastewater, the sonication process was selected for research. The use of the active operation of the ultrasonic field, which results in a number of sono-chemical phenomena, including cavitation or oxidizing processes, is a new approach to the research problem. On the basis of the obtained research results, it was found that the application of the selected method results in a high and repeatable degree of removal of selected pharmaceuticals. For ketoprofen, the decrease in the toxicity unit value ranged from 1.307 to 0.839 TU, for diclofenac from 0.966 to 0.748 TU, and the mixture from 1.722 to 1.151 TU.

Keywords: Non-steroidal anti-inflammatory pharmaceuticals (ketoprofen and diclofenac); Wastewater; Conditioning; Ultrasonic field; Bioindication tests; Toxicity

1. Introduction

Currently, more and more attention is paid to micro-pollutants of surface water, drinking water, and micro-pollutants entering the environment together with treated wastewater [1–3].

A significant number of micro-pollutants of anthropogenic origin, mainly chemical pollutants introduced into urban wastewater, enter the water and wastewater environment. The main groups of micropollutants are shown in Fig. 1.

The emergence of residues of pharmaceuticals and their metabolites in treated wastewater and the aquatic environment is one of the research issues currently undertaken by scientists in the field of engineering and environmental protection.

It should be noted that in Polish, European, and even global legislation there are no documents regulating the permissible concentrations of specific pharmaceuticals delivered with wastewater to treatment plants [4].

To date, scientists around the world have analysed about 500 different medicinal substances, mainly found in surface water, demonstrating their harmful effects on animals and microorganisms [5–11].

Non-steroidal analgesics and anti-inflammatory drugs (NSAIDs) are the most commonly used pharmaceuticals in the world. The most popular are salicylic acid derivatives, propionic acid derivatives, and phenylacetic acid derivatives [12].

The presence of NSAIDs and their metabolites in the soil and water environment is the subject of research both in the European Union and worldwide. Due to

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structural differences within NSAIDs, these substances are not removed to the same extent in wastewater treatment or water treatment [13,14]. Lindqvist et al. [13] achieved the highest purification efficiency in the water treatment process for ibuprofen and which ranged from 84%–99%, and the lowest for diclofenac from 9% to 43%.

In general, it should be noted that in most European countries the most abundant group of pharmaceutical substances in wastewater are non-steroidal anti-inflammatory drugs [15].

Stumpf et al. [16] observed that the degree of removal of pharmaceuticals in treated wastewater from 10 selected South American treatment plants ranged from 12% to 90%. As a consequence of the incomplete removal of pharmaceutical residues from wastewater during the treatment process, the river water was contaminated. The average concentration of NSAIDs ranged from 0.02 to 0.04 μg/L, and maximum values were observed up to 0.5 μg/L. The most common substances were: ibuprofen 0.1 μg/L naproxen 0.2 μg/L, ketoprofen 0.2 μg/L, diclofenac 0.2 μg/L.

The main sources of pollution are households and hospitals [17]. In unchanged form or after a slight transformation, as polar molecules, pharmaceuticals are excreted from the body.

Ibuprofen is the most abundant pharmaceutical contaminant in wastewater [18–20].

Table 1 shows the concentration values of selected pharmaceuticals in drinking water.

Since pharmaceuticals do not undergo 100% metabolic processes, they are therefore present in wastewater both in the basic form and as metabolites [27]. The transformation products of pharmaceuticals have properties other than the initial drug, they are most often characterized by water solubility and polar properties, and may be more toxic. It was also noted that some metabolites may undergo hydrolysis in the environment and return to the basic form of the drug [28]. Dangerous pharmaceuticals load enters the environment in the form of leachate from landfills, hospital wastewater, and leachate from necropolis areas. Veterinary pharmaceuticals may also enter the environment in the form of excrement, manure, and leachate from fields and cattle breeding areas. These pharmaceuticals enter groundwater along with wastewater or directly through the soil. A large part of these pollutants goes to the wastewater treatment plant, from where they enter the treated wastewater and with it to the natural environment. This happens because existing treatment plants are not designed to remove chemicals, including pharmaceuticals, from wastewater. In order to improve the efficiency of removing the above-mentioned substances from wastewater, advanced treatment methods can be used, such as ozonation or sonication with simultaneous exposure to UV lamps, or the use of active carbons absorbing chemicals [10,29].

Despite the intensification of wastewater treatment processes through the use of new methods and reactors, for example, sequential biological reactors, it was not possible to obtain (apart from ketoprofen) the complete removal of selected pharmaceuticals from wastewater [17,30–32].

In many research centres, experiments are carried out to improve the existing state of affairs, that is, to find an effective method of removing pharmaceutical residues from the water and soil environment. A promising solution leading to wastewater treatment and water disinfection is the use of an active ultrasonic field, causing physical (shear forces and shock wave) and chemical (cavitation phenomenon) effects conducive to the degradation of pharmaceutical residues [33].

The new approach to the research problem consists in supporting the removal of toxic substances (pharmaceuticals) from wastewater through the active action of

Table 1
Concentration of selected pharmaceuticals in drinking water

Pharmaceutical	Concentration in drinking water, ng/L	Country	References
Bezafibrate	27	Germany	[21]
Estradiol	11.6	France	[22]
	0.3–2.1	Germany	[23]
Diclofenac	6–35	Germany	[21]
	4	Poland	[24]
Carbamazepine	30	Germany	[25]
Clofibric acid	10	Germany	[26]
	5.3	France	[21]
Naproxen	13	Poland	[24]

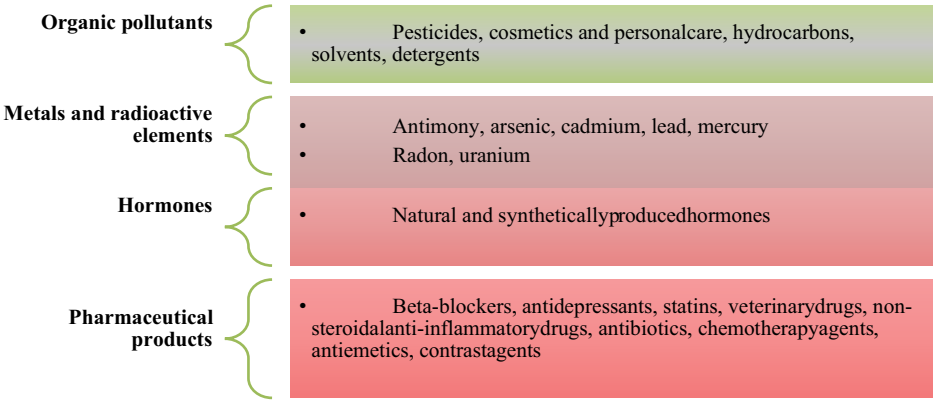

Fig. 1. Main groups of micropollutants in the aquatic environment [4].

the ultrasonic field and optimizing the process in terms of obtaining a decrease in wastewater toxicity. Moreover, it should be noted that the use of sonication to support the removal of selected pharmaceuticals from wastewater is a promising solution, both due to the lack of harmful wastewater treatment products, the possibility of modernizing existing technological lines, and the lack of the need to use additional chemicals necessary to conduct the process. Like any technological process, sonication has limitations, for example, regarding energy requirements, which is why it is necessary to optimize also in this respect.

During the sonication of the solutions, three zones are formed in which cavitation-initiated reactions may occur, namely: a cavitation bubble, the interphase boundary (gas–liquid phase boundary), and the proper solution. The ongoing degradation processes differ between the three zones. Some studies found that hydrophilic and non-volatile compounds decomposed mainly in solution, while hydrophobic, non-polar, and volatile compounds reacted in all three zones.

The literature review indicates the need to remove pharmaceuticals and other micropollutants from wastewater. Significant discrepancies in the previous test results justify conducting tests using an ultrasonic field as a factor ensuring a moderate but constant and repeatable decrease in the concentration of selected pharmaceuticals in urban wastewater. Therefore, the purpose of the conducted research was to determine the possibility of removing from the wastewater selected non-steroidal anti-inflammatory pharmaceuticals (ketoprofen and diclofenac) posing a threat to the water and wastewater environment. The use of the active operation of the ultrasonic field, which results in a number of sono-chemical phenomena, including cavitation or oxidizing processes, is a novelty to the research problem.

2. Experimental part

2.1. Substrate

The substrate for the study was wastewater from a mechanical–biological wastewater treatment plant with the use of highly effective methods of removing biogenic compounds. In unmodified wastewater samples, the sizes of ketoprofen particles in the range of 45–478 μm were observed, the largest percentage share in the given size distribution had particles of the following sizes: 79 μm (17.77%), 91 μm (18.68%) and 104 μm (14.56%). On the other hand, in the case of diclofenac, particle sizes in the range of 30–954 μm were observed, the largest percentage share in the given size distribution had particles with the following sizes: 630 μm (10.72%), 549 μm (10.29%) and 724 μm (10.15%).

The research covered diclofenac (CAS 15307-79-6) and ketoprofen (CAS 22071-15-4), which belong to the group of non-steroidal anti-inflammatory pharmaceuticals, differing significantly in their susceptibility to removal, that is, low and high susceptibility, respectively. Table 2 presents the characteristics of the pharmaceuticals selected for testing. Models of solutions containing the above pharmaceuticals were created. A mixture of selected pharmaceuticals was made by mixing 1 g ketoprofen with 1 g diclofenac, and an aqueous solution was prepared. Table 2 shows characteristics of selected pharmaceuticals.

For the Test DAPHTOXKIT FTM, the medium and dilution medium were aerated for 15–30 min to ensure adequate oxygenation of the solutions. In order to prepare the medium in which the organisms are placed, solutions of $NaHCO_3$, $CaCl_2$, MgSO4 and KCl were added to distilled water. After mixing all ingredients, 2 L of standard medium (medium) was obtained. C1-C5 measuring flasks (100 mL capacity) were prepared and the solution with pharmaceuticals was diluted according to accredited DAPHTOXKIT FTM (*Daphnia magna*) tests. 5 test series were carried out, each consisting of 6 types of samples: 1 - diclofenac solution, 2 - sonicated diclofenac solution, 3 - ketoprofen solution, 4 - sonicated ketoprofen solution, 5 - mixture of diclofenac and ketoprofen (1:1), 6 - mixture diclofenac and ketoprofen (1:1) sonicated.

2.2. Research methodology

2.2.1. Ultrasonic conditioning of samples

In order to select the most favourable operating parameters of the ultrasonic removal of pharmaceuticals from model solutions and conditioning of wastewater with an ultrasonic field, the disintegrating effect of the ultrasonic field was examined. During the tests, the exposure time was extended at constant power and vibration amplitude, which were selected for research on the basis of preliminary research. The selection of the most optimal time of ultrasonic sonication was based on the study of particle size distribution in model solutions and aqueous wastewater solutions. This was done based on the percentage fraction of particles of a given size (Figs. 3 and 4). For each drug, 5 test cycles were carried out using a specific time of sonication.

Samples with a constant volume and concentration of pharmaceuticals selected for testing were subjected to an ultrasonic field with a power of 750 W, a vibration frequency of 20 kHz and a vibration amplitude of 12 μm and an exposure time of 30–240 s. The SONIC ultrasonic generator VIBROCELL VC750 was used for testing. The intensity of the ultrasonic field was approx. 177 W/m^2.

The concentrations of samples subjected to ultrasonic treatment for each type of test in the case of laser particle size analysis were 5 mg/L for ketoprofen and diclofenac; in the case of gas chromatography: 15 mg/L for ketoprofen and diclofenac.

Table 2
Characteristics of selected pharmaceuticals [34–38]

Pharmaceutical	CAS number	Water solubility, mg/L	Biological half-life in water, d	$\log K_{ow}$	$\log K_{oc}$
Diclofenac	15307-79-6	2.4	8	4.51	2.92
Ketoprofen	22071-15-4	51	n.d.	3.12	2.46

2.2.2. Laser particle size analysis

Laser particle size analysis was used to present the size distribution of all particles in the solid–liquid system. Thanks to this method, a quantitative analysis of pharmaceuticals dissolved in aqueous solutions was carried out. The changes in the size of individual fractions of particles in the samples before and after the sonication process were compared. The tests were performed with the Malvern Mastersizer 2000 device.

The final result of the laser diffraction measurement is a volumetric particle size distribution. Laser diffraction does not allow the counting of the tested particles. The recorded distribution is a relative volume distribution, representing the percentage content of individual fractions in the entire volume of the sample [39]. In order to perform the test properly, the samples were tested *n*-fold. The diagram of the laser particle size analysis test bench is shown in Fig. 2.

2.2.3. Bioindication tests

In order to assess the course and effectiveness of ultrasonic degradation of selected pharmaceuticals in wastewater and model solutions, toxicity tests based on indicators (living organisms) were carried out on a laboratory scale. The tests were carried out on licensed, accredited DAPHTOXKIT FTM (*Daphnia magna*) tests [40].

Selected acute toxicity tests are based on the principle of Shelford tolerance, which states that the development of the organism may be disturbed by both the lack and excess of any of the factors [41]. In the case of selected non-steroidal anti-inflammatory pharmaceuticals, the influence of excess (presence) on test organisms was examined, which allowed for determining the tolerance range of the organisms.

24-h daphnia (*Daphnia magna*) organisms, supplied with the purchased test in the form of cysts and from own breeding, were used for the tests. The culture of test organisms was continuous, taking into account the daily irradiation rhythm and darkness: 12 h day and 12 h night. The culture temperature was constant at 20°C–22°C. All organisms used in bioindication tests showed no signs of disease. Own-bred organisms were fed with powdered spirulina (*Arthrospira platensis*).

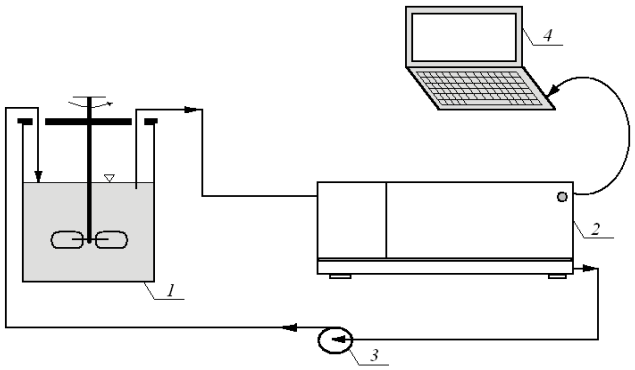

Fig. 2. Test bench for particle size distribution analysis: (1) reaction tank with agitator, (2) particle size analyzer, (3) pump, (4) computer unit, [own elaboration].

The 48-h test selected for the research allows for the determination of both immobilisation (EC_{50}) and mortality (LC50). The DAPHTOXKIT FTM test complies with the recommendations and guidelines of the OECD Guideline 202 and ISO 6341, which allows for easy interpretation of the results and its adaptation to European Standards [42,43]. The number of immobilized and dead individuals was calculated after 24 and 48 h of the test [44].

The effectiveness of the tested methods of pharmaceutical degradation was estimated based on the degree of reduction of the toxic effect of ketoprofen and diclofenac, and their mixture (in a 1:1 weight ratio). For each drug and their mixtures, 10 test cycles were carried out with a predetermined sonication time: 30–240 s. In order to determine the degree of reduction, the EC_{50} values obtained from the samples subjected to sonication were compared with the unmodified samples, considered as a reference point in the determination of toxicity reduction.

3. Results and discussion

3.1. Effect of sonication on particle size and concentration of selected pharmaceuticals

It was observed that the size of the particles conditioned within the ultrasonic field decreases during the sonication to a certain time, defined as the most effective and beneficial time of sonication of the samples.

According to the literature data [45,46], there are processes that may be an alternative solution to those currently used methods of wastewater treatment, for example, use of Fenton's reagent, hydrogen peroxide oxidation, also playing a supporting role in relation to the applied solutions, especially those based on intensive oxidation. The effectiveness of the sonication process depends on the power of the generator and the time of sonication of the samples, as well as the additives used [47].

Figs. 3 and 4 show the relationship between the size of ketoprofen and diclofenac particles and the exposure time of the ultrasonic field of 0, 30, 60, 120, and 240 s.

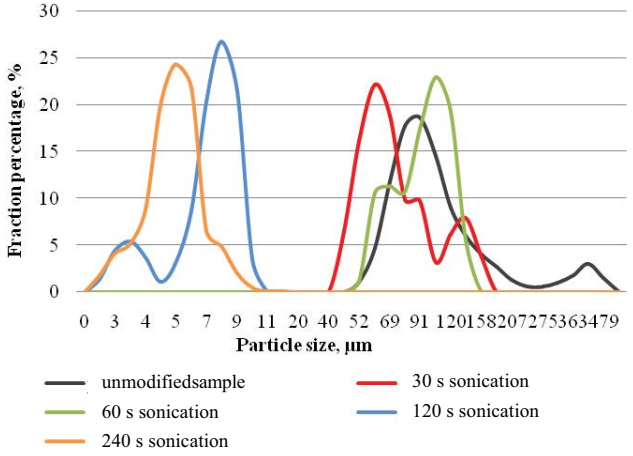

Fig. 3. The relationship of changes in the size of pharmaceutical particles (ketoprofen) on the exposure time of the ultrasonic field.

In non-sonicated samples containing ketoprofen, particle sizes in the range of 45–478 μm were observed, the largest percentage in a given size distribution were particles of 79 μm (17.77%), 91 μm (18.68%) and 104 μm (14.56%). In samples subjected to 30 s sonication, particle sizes decreased to 39–158 μm, and the largest proportion was 22.18% for the 60 μm molecule. During the 60 s sonication, the particle size range decreased: 52–138 μm, with the largest proportion of particles: 104 μm (22.19%), 120 μm (19.26%), and 91 μm (17.43%). During the 120 s sonication, a noticeable reduction in particle size to 2–11 μm was observed, the particle with the highest percentage was 7 μm (26.77%). After a 240 s sonication, particle sizes ranged from 2 to 13 μm, with the largest having 5 μm (24.32%).

Particle sizes in the range of 30–954 μm were observed in non-sonicated samples containing diclofenac, the largest percentage in a given size distribution were 630 μm (10.72%), 549 μm (10.29%) and 724 μm (10.15%). In samples subjected to 30 s sonication, the particle sizes decreased to a size of 45–104 μm, and the largest proportion was 30.93% for the 79 μm molecule. During the 60 s sonication, the particle size range decreased significantly: 5–11 μm, with the largest proportion of particles: 7 μm (39.47%) and 6 μm (25.56%). During the 120 s sonication, particle sizes in the range of 5–45 μm were observed, the particle with the highest percentage was 34 μm (25.17%). After a 240 s sonication, particle sizes ranged from 5 to 11 μm. This sample was the most homogeneous, with the largest proportion of 5 μm particles (as much as 45.79% of all particles).

For the selected exposure times, the most favourable particle size decreases were obtained compared to their initial value:

Ketoprofen – the most favourable exposure time was 240 s, particle size decreased from 45,709–47,863 μm to 2,512–13,183 μm. However, the size of the most numerous particles in the size distribution decreased from 91.201 μm (with a proportion of 18.68%) for the unmodified sample to 5.012 μm (24.32%).

Diclofenac – the most favourable exposure time was 240 s, particle sizes decreased from 30,199–954,992 μm to 5,011–11,481 μm. However, the size of the most numerous particles in the size distribution decreased from 630.957 μm (10.72%) for the unmodified sample to 5.754 μm (45.79%). Sonication for 60 s was also favourable for diclofenac, particle sizes were in the range of 5.754–11.481 μm, and the largest proportion (39.47%) was 7.785 μm. Despite the equally favourable effect in the case of 60 s sonication, in order to unify the methodology, the same sonication time for both investigated pharmaceuticals was used, amounting to 240 s.

The changes in the percentage concentration of the test substance over time were also measured. Fig. 5 shows the concentration values for ketoprofen and diclofenac at the time of sonication for the three-test series.

It was found that subjecting pharmaceuticals to exposure in an ultrasonic field contributed to changes in the concentration of the test substances (for each test sonication time). It was noted that with the extension of the sonication time for all test series, a decrease in the concentration of selected pharmaceuticals was observed.

The scope of possibilities of using the active action of the ultrasonic field to remove micropollutants from wastewater and the water-sewage environment has also been included in the research by other authors [48–54]. A high degree of removal was obtained for long sonication times, for example, 30 or 90 min (up to 100%), as well as in the case of using the additive 30% H_2O_2 (the degree of reduction was 40–60%) [55,56]. Zupanc et al. [57] conducted similar research, based on the ultrasonic decay of diclofenac, where sonication was carried out for 60 min using a probe with parameters: 400 W and 20 kHz. A 55% reduction in diclofenac was obtained, with an initial concentration of 80 mg/L. Despite the much longer sonication time, a lower degree of reduction was obtained, which could be due to the use of a device with lower power. Nie et al. [58] also obtained comparable results, the degree of reduction during 15 min of sonication was 32% for diclofenac and 0% for ketoprofen. The lower degree of removal of the pharmaceuticals may have been caused by the way the sample flowing through the device was sonicated over time, rather

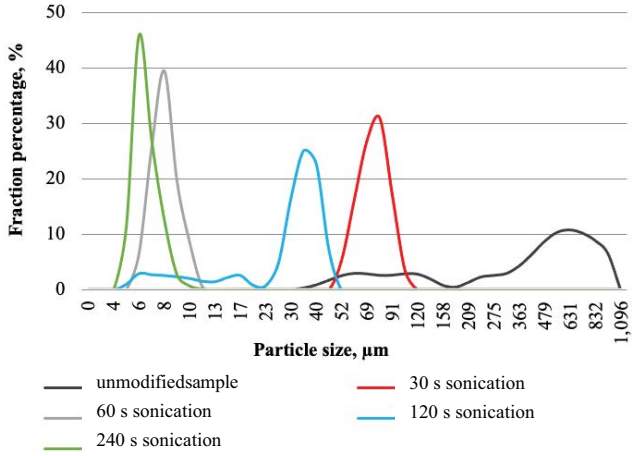

Fig. 4. The relationship of changes in the size of pharmaceutical particles (diclofenac) on the exposure time of the ultrasonic field.

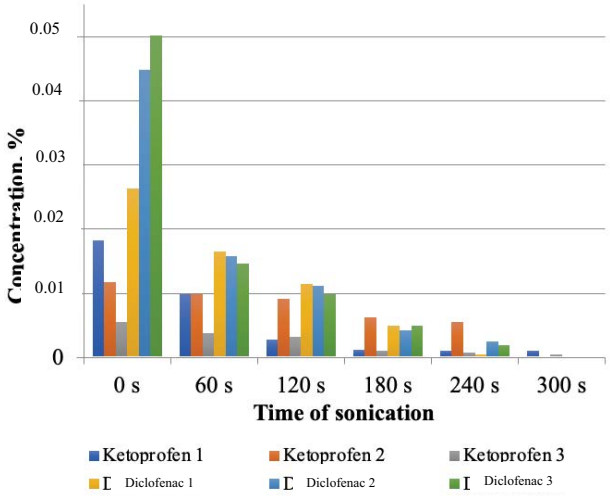

Fig. 5. Change in ketoprofen and diclofenac concentrations during sonication for three-testseries.

than a static liquid tank. The degree of removal of pharmaceuticals increased significantly after the combination of the hydrocavitation method with the oxidizing agent H_2O_2 and amounted to 36% and 20%, respectively, for the same sonication time and 77% and 52% for 30-min sonication.

Diclofenac is a non-volatile hydrophilic compound, therefore the effect of $OH^•$ on the diclofenac molecule in the proper solution was considered to be the main reaction occurring in the ultrasonic field. Since the test pharmaceutical molecules contain polar and non-polar groups, they may behave differently in three zones of the reaction solution when exposed to the ultrasonic field. For example, diclofenac contains one non-polar moiety (2,6-dichloroaniline) and one polar moiety (phenylacetic acid) [59]. According to the literature data [60], the sonication of the diclofenac solution causes the release of Cl ions. The concentration of released Cl^- ions produced by sonication corresponds to the primary growth curve. Since there are two chlorine atoms in each diclofenac molecule, this indicates that during ultrasonic degradation of diclofenac, the first and main reaction was dechlorination. So far, there is little information on the ultrasonic degradation of ketoprofen. Successful degradation of ketoprofen was observed in advanced oxidation processes (AOP), combining UV irradiation, chemical additives, for example, H_2O_2, Fenton's reaction, and the ultrasonic field. It was observed that the use of ultrasounds increases the formation of radicals, including hydroxyl ones [61]. Since sonication is also part of advanced oxidation processes, a similar mechanism of ultrasonic degradation of ketoprofen can be adopted based on the known mechanisms of ketoprofen degradation in other processes (AOP). In addition, it is known that there are two main mechanisms for removing organic pollutants during sonolysis: pyrolysis reactions in cavitation bubbles and radical reactions of $H^•$, $OH^•$ radicals formed during sonolysis of water [62].

3.2. Toxicity of pharmaceuticals treated with ultrasonic fields, based on EC_{50} fluctuations

Toxicological tests performed on *Daphnia magna* included acute toxicity studies and lethality analysis for a given concentration of the toxic substance (selected NSAID). It was observed that subjecting pharmaceutical solutions to the ultrasonic field conditioning process contributed, for each sonication time, to changes in the level of toxicity. It was noted that the longer the ultrasonic field was used, the more significant the toxicity drops were: from 2% for 60 s of sounding, up to about 30%–50% for 240 s of exposure. Minimal deterioration of sample parameters and an increase in toxicity were observed only in the case of 30 s sonication. A comparison of changes in the EC_{50} concentration value of non-steroidal anti-inflammatory pharmaceuticals for *Daphnia magna* for all sonication times in subsequent test series is shown in Fig. 6.

Table 3 shows the toxicity results for *Daphnia magna* for unmodified samples.

The average EC_{50} value of, out of 10 test series, unmodified samples containing ketoprofen was 76.3 mg/L, while for diclofenac it was 103.4 mg/L. The highest toxicity was characterized by a mixture of both pharmaceuticals in a 1:1 weight ratio, with an average EC_{50} value of 58 mg/L. Assuming a 95% confidence interval, it can be assumed that the toxicity value for individual pharmaceuticals was in the range of: 1.27–1.34 TU for ketoprofen, 0.95–0.98 TU for diclofenac and 1.69–1.75 TU for a mixture of both pharmaceuticals.

There is relatively little data in the literature on the effects of diclofenac and ketoprofen on the fauna and flora inhabiting aquatic ecosystems. Haap et al. [63] in his study obtained an EC_{50} diclofenac score of about 68 mg/L. Czech et al. [64] reported similar toxicity of diclofenac at 70 mg/L. Barceló et al. [65] obtained a much lower EC_{50} value of 3.2 mg/L, which indicates significantly higher toxicity than other sources. According to Nosek [66] EC_{50} of diclofenac was equal to 22 mg/L, which indicates much higher toxicity.

The toxicity value for the mixture was greater than the predicted value which is the sum of the toxicity of the pharmaceuticals in the mixture, which may indicate synergy. The synergy effect can be described as the interaction of different factors, the effect of which is greater than the sum of the individual separate actions. In the case of the toxicity tests performed, it was found that the two pharmaceuticals that had little effect on the test organism, when applied together, gave a strong effect. Synergy in this case may consist both in the interaction of the active substances of individual pharmaceuticals at the biochemical level and in the synergistic response of the organism to the toxin (at the physiological level).

The sonication of the samples for 240 s produced the best results and resulted in the greatest decrease in the toxicity effect of ketoprofen, diclofenac, and their mixture. The mean value of EC_{50}, out of 10 test series, for the sonicated samples containing ketoprofen was 107.8 mg/L, while for diclofenac it was 133.7 mg/L. The highest toxicity was characterized by a mixture of both pharmaceuticals in a 1:1 weight ratio, with an average EC_{50} value equal to 86.9 mg/L. Assuming a 95% confidence interval, it can be assumed that the toxicity value for individual pharmaceuticals was in the range of: 0.58–1.10 TU for ketoprofen, 0.74–0.76 TU for diclofenac and 1.13–1.17 TU for a mixture of both pharmaceuticals. A decrease in toxicity of approximately

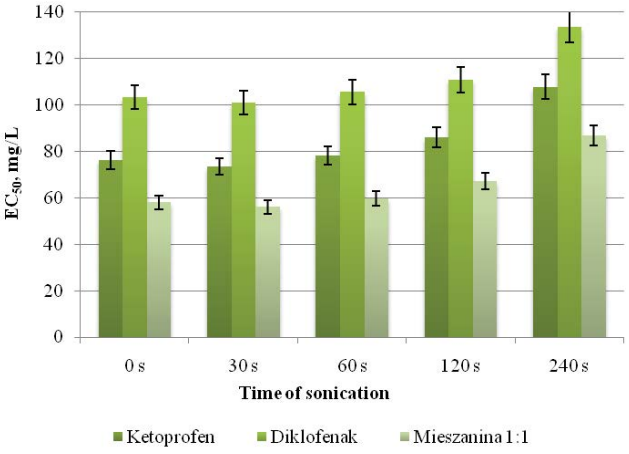

Fig. 6. Changes in EC_{50} concentration values of (average of 10 series) non-steroidal anti-inflammatory pharmaceuticals for *Daphnia magna* in subsequent test series, sound time: 0, 30, 60, 120, 240 s.

41.28% was observed for ketoprofen, 29.3% for diclofenac, and 49.82% for their mixture. The results from the samples sonicated for 240 s are presented in Table 4.

For the selected exposure times, the most favourable decreases in EC_{50} concentration compared to their initial value were obtained:

Ketoprofen – the most favourable exposure time was 240 s, the average value of the effective EC_{50} concentration increased from 76.3 mg/L for non-modified samples to 107.8 mg/L for samples subjected to 240 s sonication, which indicates a decrease in the value of the unit of toxicity from 1.307 to 0.839 TU.

Diclofenac – the most favourable exposure time was 240 s, the average value of the effective EC_{50} concentration increased from 103.4 mg/L for non-modified samples to 133.7 mg/L for samples subjected to 240 s sonication, which indicates a decrease in the value of the unit of toxicity from 0.966 to 0.748 TU.

Mixture – the most favourable exposure time was 240 s, the average value of the effective EC_{50} concentration

Table 3
Results of toxicological tests for *Daphnia magna*

Series	Pharmaceuticals					
	Ketoprofen		Diclofenac		Mixture 1:1	
	EC_{50}, mg/L	TU	EC_{50}, mg/L	TU	EC_{50}, mg/L	TU
1	75	1.33	100	1.00	60	1.67
2	79	1.26	102	0.98	57	1.75
3	76	1.31	106	0.94	59	1.69
4	77	1.29	103	0.97	56	1.78
5	73	1.37	104	0.96	58	1.72
6	76	1.31	103	0.97	58	1.72
7	78	1.28	105	0.95	57	1.75
8	74	1.35	101	0.99	57	1.75
9	76	1.31	104	0.96	58	1.72
10	79	1.26	106	0.94	60	1.67
$\bar{x}$	76.3	1.31	103.4	0.97	58	1.72
σ	1.90	0.034	1.907	0.019	1.264	0.034
P	74.49–78.10	1.27–1.34	101.59–105.21	0.95–0.98	56.80–59.20	1.69–1.75

$\bar{x}$ ·mean value, σ standard deviation, P confidence interval.

Table 4
Results of toxicological tests for *Daphnia magna* after 240 s sonication

Series	Sonicated pharmaceuticals					
	Ketoprofen		Diclofenac		Mixture 1:1	
	EC_{50}, mg/L	TU	EC_{50}, mg/L	TU	EC_{50}, mg/L	TU
1	106	0.94	130	0.77	90	1.11
2	110	0.01	133	0.75	86	1.16
3	109	0.92	136	0.73	89	1.12
4	109	0.92	134	0.75	84	1.19
5	104	0.96	135	0.74	87	1.15
6	108	0.93	132	0.76	85	1.18
7	107	0.93	133	0.75	88	1.14
8	109	0.92	134	0.75	87	1.15
9	108	0.93	134	0.75	87	1.15
10	108	0.93	136	0.73	86	1.16
$\bar{x}$	107.8	0.84	133.70	0.75	86.9	1.15
σ	1.66	0.28	1.73	0.011	1.7	0.023
P	106.22–109.38	0.58–1.10	132.05–135.35	0.74–0.76	85.28–88.51	1.13–1.17

$\bar{x}$ average value, σ standard deviation, P confidence interval

increased from 58 mg/L for unmodified samples to 86.9 mg/L for samples subjected to 240 s sonication, which indicates a decrease in the value of the unit of toxicity from 1.722 to 1.151 TU.

Most of the research currently carried out by scientists is carried out under strictly defined laboratory conditions, and the microorganisms or fish studied come from their own breeding or from sterile surviving eggs, which may affect the results of the conducted experiments [67]. Fent et al. [68,69] identified the risk resulting from the toxicity of pharmaceuticals to fauna and flora as unlikely. In addition, it was noted that acute toxicity studies are usually conducted at concentrations of pharmaceuticals 100–1,000 times higher than those observed in the aquatic environment. For example, the toxicity of diclofenac towards plankton was determined as 14.5–22.43 mg/L (EC_{50}), while the confirmed presence of this drug in wastewater is several ng/L [70]. The exceptions are, for example, hormones, diazepam, or neurological pharmaceuticals, which show toxicity already in concentrations of several ppm [67]. However, it should be emphasized that a significant disadvantage of the conducted tests is a very short exposure to a toxic agent, causing disorders of vital functions and lethality, rarely allowing to observe changes occurring from generation to generation. In individual cases, however, the phenomenon of bioaccumulation of pharmaceuticals in animal organisms was confirmed. This applies, among others, to diclofenac, whose presence has been confirmed in the kidneys of a vulture [71] and the liver, kidneys, and gills of rainbow trout [72]. In order to assess the full risk posed to man and the environment by pharmaceuticals, a series of tests should be carried out, taking into account the multigenerational exposure and mapping the complex environmental conditions as closely as possible. Fent et al. [67] showed that standard tests may underestimate the risk, because the toxicity of the pharmaceutical mixture may be significantly higher than the individual toxicity of each drug.

The effectiveness of the studied sonication process may be lower than in the case of absorption processes, membrane techniques or oxidation/reduction processes, but it creates greater possibilities when transferring the phenomenon to a technical scale, especially in the case of older wastewater treatment plants (ease of implementation in an already existing technological line) [60].

4. Summary and conclusions

The presence of pharmaceuticals in the water and wastewater environment is an issue that requires a wider diagnosis and, consequently, the implementation of the method of their effective removal in the technological process of wastewater treatment.

Based on the review of the literature data and the conducted own research, the following conclusions were formulated:

- The exposure of organic micropollutants (NSAIDs) to the ultrasonic field can lead to degradation of these substances and reduce their concentration, providing an effective method of removing pharmaceuticals from wastewater.
- As a result of subjecting the tested pharmaceuticals to sonication (ultrasonic field parameters: 750 W power, 20 kHz frequency and 12 μm vibration amplitude), the largest, more than 200-fold decrease in particle size.
- In a solution from 1,096 to 5 μm and a decrease in the concentration of pharmaceuticals was obtained. For ketoprofen there was a reduction in concentration from 12 to 0.1 mg/L, and for diclofenac from 15.1 to 0.23 mg/L.
- For selected, most favourable sonication conditions, a decrease in the value of the toxicity unit was obtained for ketoprofen from 1.307 to 0.839 TU, for diclofenac from 0.966 to 0.748 TU, for the mixture from 1.722 to 1.151 TU.

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