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Ecotoxicological effects of disinfection of treated wastewater

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ABSTRACT

Treated wastewater may provide an effective alternative for meeting agriculture's demands and increase freshwater resources for other needs. Disinfection of treated wastewater protects the receivers; nevertheless, hazards of handling disinfectants, and the fact that some of them do not remove certain pathogenic and opportunistic organisms, are two main reasons for undertaking research in this field. Ecotoxicity of effluent from a full-scale wastewater treatment plant, subjected to disinfection by chlorination, ozonation and UV irradiation, was investigated under laboratory conditions. The efficiency of bacterial inactivation was examined: microorganisms were sensitive to chlorine and UV disinfection, however, they were more resistant to ozonation. Ecotoxicity was evaluated on samples before disinfection and on disinfected samples in which the bacterial inactivation level was similar. Immobilization, growth and enzymatic ecotoxicological tests were performed using consumers, producers and decomposers, respectively. UV irradiation had the least negative impact on the tested bioindicators. Although some studies have shown opposite trends, it has been proved that ozonation and chlorination increase the toxicity of treated wastewater. This study suggests that the wide application of disinfectants to wastewater should be reviewed because, under the experimental conditions tested, they were able to cause harmful effects on one or more of the species tested and they could adversely affect biodiversity in the environment.

Keywords: Ozonation; Chlorination; UV disinfection; Treated wastewater ecotoxicity

1. Introduction

Global population growth and economic development are causing significant risks of freshwater shortages [1]. Treated wastewater, as an alternative water resource, may be used as a part of sustainable water management in many human activities, for example, for agricultural irrigation, aquaculture or replenishing surface water and groundwater for indirect and direct potable reuse [2–4]. In many countries disinfection of secondary treated domestic wastewater is routinely used, but there are still many places (including Poland) where municipal wastewater treatment plant (WWTPs) do not disinfect effluents. However, it is expected that in the near future disinfection of treated wastewater cannot be omitted and the number of WWTPs that employ post-secondary treatment, including disinfection, will increase [5]. Among many disinfection strategies, chlorination, ultraviolet irradiation (UV), ozonation and membrane filtration are the most widely used. There is also strong and growing interest in the application of peracetic and performic acids [6]. However, some disinfectants, if overdosed or used inappropriately, can trigger the formation of disinfection by-products (DBPs), causing detrimental environmental effects and adverse health effects in humans [3,7,8]. Despite these concerns, the ecotoxicity of disinfected secondary effluents is often not considered in feasibility studies for reuse of reclaimed effluents [2]. Technical advances and practical applications of

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disinfection of treated wastewater by ozonation, chlorination and UV irradiation have been carefully examined and summarized [9–11]. Despite many attempts to assess the ecotoxicity of disinfected wastewater, the impact of various disinfection methods on the quality of effluents discharged into the environment is still unclear, as the results obtained are contradictory and ambiguous. Previous research [3,12,13] has provided the means to calculate toxicity of disinfected effluents based on the concentrations of detected DBPs. In contrast, in this study we applied the whole effluent approach to investigate and compare the ecotoxicity of effluent from a full-scale WWTP, disinfected by three methods, to assess the hazard when being discharged into the aquatic environment. Whole effluent toxicity is a useful parameter for assessing impacts caused by cumulative toxic effects of a mixture of toxicants in wastewater. It is particularly important when interactions between toxicants are not only additive, as in the case of DBPs formed during the disinfection of treated wastewater. Synergism, antagonism, or potentiation between compounds in the mixture cannot be resolved when toxicity of disinfected effluents is calculated from DBPs concentrations. The whole effluent approach applied in this study detects toxicity most likely caused by DBPs, which are not commonly analyzed by chemical testing, and also addresses the bioavailability of products formed during disinfection. It is noteworthy that a similar approach was used in other studies, however, only one bioassay was applied to test ecotoxicity of effluents [14–16], or the studies did not cover all food chain representatives [17,18]. When a complete battery of tests was used, including decomposers, producers, and consumers, research was limited to only one or two disinfection methods [19], or residual disinfectants were not removed from effluents prior to toxicity testing [20]. Because different bioindicators used in ecotoxicity tests have different sensitivities, the above studies showed opposite trends; either no toxicity or higher toxicity of effluents after treatment, as the result of DBPs formation.

Our main goal was to assess and compare the ecotoxicity of effluent from a full-scale WWTP, disinfected by three methods at laboratory scale, to assess the hazard when discharged into the aquatic environment. Three methods for treated wastewater disinfection were tested: chlorination with sodium hypochlorite, ozonation, and UV radiation. This study is the first such comparison using the whole effluent approach and we believe that it gives an important hint concerning ecologically safe options for treated wastewater disinfection. The findings are especially important for the case of micropollutants, potential precursors of DBPs in wastewater, which are sometimes unidentified and difficult or even impossible to detect by chemical analyses.

2. Materials and methods

2.1. Samples of treated wastewater and preliminary tests

Samples were collected between October 2017 and June 2018 from a municipal WWTP (Stare Babice near Warsaw, Poland, Fig. 1).

Fresh effluent was used for the tests, collected in the morning of the same day when the given disinfection

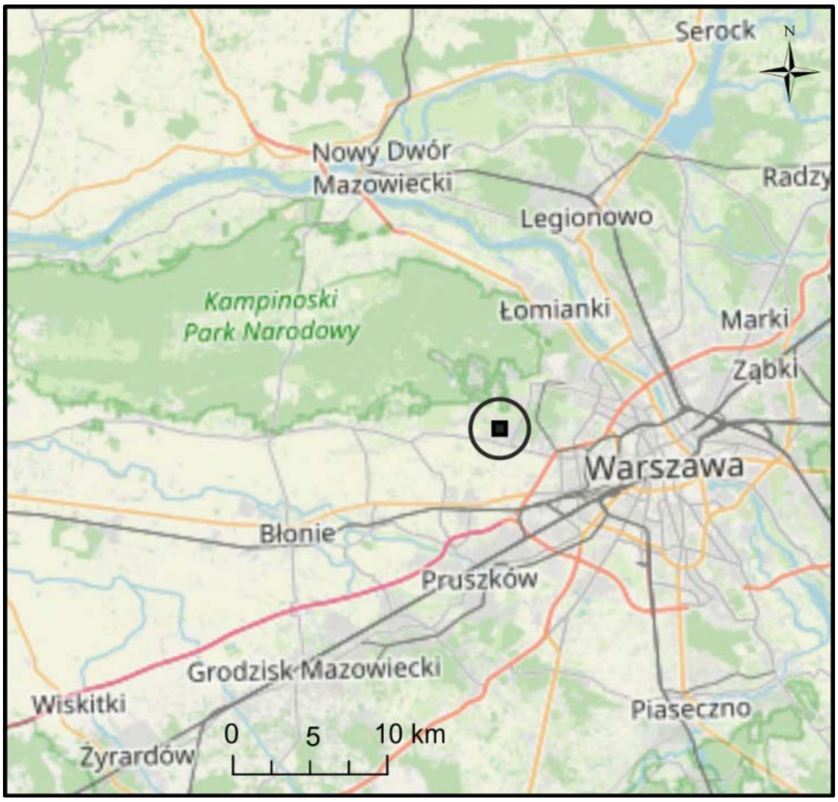

Fig. 1. Sampling site location (WWTP in Stare Babice), Basemap: OpenStreetMap.

method was examined. The WWTP received typical domestic wastewater, corresponding to an 18 000 population equivalent. The facility has an A_2O configuration (anaerobic-anoxic-aerobic), without primary settling but with an additional post-denitrification tank, placed after the nitrification tank. Treated wastewater was released into the catchment of the Kampinoski National Park, so the WWTP complied with the stringent effluent limits; the samples of effluent subjected to disinfection were characterized, according to the plant operator, by low pollution parameters (Table 1). Samples of treated wastewater were delivered to the laboratory within 2h and subjected to disinfection by ozonation, chlorination and, UV irradiation. The final concentrations of disinfectants and contact times were carefully selected based on the dose range commonly used in WWTPs [21,22] and the results of preliminary tests. The preliminary tests were designed to achieve a target output (disinfection level) appropriate for comparing ecotoxicity of the three methods of disinfection. In the preliminary tests, the following parameters for each disinfection process were: duration of ozonation (15 min, 30 min, 1 h, 2 h, 5 h), duration and disinfectant dose of chlorination (10 min, 30 min; 0.5 mg/L, 2 mg/L) and duration of UV irradiation (30, 60, 120 s). Each bacterial enumeration was done in triplicate. Finally, each disinfection process with selected parameters (described below) was repeated at least three times, after which the disinfected samples were subjected to ecotoxicity tests.

2.2. Ozonation

The reactor and ozone generator was a ready-made construction purchased from "Korona" Laboratory, Piotrków Trybunalski (Poland) and it was used in this study without any additional modifications. The 12 L reactor (internal diameter of the base: 0.217 m, height of the wastewater column: 0.325 m) was connected with the Korona L 20 SPALAB ozone generator, supplied with atmospheric air and operated in continuous operation mode with constant gas flow (3 L/min). Its efficiency averaged 7.4 ± 0.9 mg O_3/min (measured by us using iodometric method). Ozone was introduced into treated wastewater from the bottom of the reactor, through a diffuser. Tests lasted 2 h; the reaction was stopped by adding sterile 0.2 N sodium thiosulphate solution (analytical grade, POCH S.A., Gliwice, Poland).

2.3. Chlorination

The experiment was carried out in 1 L glass vessels (Chemland, Stargard, Poland), the contents of which were slowly stirred on magnetic stirrers. Sodium hypochlorite solution (0.1% Cl_2) was purchased from Chempur (Piekary Śląskie, Poland). Free chlorine was determined by the orthotolidine test and expressed as mg Cl_2/L. The volume of aqueous sodium hypochlorite solution was determined experimentally to meet the wastewater demand for chlorine (the amount of chlorine needed to oxidize organic and inorganic substances contained in wastewater, not available for disinfection), as well as to ensure a final test concentration (free chlorine) of 2 mg Cl_2/L to inactivate bacteria. Therefore, each time a new batch of wastewater was tested, the chlorine demand of the wastewater was determined prior to the actual disinfection test, using the orthotolidine test. The disinfection tests lasted 10 min and the reaction was stopped by adding a sterile 0.2 N sodium thiosulphate solution (POCH S.A., Gliwice, Poland).

2.4. UV irradiation

Disinfection was carried out using a Katadyn low-pressure UV radiator (120 mJ/cm^2) with a quartz shield (CH-8304, Wallisellen, Switzerland) in a disinfection chamber (0.7 L). The disinfection chamber was filled each time with a new batch of treated wastewater using a peristaltic pump (type 372.C, Poland), then wastewater was irradiated for 30 s under static conditions and withdrawn from the chamber by the peristaltic pump.

2.5. Microbiological analyses

Numbers of culturable psychrophilic and mesophilic bacteria were determined using the pour-plate technique in accordance with PN-EN ISO 6222, with incubation at 22°C and 37°C, respectively [23]. Enumeration of *Escherichia coli*, *Clostridium perfringens* and *Enterococcus faecalis* was performed in accordance with PN-EN ISO 9308-3, PN-EN ISO 14189:2016-10 and PN-EN ISO 7899-1, respectively [24–26].

E. coli is widely used as an indicator of the presence of potential pathogens. *C. perfringens* is spore-forming bacteria that survives longer in water than coliform bacteria and has proved to be a good indicator of pollution of water with *Giardia* and *Cryptosporidium* protozoa.

Table 1
Effluent limits according to local legislation and the actual values of parameters of treated wastewater from WWTP in Stare Babice over the investigated period (mean values ± standard deviations and ranges in parentheses)

Parameter	Effluent limits	Actual values
BOD_5 (mg/L)	8	0.6 ± 0.8 (0–2.0)
COD (mg/L)	70	27 ± 2 (21–31)
Total suspended solids (mg/L)	30	2.7 ± 1.4 (0–5.2)
N_{tot} (mg/L)	10	6.5 ± 1.0 (3.9–8.3)
P_{tot} (mg/L)	0.25	0.20 ± 0.03 (0.11–0.26)

Data provided by the plant operator.

Enterococci (*E. faecalis*) also survive longer in water and are more resistant to disinfection (e.g., chlorine). Their presence in water is more strongly correlated with the number of pathogenic bacteria in municipal wastewater than the presence of coliform bacteria [27].

2.6. Ecotoxicity tests

The whole effluent approach was applied to measure the aggregate toxic effect of the toxicants contained in treated wastewater before and after disinfection. Ecotoxicity tests were carried out on samples of disinfected wastewater stored for 24 h at 2°C–6°C. Growth, immobilization, and enzymatic assays were performed using green algae *Desmodesmus quadricauda* (CCALA 463), crustaceans *Daphnia magna* and bacteria *Aliivibrio fischeri*, respectively. Growth tests with *D. quadricauda* were performed in accordance with PN-EN ISO 8692; growth inhibition was assessed by cell densities after 72 h exposure to wastewater samples [28]. The immobilization assay with *D. magna* was performed in accordance with PN-EN ISO 6341 [29]. The immobilized organisms were counted after 48 h incubation with wastewater samples. Bioluminescence inhibition (test with *A. fischeri*) was measured after 5 min exposure to wastewater samples using a portable device, DeltaToxII (Modern Water, UK). Controls were performed for each test in line with the ISO standards. Sodium thiosulphate solution was tested in parallel to exclude its potential contribution to ecotoxicity of wastewater samples disinfected by ozonation and chlorination.

Effect concentrations (EC_{50}) were determined using probit analysis with 95% confidence intervals [30]. The ecotoxicity assessment was based on the toxicity classification system for wastewater discharged into the aquatic environment, developed by Persoone et al. [31]. For this purpose, EC_{50} values obtained with each test were transformed into toxic units (TU) using Eq. (1):

$$\text{TU} = \frac{1}{\text{EC}_{50}} \times 100 \tag{1}$$

Then, for each wastewater sample examined, a test score was allocated that reflects the toxic effect, according to the following scale:

- score 0: no significant toxic effect (i.e., a toxic effect that is not significantly higher than that in control),
- score 1: significant toxic effect (the 20% effect level, corresponding to 0.4 TU, was considered as the lowest effect to have a significant toxic impact), but <1 TU,
- score 2: toxic effect (≥1 TU and <10 TU),
- score 3: high toxic effect (≥10 TU and <100 TU),
- score 4: very high toxic effect (≥100 TU).

Based on the highest TU value obtained with any of the tests, wastewater samples were classified into the following categories:

- class I (no toxicity): none of the tests showed a toxic effect significantly higher than that in the controls;
- class II (slight toxicity): a toxic effect significantly higher than that in the controls (at least 20%, corresponding to 0.4 TU) was observed in at least one test, but it did not exceed the 50% toxic effect (<1 TU);
- class III (toxicity): at least 50% toxic effect (1 TU) was observed in at least one test, but ecotoxicity did not exceed 10 TU;
- class IV (high toxicity): ecotoxicity of at least 10 TU (but not more than 100 TU) was observed in at least one test;
- class V (very high toxicity): ecotoxicity of at least 100 TU was observed in at least one test.

Class weight score (CWS) and the percentage class weight score ($CWS_{\%}$) were calculated for each wastewater sample using Eqs. (2) and (3), respectively:

$$\text{CWS} = \frac{\sum_{i=1}^{n} \text{scores}}{n} \tag{2}$$

$$\text{CWS}_{\%} = \frac{\text{CWS}}{\text{CWS}_{\max}} \times 100, \tag{3}$$

where n = number of tests performed, and CWS_{max} = maximum class weight score (the highest value of all test scores obtained for a given wastewater sample). The higher the percentage class weight score, the more the score expresses the ecotoxicity of the wastewater sample in the concerned class.

3. Results and discussion

The primary aim was to examine the ecotoxicity of the effluent from the full-scale WWTP, subjected to different disinfection procedures (chlorination, ozonation, and UV radiation), in order to predict the ecotoxicological consequences of disinfected wastewater discharges into aquatic ecosystems. Enumeration of psychrophilic and mesophilic bacteria, *E. coli*, *C. perfringens* and *E. faecalis* in the disinfected and non-disinfected wastewater was carried out to evaluate the efficiency of the processes (Table 2).

Disinfection by ozonation is achieved by free radicals as oxidizing agents. It is reported to be an effective technology not only in the inactivation of microorganisms, but also for micropollutant removal [32]. Ozonation is more effective against viruses, bacteria and helminths than chlorination, but as with other disinfection methods, problems with effective bactericidal action occur when conditions are not ideal. There are some microorganisms that show resistance to its use, for example, *Cryptosporidium parvum* [9,33–35]. Chemical substances formed in the ozonation process, such as brominated DBPs, aldehydes, ketones, and carboxylic acids may be harmful to the environment and there is a shortage of information on residual bioactivity of transformation products formed during ozonation of secondary treated wastewater [32]. In our study, high inactivation (93%–100%) of each bacterial group was observed only after 2 h ozonation. Shorter contact times were insufficient to achieve satisfactory results (data not shown). The relatively long time needed for bacterial

Table 2
Number of culturable bacteria (CFU/mL) in treated wastewater before and after disinfection by ozonation (2 h), chlorination (2 mg Cl_2/L, 10 min) and UV irradiation (30 s)

Bacteria	Ozonation		Chlorination		UV irradiation	
	before	after	before	after	before	after
Psychrophilic	31×10^3	1.8×10^3 (94; 1.2)	30×10^3	0.1×10^3 (100; 2.5)	30×10^3	3.8×10^3 (88; 0.9)
Mesophilic	9.8×10^3	0.7×10^3 (93; 1.1)	5.1×10^3	0.4×10^3 (93; 1.2)	15×10^3	1.5×10^3 (90; 1.0)
Escherichia coli	19×10^1	0.6×10^1 (97; 1.5)	0.9×10^1	n.d.[a] (100; n.a.[b])	19×10^1	1.1×10^1 (94; 1.2)
Clostridium perfringens	1.33	0.07 (95; 1.3)	0.77	0.04 (95; 1.3)	1.33	0.07 (95; 1.3)
Enterococcus faecalis	0.77	n.d.[a] (100; n.a.[b])	0.20	n.d.[a] (100; n.a.[b])	0.77	n.d.[a] (100; n.a.[b])

[a] n.d. – not detected;
[b] n.a. – not available;
Inactivation (in percentage and log) is presented in parentheses for each disinfection method.

inactivation may be due to ozone consumption by the oxidation of chemical compounds contained in treated wastewater [31] and/or the presence of bacterial cells in a form shielded and protected against the action of a disinfectant (e.g., small flocs of activated sludge) [19,36].

Chlorination can be performed using several forms of chlorine, among others sodium hypochlorite. The disinfection process occurs primarily through oxidation of cell walls leading to cell lysis or inactivation of functional sites on the cell surface. The free chlorine radicals remain in wastewater and continue to inactivate most microorganisms. While it is advantageous for drinking water, chlorine can adversely impact aquatic life producing a significant amount of toxic halogenated DBPs, such as trihalomethanes and haloacetic acids [9,33,37]. High inactivation (nearly 100%) of psychrophilic bacteria and complete inactivation of fecal coliforms and enterococci was observed during chlorination in our research. *C. perfringens* turned out to be slightly more resistant (95% inactivation). Inactivation of microorganisms was effective, suggesting that the reaction time and/or disinfectant dose can be reduced in further studies.

UV irradiation causes double-stranded DNA damage and the formation of toxic photooxidation by-products that inactivate microorganisms prior to effluent discharge. The efficiency of UV disinfection depends on the physical and chemical quality of wastewater and sometimes has to be preceded by filtration [9,33]. UV systems are effective against a variety of pathogenic microorganisms [38,39], with minimal DBPs production and no need to generate, handle, or transport hazardous or corrosive chemicals [22,40]. In this study, high inactivation (88%–100%) of bacteria was observed after 30 s of UV irradiation. Extending the disinfection time did not significantly reduce the number of bacteria in the treated wastewater (data not shown). Psychrophilic bacteria were the most resistant to this method of disinfection, probably representing typical mechanisms of resistance, such as production of pigments (reviewed by De Maayer et al. [41]).

The primary methods for assessing WWTP effluent quality in many countries still rely on measuring fecal indicator bacteria, and largely ignores other microorganisms, genes, and many chemical contaminants [42]. However, it is claimed that a variety of other bacteria can survive and remain active in WWTP effluent following disinfection. These organisms can persist in the environment and enhance the metabolic potential of the biocenosis, such as nitrification and antibiotic resistance [5]. Routine microbiological monitoring should be therefore expanded to include culture-independent methods of bacterial enumeration and identification.

An unintended consequence of disinfection processes is the reaction of disinfectants with anthropogenic contaminants, bromide/iodide or natural organic matter to form DBPs. Many DBPs are cytotoxic, neurotoxic, mutagenic, genotoxic, carcinogenic and teratogenic [7]. Table 3 presents the results of growth, immobilization and enzymatic ecotoxicological tests performed on the samples of treated wastewater before and after disinfection processes. Producers, consumers and decomposers were used in the bioassays to cover the entire food chain.

Undiluted treated wastewater, not subjected to disinfection, was not toxic to bioindicators. According to the classification system, treated wastewater belonged to class I (no toxicity), because the 20% effect level was not reached in any of the tests (test scores 0, CWS = 0) and $CWS_{\%}$ was 100% (Table 3). It can be therefore concluded that biologically treated domestic wastewater, not subjected to disinfection, did not contain toxic substances. On the contrary, samples of treated wastewater ozonated for 2 h completely inhibited the growth of green algae at concentrations of 90%–12.5%, which reflects high toxic effect (test score at least 3). Also, the *A. fischeri* bioluminescence inhibition test showed strong effects of treated wastewater disinfected with ozone. These results indicate increasing ecotoxicity of effluent during ozonation (increase in toxicity class from I to III). Because the $CWS_{\%}$ is very high (67%, Table 3), ozonated effluent should be considered highly hazardous and toxic to aquatic biocoenoses, most likely due to the formation of toxic DBPs. The observations are in line with our previous findings [19] and also with Stalter et al. [43], in which the rainbow trout early life stage toxicity test revealed considerable developmental retardation of test organisms exposed to ozonated effluents. However, the same authors observed the removal of estrogenic activity by ozonation and reduced toxicity after sand filtration. Bhuvaneshwari et al. [44] reported that ozonation could degrade genotoxic compounds in some effluents, but the cytotoxic potential of wastewater effluents may increase with ozonation time. Graça et al. [45] combined ozonation with ultrafiltration

Table 3
Ecotoxicity assessment of treated wastewater before and after disinfection by ozonation (2 h), chlorination (2 mg Cl_2/L, 10 min) and UV irradiation (30 s), based on the toxicity classification system for wastewater discharged into the aquatic environment [31]

Wastewater sample	Parameter	*Desmodesmus quadricauda*	*Daphnia magna*	*Aliivibrio fischeri*
Before disinfection	TU	0	0.2	0
	Score	0	0	0
	Toxicity class	I (no toxicity)		
	CWS ($CWS_{\%}$)	0 (100%)		
After ozonation	TU	>16	0.6	3.3
	Score	3	1	2
	Toxicity class	IV (high toxicity)		
	CWS ($CWS_{\%}$)	2.00 (67%)		
After chlorination	TU	4.0	0.2	1.1
	Score	2	0	2
	Toxicity class	III (toxicity)		
	CWS ($CWS_{\%}$)	1.33 (67%)		
After UV irradiation	TU	0	0	0
	Score	0	0	0
	Toxicity class	I (no toxicity)		
	CWS ($CWS_{\%}$)	0 (100%)		

and observed comparable treated wastewater biological effects (measured by viability of cell lines and yeast estrogenicity tests) before and after treatment, which means that this process triggered no cytotoxicity or estrogenicity. However, the authors admit that the process should be improved to obtain better prevention of bacterial regrowth.

Undiluted treated wastewater disinfected with UV irradiation was not toxic to the tested bioindicators, and belonged to class I (no toxicity, CWS = 0) with a high level of probability ($CWS_{\%}$ = 100%) (Table 3). Stimulation of algal growth and bacterial bioluminescence (compared to controls) was observed in undiluted samples, similarly to treated wastewater not subjected to disinfection. Therefore it can be concluded that this method of disinfection did not increase ecotoxicity of wastewater. However, in the study of da Costa et al. [7], *D. rerio* juveniles, exposed to effluents treated with UV, showed low survival compared with non-disinfected effluent. There is some evidence that even UV irradiation, when used to disinfect wastewater, modifies the bacterial community in the receiving waters [46] and can stimulate the growth of some antibiotic resistant bacteria and genes, while removing others [47].

Disinfection by chlorination (10 min, 2 mg Cl_2/L) increased toxicity of treated wastewater for some bioindicators. Disinfected wastewater inhibited algae proliferation (EC_{50}-72h = 25%, which corresponds to 4 TU) and bioluminescence of bacteria *A. fischeri* (EC_{50}-5min = 93%, 1.1 TU), probably due to the formation of toxic DBPs during chlorination. According to the classification system, this sample belonged to class III (toxicity) with high $CWS_{\%}$ (67%). However, CWS was 1.33, and was lower than CWS for treated wastewater disinfected by ozonation, because chlorination did not increase ecotoxicity for *D. magna*: after 48 h exposure, the toxic effect did not exceed 10%, which corresponds to 0.2 TU. Based on literature data [3,48–50], it was expected that DBPs of chlorination could be harmful to aquatic ecosystems and, consequently, could lead to changes in biodiversity. Our study confirmed this thesis; however, harmfulness of chlorinated treated wastewater was not as high as might be expected, in that the applied parameters did not increase ecotoxicity for all bioindicators.

There was some ambiguity in determining which of the disinfected wastewater samples showed the most adverse impact on the organisms tested. Despite the slightly higher CWS value for ozonated wastewater than for the chlorinated sample, $CWS_{\%}$ was the same in both cases (67%). However, UV disinfection of treated wastewater turned out to be the least harmful to bioindicators. Bhuvaneshvari et al. [44] suggest that the formation of genotoxic and cytotoxic DBPs may be followed by their eventual disappearance. Du et al. [51] showed that genotoxicity measured by umu-test and estrogenic activity both decreased after chlorination because of destruction of toxic chemicals. Many studies [39,52,53] reported declines in the reproduction rate of the crustacean *Ceriodaphnia dubia* and survival of *D. magna* after exposure of these organisms to wastewater disinfected by chlorination and ozonation. Nevertheless, others [54–56] observed reduced ecotoxicity for *D. magna* after wastewater ozonation. Bhuvaneshvari et al. [44] emphasized that toxicity can differ among wastewater effluents, reflecting variability in effluent composition. Ozonation significantly increased the cytotoxicity and genotoxicity of effluents spiked with low bromide concentrations, compared to the bromide-free effluent [57]. Effluent from a single full-scale WWTP was examined in our work, thus further detailed research is required to cover more plants from different geographical regions to confirm the findings. Nonetheless, the effluent disinfection experiments provided insights into the effects of ozonation, chlorination, and UV irradiation on bacterial inactivation, ecotoxicity of DBPs and the environmental implications of these processes.

4. Conclusions

The main findings of this work, investigating the ecotoxicity of treated wastewater disinfected with chemical and physical agents, can be summarized as follows: (1) bacteria present in treated wastewater are somewhat resistant to disinfection with ozone, but sensitive to chlorine and UV irradiation, (2) ozonation is the most harmful disinfection method to the tested organisms, followed by chlorination, (3) disinfection with UV irradiation did not increase the ecotoxicity of the effluent and, given the experimental conditions, this method seems to be the ecologically safest solution for municipal WWTPs in Poland.

The present study demonstrated that under the experimental conditions tested, two disinfection methods were capable of producing harmful effects on one or more tested species, clearly indicating that there is potential hazard associated with the increasing ecotoxicity following disinfection of treated domestic wastewater. Moreover, the ecotoxicological tests applied in the study were only short-term acute bioassays, and even the results, indicating low toxicity, cannot be conclusive [58]. Wastewater may still contain harmful micropollutants, such as pharmaceuticals, personal care products or disinfection by-products, which can be toxic, especially to higher aquatic organisms and with long-term exposure [59–61]. Monitoring the toxicity of chlorinated and ozonated effluents and identification of various toxicity mechanisms are therefore required to protect the biodiversity in water ecosystems.

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Authors' contributions

Conceptualization, A.M., K.A. and M.Z.-R.; methodology, A.M. and K.A.; validation, M.Z.-R.; investigation, A.M., K.A. and N.D.; resources, A.M., K.A. and N.D.; data curation, A.M.; writing—original draft preparation, K.A.; writing—review and editing, A.M., K.A., M.Z.-R. and N.D.; visualization, K.A. and A.M.; supervision, M.Z.-R.; project administration, K.A.; funding acquisition, K.A. All authors have read and agreed to the published version of the manuscript.

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