

Formation of micropollutant decomposition by-products during oxidation processes supported by natural sunlight

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Received 14 November 2019; Accepted 18 January 2020

ABSTRACT

The paper presents the comparison of micropollutant removal degrees after the O_3 and O_3/H_2O_2 processes supported by sunlight radiation. These tests were conducted on selected organic micropollutant water solutions prepared on the base of deionized water. The examined compounds belong to the group of pharmaceuticals (benzocaine, caffeine, carbamazepine (CBZ), diclofenac sodium salt, ibuprofen sodium salt (IBU)), hormones (β -estradiol, 17α -ethinylestradiol, mestranol, progesterone), pesticides (trilalate, triclosan, oxadiazon), food additives (butylated hydroxytoluene (BHT)) and dyes (acridine). Processes were carried out at various O_3 doses: 1, 2, 3, 5 and 10 mg/L. The reaction vessels containing the test solutions were exposed to solar radiation for 10, 20, 30 and 60 min. The process effectiveness and the identification of the formed compound decomposition by-products were assessed by the use of gas chromatography coupled with mass detection based on the NIST v17 mass spectra database. It was noted that the presence of another type of oxidant in the form of H_2O_2 during the O_3 /sun light process results in the reduction of the half-life of CBZ, IBU, BHT, and all tested hormones and pesticides. On the other hand, the presence of H_2O_2 affected the type and the number of generated micropollutant intermediates. The highest compound removal degrees, which exceeded 92%, were noted during the implementation of sunlight supported process for micropollutants belonging to the group of hormones and pesticides. Also, it was observed that the irradiation of samples with sunlight resulted in a decrease in the number of formed decomposition by-products. The conducted toxicological analysis confirmed the reduction of the toxic nature of samples subjected to sunlight assisted by the O_3/H_2O_2 process compared to samples treated by the action of O_3/H_2O_2 performed in a dark chamber.

Keywords: Organic micropollutants; Decomposition by-products; Sunlight; Toxicity

1. Introduction

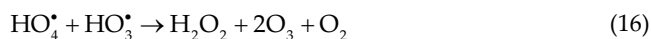
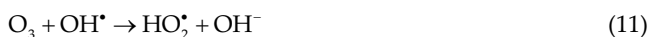
Pollution of the water environment with different types of contaminants in the light of the constantly decreasing availability of drinking water resources become one of the major problems of modern humanity. Micropollutants detected in surface and groundwater consists of various materials and can contain different types of metabolized or non-metabolized compounds such as pharmaceuticals, personal care products, hormones, pesticides, flame retardants,

waterproofing agents, plasticizers, food and industrial additives [1–3]. Effluents from wastewater treatment plants based on activated sludge treatment methods are considered to be the main sources of several types of micropollutants that is, inorganic and organic compounds [4] and microplastic [5] in the environment. Hey [6] reported that on average 70% of pharmaceutical compounds in wastewater come from a household, 20% originates from livestock farms, only 5% comes from hospital wastewater, and 5% is introduced in the form of runoff from nanoparticulate sources. Pesticides enter

the environment with surface runoff from agricultural areas, which for example constitute about 43% of the total land area of the European Union countries [7]. The most often used classes of pesticides are herbicides, fungicides, bactericides and insecticides [8].

The implementation of advanced oxidation processes based on the action of O_3 or H_2O_2 allowed the removal of a large number of hardly-biodegradable compounds [9,10]. On the other hand those in-depth oxidation processes lead to the generation of intermediates, which are the main reason for water quality deterioration [11]. The newly formed compounds increase the toxicity of the treated water matrix [12] and cause sublethal biological responses of organisms that stay in contact with water [13,14].

Because O_3 molecules are very unstable in water media, the introduction of this oxidant to water initiates different reactions from (1) to (16), leading to the transformation of O_3 to O_2 and also to the formation of reactive radicals [15]. The strongest oxidation potential after fluoride, with a standard redox potential equal to 2.8 V/SHE, and a nonselective character is attributed to OH^\bullet radicals [16].



The process of ozonation can be carried out as an effective single method or combined with other oxidizing agents or UV radiation in order to increase its efficiency and reduce process times. O_3 -based processes can be used for the treatment of industrial wastewater, for example, Lucas et al. [17] showed that O_3/UV and $O_3/UV/H_2O_2$ processes can treat winery wastewaters. Kim and Tanaka [18] reported that the comparison of O_3/H_2O_2 and O_3/UV by an O_3 dose equal to 6 mg/L and a 15 min contact time, can reduce the concentration of caffeine (CAF), N,N-diethyl-meta-toluamide and cyclophosphamide by 84%, 89%, and 46%, respectively, Chávez et al. [19] tested the process of UVA-LED photocatalytic ozonation and also achieve a nearly 90% removal of micropollutants occurring in municipal wastewater secondary effluent. Also, the process of heterogeneous catalysis can be applied in various branches of environmental chemistry because it allows for obtaining a different kind of species using various types of catalysts [20]. For example, the use of TiO_2 in the process of heterogeneous photocatalysis is one of the best-known processes for the decomposition of many types of contaminants [21,22]. However, it should be noted that the combination of some well-known processes in water treatment technologies like chlorination with other advanced processes can lead to the formation of intermediates with a larger number of Cl atoms in their structure than in the structure of the parent organic pollutant [23]. Newly formed compounds can have high biological activity and can be considered very toxic to the environment.

Some researchers classify micropollutants of anthropogenic origin to the group of compounds of emerging concern, which can have a possible harmful effect on water organisms and human health. Therefore it is necessary not only to develop methods for their removal but also methods, which guarantee the mineralization of the risk to form biologically active decomposition by-products. There is a need to evaluate the removal degrees of each type of compound in different conditions with special attention to toxicological assessment (acute and chronic toxicity) of post-processed waters carried out by the use of toxicity tests using different indicator organisms.

The paper presents the comparison of removal degrees of selected organic micropollutants most commonly occurring in the water environment, subjected to the O_3 and O_3/H_2O_2 process supported by sunlight. The obtained results were compared to the O_3/H_2O_2 conducted in a dark chamber. The influence of the O_3 dose and the time of sunlight exposure on the generation of possible compound decomposition by-products were assessed. Also, toxicological analyzes of post-process water solutions were carried out using three biotests: Microtox[®], DAPHTOXKIT F magna, and Lemna sp. Growth Inhibition Test (GIT).

2. Material and methods

2.1. Material and reagents

The analytical standards of all tested compounds that is, pharmaceuticals: benzocaine (BE), CAF, carbamazepine (CBZ), diclofenac sodium salt (DCF) and ibuprofen sodium

salt (IBU); hormones: β -estradiol (E2), 17 α -ethinylestradiol (EE2), mestranol (EEME) and progesterone (P4); pesticides: triallate (TRI), triclosan (TCS) and oxadiazon (ODZ); food additives: butylated hydroxytoluene (BHT); and dyes: acridine (ACR) of a purity grade >97% were supplied by Sigma-Aldrich (Poznań, Poland). Table 1 summarizes the chemical characteristic of the tested micropollutants. Organic solvents in the form of methanol (MeOH), dichloromethane (DCM) and acetonitrile (ACN) with a purity over 99.5% were used during the preparation of compound standard solutions and the process of solid-phase extraction (SPE) were obtained from Avantor Performance Materials Poland S.A. (Gliwice, Poland). SPE cartridges Supelclean™ ENVI-8 and ENVI-18 equipped with a silica gel base material with C₈ (octyl) and C₁₈ (octadecyl) bonding respectively were supplied by Sigma-Aldrich (Poznań, Poland). The bed weight of both types of SPE cartridges was equal to 1,000 mg with a 60 Å pore size and a total surface area of 475 m²/g.

2.2. Decomposition processes

Both, O₃ and O₃/H₂O₂ sunlight supported compound decomposition processes were carried out in glass batch reaction vessels with a volume of 1.0 L. The vessels were placed on a magnetic stirrer to ensure continuous mixing of the reaction mixture. In order to estimate the influence of sunlight on the course of the decomposition reactions, the O₃/H₂O₂ process was carried out comparatively in a dark chamber. The O₃ used in the experiments was generated from fresh air in the Ozone FM500 generator by WRC Multiozon (Sopot, Poland). The generated O₃ was introduced to the reaction vessels through a ceramic diffuser, which was placed 1 cm above the bottom of the vessel. The processes were carried out at different O₃ doses: 1.0, 2.0, 3.0, 5.0 and 10.0 mg/L (O₃ dose measured immediately after its introduction to the water matrix). The concentration of O₃ was measured photometrically using the Spectroquant® Ozone Test by Merck KGaA (Darmstadt, Germany) in a sample taken from the middle of the reaction mixture. The dose of H₂O₂ was determined during preliminary studies and set on 9 mg/L. The reaction vessels containing the test solutions were exposed to sunlight radiation for 10, 20, 30 and 60 min. The experiments were conducted during the European summer period between July and August, where the average illumination intensity was 850±45 mW/cm² and the measured air temperature ranged from 22.2°C to 29.4°C. The temperature of water solutions exposed to O₃ and O₃/H₂O₂ supported by the action of sunlight ranged from 24°C to 26°C. Whereas the temperature of the solution in the dark chamber experiment was kept at a level of 24°C±1°C. The ozonation reaction was stopped by the introduction to the reaction mixture 24 mmol/L of Na₂SO₃ by Sigma-Aldrich (Poznań, Poland).

Experiments for all tested micropollutants were carried out separately.

2.3. Water samples

The tested micropollutant water solutions were prepared based on the deionized water with the addition of compound standard solutions. The standard solutions were prepared

by dissolving 10 mg of each tested compound in 10 mL of MeOH. The micropollutant concentration in the prepared water solution was set on 0.5 mg/L. This high concentration allowed for the proper identification of the generated compound decomposition by-products. The pH of all water solutions was adjusted to 7.0 using 0.1 mol/L NaOH. The used volume of NaOH did not show any significant effect on the decomposition of the tested compounds before the implementation of the sunlight supported O₃ and O₃/H₂O₂ processes.

2.4. Analytical procedure

The concentration of the tested micropollutants in the solutions before and after the implementation of selected decomposition processes as well as the identification of generated decomposition intermediates was estimated by the use of gas chromatography coupled with mass detection GC-MS(EI) performed by 7890B gas chromatograph by Agilent Technologies (Santa Clara, United States).

The chromatographic quantitative-qualitative analysis was preceded by solid-phase SPE of the micropollutants that occurred in the tested water solutions. They analyzed the volume of water solutions that were equal to 20 mL and the pH of each sample was adjusted to 7.0 by the use of 0.1 mol/L HCl (purity grade >99.8%) from Avantor Performance Materials Poland S.A. (Gliwice, Poland). The correction of pH was necessary especially in samples after the sunlight supported processes, during which an increase in pH to a value of up to 8.3 was observed. Details of the used SPE extraction for different compounds were summarized in Table 2. Recovery of the micropollutants after the implemented SPE conditions exceeded 96%.

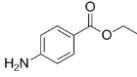

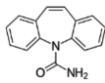
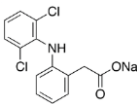
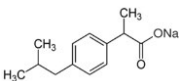
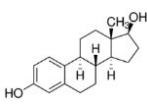
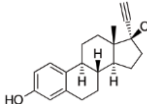
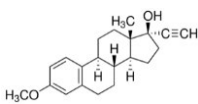
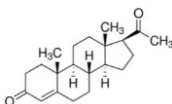
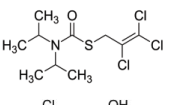
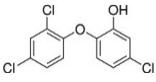
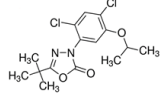
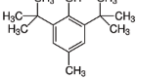

The obtained micropollutant extracts were injected into the chromatographic capillary column SLB™ - 5 ms 30 m × 0.25 mm of 0.25 µm film thickness by Sigma-Aldrich (Poznań, Poland). The carrier gas (helium 5.0) flow rate was set on 1.1 ml/min. The injector temperature was equal to 250°C. The oven temperature program started with 80°C (held for 6 min) and then the oven was heated 5°C/min up to 260°C, 20°C/min up to 300°C (held for 2 min). The temperature of the ion trap and ion source was set at 150°C and 230°C respectively. The quantitative analysis was operated in the selected ion monitoring mode, while the qualitative analysis was performed in the total ion current (TIC) model ranged from 50 to 400 m/z.

The percentage of removal of each tested micropollutant after the application of the decomposition processes was calculated by the determining of the initial C_i and post-processed C_p compound concentrations (mg/L) according to Eq. (17):

$$\text{Removal}(\%) = \frac{C_i - C_p}{C_i} \times 100 \quad (17)$$

The identification of compound decomposition by-products in the post-processed samples was made based on their mass spectra, which were compared with the United States National Institute of Standards and Technology NIST v17 Mass Spectral Library.

Table 1
Chemical characteristics of investigated micropollutants [24]

Compound	Structural formula	CAS No	Molecular weight (g/mol)	Solubility in water (mg/L)	pK _a	logK _{ow}
BE		94-09-7	165.19	1,310	2.51	1.86
CAF		58-08-2	194.19	21,600	14.0	-0.07
CBZ		298-46-4	236.30	17	2.30	2.45
DCF		15307-79-6	318.13	50	4.15	0.57
IBU		31121-93-4	228.26	100	4.91	3.30
E2		50-28-2	272.38	3.6	10.33	4.01
EE2		57-63-6	296.40	11.3	10.33	3.67
EEME		72-33-3	310.43	1.13	17.59	4.61
P4		57-83-0	314.46	8.81	18.92	3.87
TRI		2303-17-5	304.66	2.00	- ^a	4.60
TCS		3380-34-5	289.54	10.00	7.9	4.76
ODZ		19666-30-9	345.22	0.70	- ^b	4.80
BHT		128-37-0	220.35	0.6	12.23	5.10
ACR		260-94-6	179.22	38.4	5.6	3.40

^ano data; ^bnon-ionizable;

Table 2
SPE details for tested organic micropollutants

Compound	BE, CBZ, DCF, IBU, BHT	CAF, ACR, TRI, TCS, ODZ	E2, EE2, EEME, P4
Cartridge type	Supelclean™ ENVI-8	Supelclean™ ENVI-18	
Bed conditioning	5.0 mL of MeOH	5.0 mL of ACN; 5.0 mL of MeOH	3.0 mL of DCM; 3.0 mL of ACN; 3.0 mL of MeOH
Volume of deionized water used for bed washing (mL)	5.0		
Sample flow (mL/min)	1.0		
Vacuum drying time after sample filtration (min)	5.0		
Extract elution	3.0 mL of MeOH	1.5 mL of MeOH; 1.5 mL of ACN	2.0 mL of DCM; 1.5 mL of ACN; 1.5 mL of MeOH

The results with the marked error bars presented in all figures are the arithmetic average of three replicates of each experiment. The error bars' ranges were estimated based on the standard deviation and did not exceed 4%.

2.5. Toxicity tests

The toxicity of the post-process micropollutants water solution was estimated by three different biotests, that is, Microtox® test, DAPHTOXKIT F® test, and *Lemna* sp. GIT.

The Microtox® bioassay measured the changes in the behavior of bioluminescent saltwater bacteria *Aliivibrio fischeri*. The test was carried out by the use of the Microtox analyzer Model 500 by Modern Water (London, United Kingdom) according to the Screening Test procedure of MicrotoxOmni system. The Daphtokit F® test measures the immobility or mortality of freshwater crustaceans *Daphnia magna* after a 24 h exposition to the tested water samples. Whereas the *Lemna* sp. GIT base on the measurement of the number of plant fronds of freshwater vascular plants *Lemna minor*, which grows for 7 d in the tested water solution. The toxicity effect for both the Daphtokit F® test and the *Lemna* sp. Growth Inhibition Test was calculated by Eq. (18). Details of the conducted test were given in [24].

$$E = \frac{(N_c - N_T)}{N_c} \times 100 \quad (18)$$

where E – toxicity effect (%); N_c – number of lively organisms (plant fronds) in the control sample; N_T – number of lively organisms (plant fronds) in the test sample.

The obtained toxicity results were interpreted based on the toxicity classification system presented in Table 3 [25,26].

3. Results and discussion

3.1. O_3 and O_3/H_2O_2 decomposition process supported by sunlight

Fig. 1 presents the results of the first stage of the study that focused on the determination of the optimum O_3 concentration during the O_3 /sunlight process. Five different

O_3 doses (1.0, 2.0, 3.0, 5.0 and 10.0 mg/L) were tested. The micropollutant water solutions were exposed to 10 min of sun lightening after the introduction of the chosen dosage of O_3 . It was noted, that the removal degree of all tested micropollutants increased with the increase of the O_3 dose. The highest differences in compound decomposition were noted between the O_3 concentration of 5.0 and 10.0 mg/L. For example, the removal of IBU after the implementation of the process with a dose of 5.0 mg/L of O_3 was equal to 26% and increased to 52% by doubling the O_3 dose. In general, compounds from the group of pharmaceuticals were characterized by a low ozone caused removal degree compared to other tested micropollutants. The lower decomposition was noted for DCF solutions and ranged from 2% for the 1 mg/L O_3 dose to 17% for 10 mg O_3 /L. Coelho et al. [27] noted that the mineralization of DCF and IBU during the O_3 and O_3 /UV-VIS processes did not exceed 30%.

It should be noted that exposure to sun lightening had a beneficial impact on the O_3 decomposition of compounds. Previous studies [24] dedicated to the dark chamber O_3 decomposition of micropollutants indicated only a slightly decrease in their concentrations. For example, the removal degree of micropollutants by the dose of 1 mg/L of O_3 did not exceed 10% and for the dose of 10 mg/L ranged from 2% for CAF to 52% achieved for the TCS decomposition. The sunlight radiation compared with the action of O_3 and other radicals generated during the reaction from (1) to (16) mentioned above, allowed for the increase of TCS decomposition to over 81% by the use of 5 mg O_3 /L and 94% for the O_3 dose equal to 10 mg/L. Muhamad [28] indicated that the source of

Table 3
Toxicity classification system of water samples [25,26]

Effect (%)	Description	Toxicity class
<25.00	Non toxic	I
25.00–50.00	Low toxic	II
50.01–75.00	Toxic	III
75.01–100	Highly toxic	IV

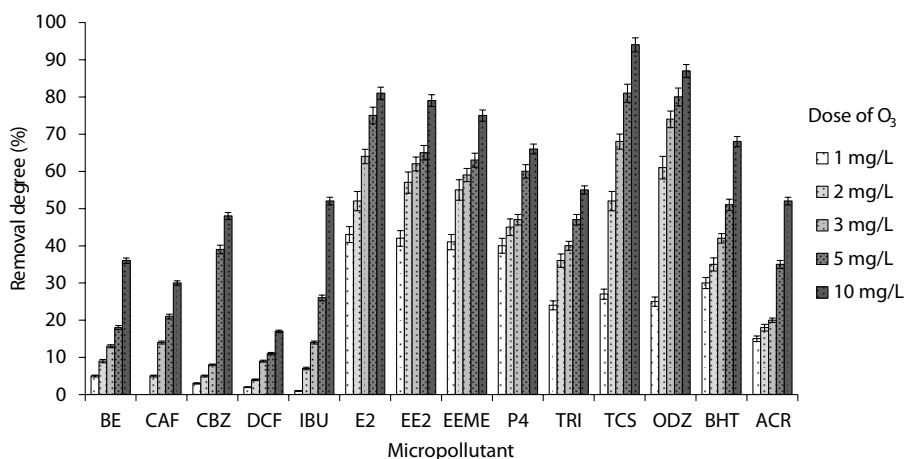


Fig. 1. Influence of the O₃ on the micropollutant removal degree during the O₃/sunlight irradiation process (10 min irradiation).

radiation plays an important role in pesticide removal and observed that pesticides occurred in water solutions exposed to UV-light decompose faster than those in samples irradiated with visible light or direct sunlight.

Due to the fact that the O₃ in a concentration of 10 mg/L causes the highest removal degrees of all tested compounds this dose was used in future experiments dedicated to the estimation of the dependence of sun lightening time on the decomposition of tested micropollutants. Fig. 2 summarizes the compound removal degrees observed during the O₃/sunlight process carried out for 10, 20, 30 and 60 min. It was shown that the elongation of the sample sun exposure time leads to a decrease in compound concentration for all tested micropollutant water solutions. The increase of the compound removal with the increasing process time was especially visible in the case of DCF solutions. The removal of this pharmaceutical increases from 17% after 10 min of irradiation to 68% after 60 min of irradiation. The least influence of sunlight irradiation time was observed for CBZ. The concentration of the compound was reduced in the range from 48% to 51%. 60 min of O₃/sunlight process duration allowed for an over 90% reduction in the concentration of all tested pesticides (E2, EE2, EEME, and P4), BHT, TCS, and ODZ. However, the studied process time intervals did not allow for a complete removal of none of the tested micropollutants.

To improve the particular compound decomposition degrees a second type of oxidant in the form of H₂O₂ was introduced to the reaction mixtures. H₂O₂ exhibits oxidant and reductant properties and can be effectively used in a wide range of temperatures and pH values [29]. The irradiation of H₂O₂ with sunlight, which is a source of UV lightening, leads to its direct photolysis and the generation of HO[•] radicals according to reaction (19) [30] and improves the oxidative capacity of the reaction mixture.



The performance of the O₃/H₂O₂/sunlight allowed for the achievement of higher removal degrees of all tested compounds except of ACR, TCS and CAF (Fig. 3). The decomposition of ACR and TCS was at the same level in both O₃/

sunlight and O₃/H₂O₂/sunlight processes, whereas the removal degrees of CAF observed in the H₂O₂ supported were lower than them noted for the single O₃/sunlight.

The simultaneous action of O₃/H₂O₂ and sunlight allowed for complete removal of E2 and EE2 after 60 min of process duration. High removal degrees reaching 98% and 99% were also noted for EEME and P4 respectively. Also, the removal of ODZ exceeded 95%. The highest removal of the compound from the group of pharmaceuticals was achieved for DCF and IBU. The concentration of both micropollutants decreased after 60 min of the O₃/H₂O₂/sunlight process by over 81% and 74% respectively. It can be assumed that the introduction of different types of oxidants and/or reactive radical's precursors to micropollutant water solutions had always a positive impact on their removal regardless of their physicochemical properties. Fernandes et al. [31] demonstrated that even volatile organic compounds can be effectively decomposed by the TiO₂/UV/O₃/H₂O₂ treatment system. However, some formed oxidative radicals like OH[•] radicals can react with O₃ molecules and accelerate their distribution to H₂O and O₂ [32].

To estimate the real impact of sunlight on the decomposition process of micropollutants the O₃/H₂O₂ process was performed in a dark chamber. The obtained results were presented in Fig. 4. The absence of the lightening source causes an explicit reduction of the decomposition effectiveness of all tested micropollutants. For example, a visible reduction in CAF concentration was noted only after 30 min of process duration and was equal to 1%. After 60 min of the dark chamber experiment, the concentration of this compound decreased by 2%. A similar low decomposition, which did not exceed 8% was noted in the case of the DCF solution. Also, low removal degrees, which did not exceed 43%, were obtained during the decomposition of hormones, which were particularly completely removed in sunlight supported processes. The highest decrease in concentration after 10 min of process duration reached 28% was noted for TCS, while the highest compound removal after 60 min was observed for the BHT solution and reached a value of 67%.

It can be assumed that sunlight supports the decomposition of a compound by the generation of highly reactive radicals, which can break the bonds occurring between the atoms of a compound molecule. A high removal degree of parent

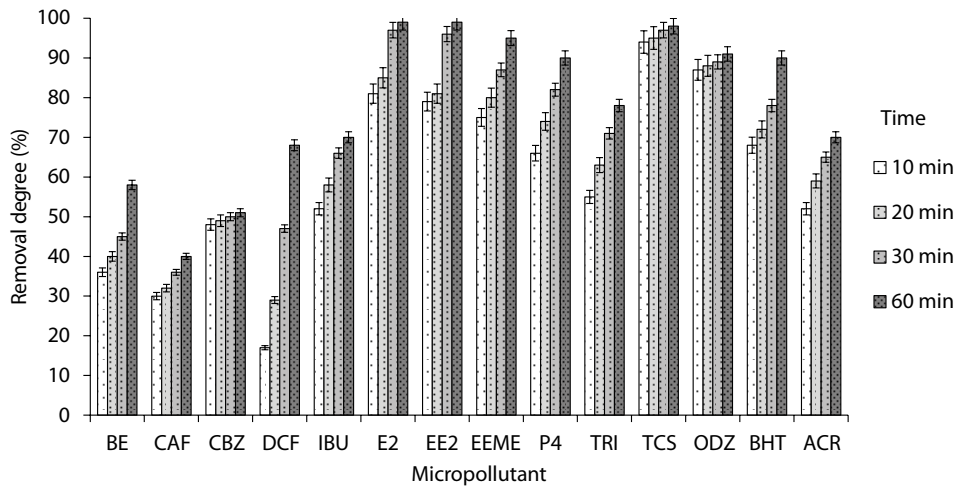


Fig. 2. Influence of the irradiation time on the micropollutant removal degree during the O_3 /sunlight irradiation process (O_3 dose equal to 10 mg/L).

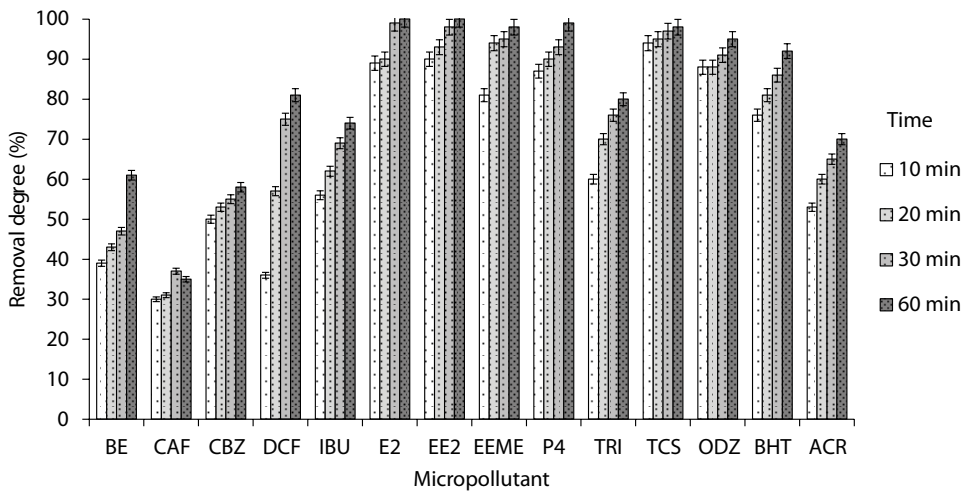


Fig. 3. Influence of the irradiation time on the micropollutant removal degree during the O_3/H_2O_2 /sunlight irradiation process (O_3 dose equal to 10 mg/L).

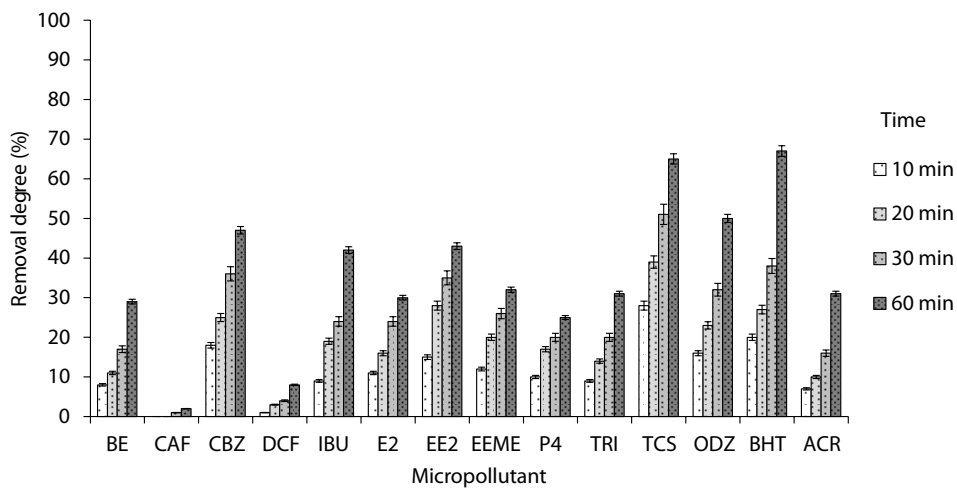


Fig. 4. Influence of the irradiation time on the micropollutant removal degree during the O_3/H_2O_2 (O_3 dose equal to 10 mg/L).

compounds is not always related to its complete mineralization. The decomposition of a micropollutant often leads to the formation of several by-products.

3.1. Identification of micropollutant decomposition by-products

The GC-MS analysis conducted in the TIC mode indicated the formation of several decomposition by-products of the tested compounds. The mass spectra of each peak noted on the chromatograms next to the peak corresponding to the parent compound was compared to the NIST v17 mass spectra library. Compound with a matching similarity over 70% were considered to be the possible formed intermediates. Table 4 summarizes all compounds matched in samples collected from both, sunlight supported and dark chamber experiments.

The largest number of decomposition by-products was observed during the DCF decomposition in the process of O_3 /sunlight irradiation. This pharmaceutical compound decomposed in the first 10 min to 7 intermediates that is, 4',5-dihydroxydiclofenac, 5-hydroxydiclofenac, 2,6-dichlorodiphenylamine, 2,6-dichloroaniline, 2,6-dichloro-4-aminophenol, 2-aminophenyl acetic acid, and 2-hydroxyphenyl acetic acid. The DCF intermediates detected during the performed oxidation processes were similar to those noted in future studies [33] and during experiments conducted by Yu et al. [34]. Also, CBZ and IBU decomposed to 5 by-products, which in the case of CBZ were: 3-hydroxycarbamazepine, 10, 11-dihydro-10-hydroxycarbamazepine, dihydrocarbamazepine-10, 11-trans-diol, 9-acridone and acridine, whereas IBU post-processed samples contained: 1-hydroxyibuprofen, 1-(4-isobutylphenyl) ethanol, 4'-isobutylacetophenone, 4-acetylbenzoic acid, and 4-ethylbenzaldehyde. During the decomposition of BE, CAF, and EE2 only one intermediate could be identified. The CAF by-product N,N'-dimethylparabanic acid was also identified by Neves et al [35], which investigated the possibility of CAF oxidation by the use of H_2O_2 catalyzed by metalloporphyrins processes. Special attention should be paid to ACR, which was formed during the CBZ decomposition. The oxidation of this compound leads to the generation of three intermediates with the same molecular formula $C_{13}H_9NO$ and therefore, also with the same molecular weight.

The formation of diallate during TRI decomposition raises concerns. This compound was classified by the Human Health Assessment Group in EPA's Office of Health and Environmental Assessment as a pesticide with possible human carcinogen possibilities [36]. Also the oxidation of P4 leads to the formation of 3 intermediates: corticosterone, aldosterone, and cortisone, which are well-known hormones. The connection of a second -OH group to the E2 compound resulted in the formation of 2-hydroxyestradiol, which was also detected by Mboula et al. [37] during the photocatalytic degradation of E2 under simulated solar light.

It was noted, that the concentration of intermediates observed in samples exposed to sunlight supported processes increases in the first 20 min of process duration. The concentration increase was estimated based on the increase of the peak areas corresponding to identify by-products. For example, the concentration of the TRI and TCS intermediates increased between 10 and 20 min of the O_3 /sunlight process

by over 20%, while the concentration of the E2 by-products 2-hydroxyestradiol and estradiol-3, 4-quinone increases in the same period by nearly 12% and 15% respectively. The continuation of the sunlight exposure resulted in a fast decomposition of the main of newly formed intermediates. Therefore the samples after 60 min of sun irradiation were characterized by a significantly lower number of decomposition by-products. An inverse relation was observed for the dark chamber O_3/H_2O_2 process. The number and concentration of intermediates increases with the increase in the processing time.

3.2. Toxicity analysis of post-processed water samples

The decrease of compounds concentrations after the conducted processes and the detection of several micropollutant intermediates force the performance of a toxicological analysis on different indicator organisms belonging to bacteria, crustaceans, and vascular plants. Figs. 5–7 compare the toxic effect of micropollutant water solutions before and after oxidation processes. The used toxicity bioassays are characterized by a different sensitivity to the tested compound groups. The estimation of the toxic effect by the use of the Microtox® test of compound solutions before decomposition shows that only the TCS solution can be classified according to the toxicity classification (Table 3) as highly toxic. The EE2 and ACR solutions were described by this test as low toxic, while other compound water solutions were found to be non-toxic. Whilst the DAPHTOXKIT F® test classified the TRI and TCS solution as toxic and the EE2 solution, similar to the Microtox® test, as low toxic (Fig. 6). The highest sensitivity against ODZ was noted for the *Lemna* sp. GTI (Fig. 7). The vascular plant classified this compound as highly toxic.

The subsection of the tested micropollutant solutions to the decomposition processes resulted in an increase of their toxicity. The observed toxicity effect corresponded to the results of the mass spectrometry analysis. The formed intermediates had a harmful effect on the tested organism's behavior. The highest toxic effect was observed for both, saltwater bacteria, freshwater crustaceans and vascular plant bioassays in samples subjected to the O_3/H_2O_2 dark chamber process. Only in the case of CAF and DCF, a higher toxic response was noted in samples after the O_3 /sunlight process. Also the *Lemna* sp. GIT was more sensitive to intermediates formed during the O_3 /sunlight decomposition of BHT than to them formed after other processes.

Irrespective of the decomposition process BE, CAF, DCF and IBU post-processed samples were classified by all toxicity tests as non-toxic. Only the pharmaceutical CBZ solution was after the O_3 /sunlight process characterized by low toxicity according to all bioassays, and highly toxic (Microtox® and DAPHTOXKIT F®test) after the O_3/H_2O_2 dark chamber process.

The toxicity results obtained for the hormone post-processed samples by the Microtox® and DAPHTOXKIT F®tests were very similar. For example, both tests classified the EEME solutions subjected to the action of the dark chamber and the sunlight O_3/H_2O_2 as toxic, whereas the O_3 /sunlight lead to the increase of the toxicity only to a low toxic level. A different toxic effect was only noted in the case of P4 solutions exposed

Table 4
Summary of micropollutant decomposition by-products

Parent compound	Identified compound	Molecular weight (g/mol)	Sunlight supported				Dark chamber		
			O ₃		O ₃ /H ₂ O ₂		O ₃ /H ₂ O ₂		
			10 min	60 min	10 min	60 min	10 min	60 min	
BE	ethyl 4-hydroxybenzoate	166.17	+	+	+	–	+	+	
CAF	N,N'-dimethylparabanic acid	142.11	+	+	+	–	+	–	
CBZ	3-hydroxycarbamazepine	252.27	+	–	+	–	–	+	
	10,11-dihydro-10-hydroxycarbamazepine	254.28	+	–	+	–	–	+	
	dihydrocarbamazepine-10,11-trans-diol	270.28	+	+	+	–	–	+	
	acridone	195.22	+	+	+	–	–	–	
	acridine	179.22	+	+	+	–	–	–	
	DCF	4',5-dihydroxydiclofenac	328.10	+	–	+	–	+	+
		5-hydroxydiclofenac	312.10	+	–	–	–	+	+
2,6-dichlorodiphenylamine		238.11	+	+	–	–	–	–	
2,6-dichloroaniline		162.01	+	+	–	–	–	–	
2,6-dichloro-4-aminophenol		178.01	+	–	+	–	+	+	
2-aminophenylacetic acid		151.16	+	+	–	–	–	–	
2-hydroxyphenylacetic acid		152.15	+	–	–	–	+	+	
2,3-dihydroxyphenylacetic acid		168.15	–	–	+	–	+	+	
IBU	1-hydroxyibuprofen	222.28	+	–	–	–	–	+	
	1-(4-isobutylphenyl)ethanol	178.27	+	–	+	–	–	+	
	4'-isobutylacetophenone	176.25	+	–	–	–	–	+	
	4-acetylbenzoic acid	164.16	+	+	+	+	+	+	
	4-ethylbenzaldehyde	134.17	+	–	–	–	+	+	
E2	2-hydroxyestradiol	288.40	+	–	+	–	–	+	
	estradiol-3,4-quinone	286.40	+	–	–	–	–	+	
	4-(1-hydroxyethyl)phenol	138.16	+	–	+	–	+	+	
EE2	4-hydroxy-ethinylestradiol	312.40	–	+	–	–	–	+	
EEME	2,6-di-tert-butylhydroquinone	222.32	+	–	+	+	+	+	
	2-hydroxy-3-methoxy-estrone	300.40	+	–	–	–	–	+	
P4	corticosterone	346.50	+	–	+	–	+	+	
	aldosterone	360.40	+	–	–	–	+	+	
	cortisone	360.40	+	–	–	–	+	+	
TRI	diallate	270.22	+	+	–	–	+	+	
	N,N-diisopropylformamide	129.20	+	–	+	–	+	+	
TCS	2,3-dichlorophenol	163.00	+	–	+	–	+	+	
	4-chlorophenol	128.56	+	–	+	–	+	+	
ODZ	5-tert-butyl-3-(2,4-dichloro-5-hydroxyphenyl)-1,3,4-oxadiazol-2 (3H)-one	303.14	+	+	–	–	+	+	
	4,6-dichlororesorcinol	178.00	+	+	+	+	+	+	
BHT	2,6-di-tert-butylhydroquinone	222.32	–	+	+	–	+	+	
	tert-butylhydroquinone	166.22	+	+	+	–	+	+	
ACR	acridone	195.22	+	+	+	–	+	+	
	acridine-10-oxide	195.22	+	+	+	–	–	+	
	2-hydroxyacridine	195.22	+	–	+	–	–	+	

+ - compound identified in the sample; – - compound not identified in the sample

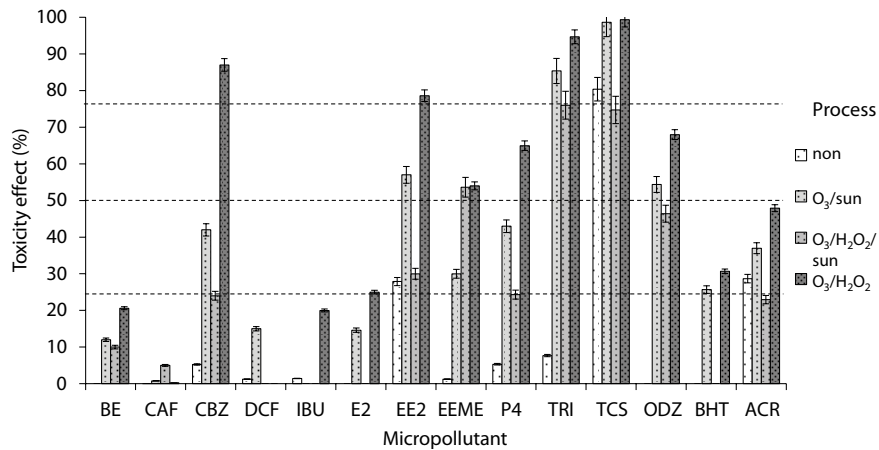


Fig. 5. Toxicity of post-processed samples after 60 min of process duration – estimated by the Microtox® test (where: non – samples before the decomposition process; O₃/sun – samples exposed to O₃ and sunlight radiation; O₃/H₂O₂/sun – samples exposed to O₃ and H₂O₂ supported by sunlight; O₃/H₂O₂ – samples subjected to the action of O₃ and H₂O₂).

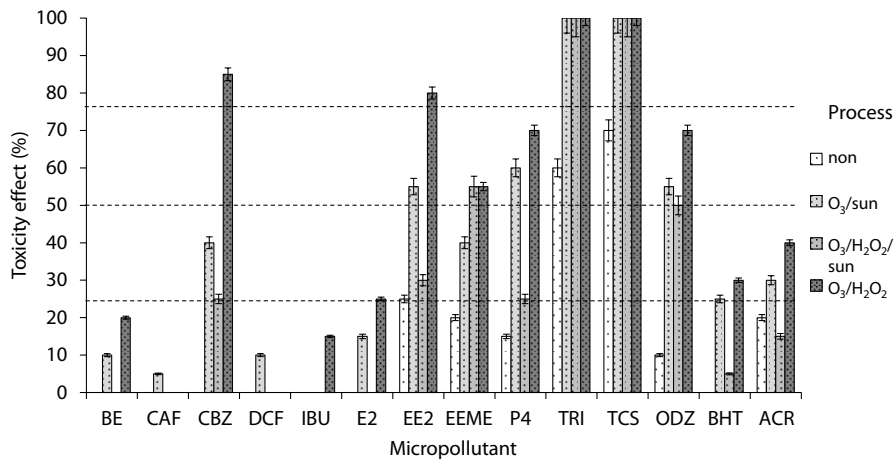


Fig. 6. Toxicity of post-processed samples after 60 min of process duration – estimated by the DAPHTOXKIT F® (where: non – samples before the decomposition process; O₃/sun – samples exposed to O₃ and sunlight radiation; O₃/H₂O₂/sun – samples exposed to O₃ and H₂O₂ supported by sunlight; O₃/H₂O₂ – samples subjected to the action of O₃ and H₂O₂).

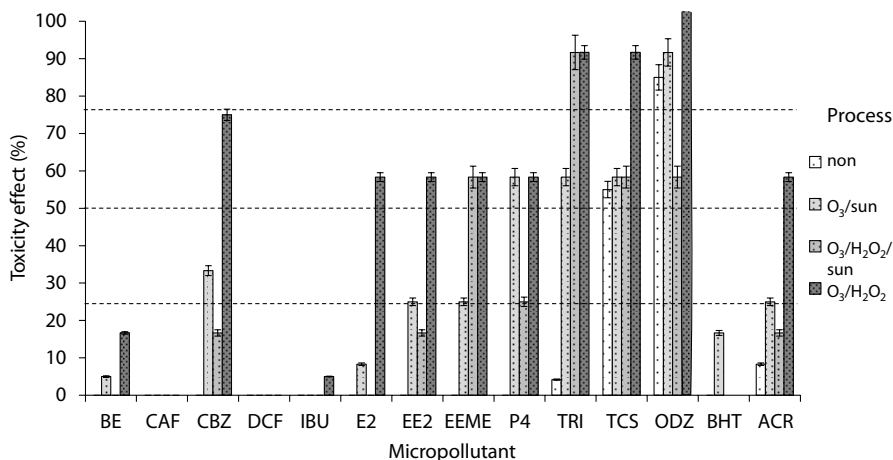


Fig. 7. Toxicity of post-processed samples after 60 min of process duration – estimated by the Lemna sp. GTI (where: non – samples before the decomposition process; O₃/sun – samples exposed to O₃ and sunlight radiation; O₃/H₂O₂/sun – samples exposed to O₃ and H₂O₂ supported by sunlight; O₃/H₂O₂ – samples subjected to the action of O₃ and H₂O₂).

to the O_3 /sunlight process, where the Microtox® test shows a low toxicity and the DAPHTOXKIT F® tests indicate a higher toxic value, which classifies the sample as toxic. The *Lemma* sp. GTI indicated an over 58% toxic effect in all hormone water samples after 60 min of the O_3 /H₂O₂/dark chamber process. It can, therefore, be assumed that the vascular plants are sensitive to the generated decomposition intermediates. Adeel et al. [38] indicated a harmful effect on different types of endocrine-disrupting chemicals occurring in the water environment on a plant, animal and human health.

Special attention should be paid to the pesticide post-processed samples, which in general had a negative impact on living organisms. For example, the Microtox® and DAPHTOXKIT F® test classified the TRI solution after all tested processes as highly toxic. Also, the TCS post-processed solutions were considered being highly toxic according to the DAPHTOXKIT F® test. Only ODZ solutions were classified by the Microtox® as low toxic or toxic, whereas the DAPHTOXKIT F® test assigned them as toxic. The *Lemma* sp. GTI show an over 92% toxic effect of all pesticide samples subjected to the O_3 /H₂O₂/dark chamber process, also the TRI and ODZ samples were classed as highly toxic after the O_3 /H₂O₂/sunlight and the O_3 /sunlight process respectively.

Differences in the sensitivity of the used bioassays were also noted in the case of ACR post-processed solutions. For example, the Microtox® test indicates a higher toxic effect of all tested samples containing this compound than the DAPHTOXKIT F®, however, both tests assigned the samples to the same toxicity classes. Only the *Lemma* sp. GTI indicated a toxic level of ACR O_3 /H₂O₂/dark chamber samples, whereas the bacterial and the crustaceans test show low toxicity of those water samples. Belmont et al. [39] indicated that acridine and acridone analogues show potential cytotoxicity. On the other hand, acridine and acridone derivatives becoming more and more interesting do to their anticancer activity [40].

4. Conclusions

The results obtained during the conducted studies indicated that sunlight strongly increases the effectiveness of O_3 decomposition of all types of studied micropollutants. The highest compound removal degrees, which exceeded 92%, were noted for micropollutants belonging to the group of hormones and pesticides. However, the removal of compounds depended on their chemical structure and the types of bond occurring between the atoms of the molecule. It was noted, that the presence of H₂O₂ during the O_3 /sunlight process resulted in faster decomposition of compounds especially CBZ, IBU, BHT, and all tested hormones and pesticides. A second type of oxidant lead also to a decrease in the number of generated micropollutant intermediates. The conduction of the O_3 /H₂O₂ process without the exposure to sunlight not only effects on a slower decomposition of tested compounds but also lead to the increase in the concentration of particular intermediates, which were more slowly oxidized than their parent compounds. The conducted toxicological analysis confirmed the reduction of the toxic nature of samples subjected to sunlight assisted O_3 /H₂O₂ process compared to samples treated by the action of O_3 /H₂O₂ performed in a dark chamber. The chosen toxicological

test showed different sensitivity to the formed toxic compound intermediates.

Acknowledgment

This work was supported by Ministry of Science and Higher Education Republic of Poland within statutory funds no. 08/040/BK_18/0093.

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