Research Article

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Degradation and ecotoxicity of favipiravir and oseltamivir in the presence of microplastics during ozonation and catalytic ozonation of synthetic municipal wastewater effluents

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Abstract

Background: Favipiravir (FAV) and oseltamivir (OSE) are antiviral agents developed against influenza and they were repurposed against SARS-CoV-2 during the COVID-19 pandemic. This study evaluated the potential of ozonation and catalytic ozonation as tertiary treatment approaches for removing FAV and OSE from municipal wastewaters, both in the presence and absence of microplastics (MPs), while comparing the ecotoxicity of untreated and treated secondary effluents to predict the ecotoxicological effects of these technologies during municipal wastewater treatment.

Results: At an initial antiviral concentration of 50 μg L^{-1}, ozonation at pH 7 with a specific ozone dose of 0.6 mg O_3 (mg DOC)$^{-1}$ yielded FAV and OSE removals of 84 and 64%, respectively, while the presence of catalyst or MPs decreased the degradation rate by 30–40%. Raising the pH to 10 had minimal impact on FAV abatement, but improved OSE reduction by 21%. Acute toxicity tests using *Vibrio fischeri* demonstrated that simultaneous ozonation of the analytes led to the accumulation of transformation products (TPs) of FAV and OSE, with their combined effect almost equal to that of the original compounds. Reproduction toxicity tests indicated that TPs of antiviral drugs generated during ozonation were less toxic to *Enchytraeus cryptticus* than the parent chemicals.

Conclusion: Ozonation proved to be a viable option for upgrading existing wastewater treatment facilities, serving as a complementary treatment to minimize the release of antivirals from municipal secondary effluents and reduce their inhibitory effect on earthworm reproduction, thereby enhancing the reuse potential of treated wastewater for irrigation.

Keywords: favipiravir; oseltamivir; ozonation; catalytic ozonation; microplastics; ecotoxicity

INTRODUCTION

Antivirals are pharmaceuticals used to treat viral infections such as acquired immunodeficiency syndrome (AIDS), influenza, hepatitis, and herpes.[1] Although antiviral drugs play a crucial role in treating various diseases, their occurrence and fate in the urban water cycle have been studied less extensively than other pharmaceuticals like antibiotics, analgesics or antihypertensive drugs.[2,3] Wastewater treatment plants (WWTPs) become a major source of antivirals, and their metabolites are released into aquatic environments as they are not specifically designed to remove drug residues.[4,5] Since the onset of the COVID-19 pandemic, there has been a substantial rise in the concentrations of antiviral drugs found in sewage, treated wastewater effluents, and surface water.[6] Some of these antivirals are present at levels that could pose significant acute or chronic toxicity to non-target organisms in the aquatic environment.[7,8]

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Among the antivirals, favipiravir (FAV) and oseltamivir (OSE) have been widely reported acting against SARS-related coronavirus (SARS-CoV-2) infection[1,9,10] and recommended as appropriate wastewater-based epidemiology (WBE) biomarkers from the perspective of in-sewer stability.[11] Kuroda *et al.*[5] pointed out that conventional WWTPs (employing activated sludge process as secondary treatment) were found to be unable to efficiently remove FAV and its metabolite (T705M1) from raw wastewater, as evidenced by predicted removal efficiencies of less than 2%. Data analysis performed by comparing the influent and effluent of the same WWTP revealed that the concentrations of OSE and its metabolite oseltamivir carboxylate (OC) remained practically unchanged throughout the treatment, both exhibiting high resistance to biodegradation.[12,13] The inadequate removal of these compounds by biological treatment systems highlights the need for complementary processes to effectively eliminate them from secondary effluents. Therefore, enhancing and updating existing conventional WWTPs by incorporating suitable tertiary treatment options is essential not only to reduce the release of antiviral drugs into the effluent,[14] but also to mitigate the risk of their uptake by plants and soils when the treated wastewater will be reused for irrigation purposes.[15]

To address the issues associated with antivirals, the attention of researchers has been focused on advanced oxidation processes (AOPs), successfully investigated as tertiary treatment methods of municipal wastewaters.[16,17] Among the AOPs, ozonation is considered as one of the most promising techniques for the removal of pharmaceuticals from secondary treated municipal effluents[18] since laboratory,[19] pilot,[20] and full-scale[21] studies revealed that ozonation could reduce the pharmaceuticals load by more than 80%. However, sole ozonation suffers from some drawbacks, including low mineralization ability, selective oxidation, and unstable treatment effect.[22] Heterogeneous catalytic ozonation can address the limitations of ozonation alone, garnering significant interest over the past two decades.[22,23] Recently, layered double hydroxides (LDHs) have gained significant attention as catalysts owing to their high anion exchange capacity, large specific surface area, abundant active sites, stability, and ease of synthesis.[24,25]

The ongoing COVID-19 pandemic has led to a significant rise in the use of plastics for personal protective equipment and sanitizer containers, with an estimated 40% global increase in plastic usage.[26] WWTPs play a crucial role in the transfer of MPs from human life to the natural environment and are key to controlling the entry of MPs into the material cycle.[27] The impact of MPs on tertiary treatment of urban wastewater remains largely unexplored.[28] The ozone disinfection efficacy is negatively affected by the presence of MPs as the log reductions of *E. coli* bacteria decreases with the increase of MPs concentration and a complete inactivation can only be achieved at prolonged contact times.[28] MPs can act as scavenger of ozone molecules and hydroxyl radicals by oxidizing their surfaces, thereby reducing the amount of them available for the effective inactivation of pathogenic bacteria[29]; which highlights the importance of studying the effect of MPs on tertiary treatment by ozonation. During the ozonation process, the surface of MPs can undergo oxidation, leading to the formation of oxygen-functional groups (i.e., carbonyl groups) and, thus, to an increase in the hydrophilicity of MPs and adsorption sites.[30] As a result, the oxidized MP surface can demonstrate an enhanced ability to adsorb hydrophilic compounds.[31,32]

Despite the increasing interest in the utilization of LDH compounds as catalysts, there has been limited research on the catalytic activity of LDHs in the ozonation process.[33] Besides, the majority of previous catalytic ozonation studies focused on the degradation of a single pharmaceutical compound,[34] which cannot reflect the real performance of catalysts due to the coexistence of multiple pharmaceuticals during actual wastewater treatment as the intermediates formed by the joint degradation of multi-pollutants may affect the degradation efficiency of the catalysts along with the toxicity of the resulting products. Based on the review of existing literature, it is also evident that the effect of MPs on the degradation of FAV and OSE, as well as on aquatic and soil organisms, through ozonation alone and catalytic ozonation processes has not yet been examined. In this context, this study aimed to fill the above-mentioned knowledge gaps by assessing the potential of ozonation and catalytic ozonation processes for the removal of FAV and OSE from synthetic municipal wastewater effluents, both in the presence and absence of MPs. Another equally important objective was to comparatively evaluate the ecotoxicity of raw and treated (ozonated and catalytic ozonated) effluents in order to predict the ecotoxicological consequences of the application of tertiary treatment technologies in municipal wastewaters.

MATERIALS AND METHODS

Experimental approach

Synthetic municipal wastewater effluent (SMWE) samples with two different concentrations of FAV and OSE were used during the course of ozonation and catalytic ozonation tests. Sample 1 was defined as the SMWE with 50 μg L^{-1} concentration of FAV and OSE, whereas Sample 2 represented the SMWE in combination with 500 ng L^{-1} concentration of antivirals. The removal of antiviral drugs was assessed by testing three different specific ozone doses (0.2, 0.6, and 1 mg O_3 (mg DOC)$^{-1}$) both in the presence and absence of MPs (0.1 mg L^{-1}). Polyethylene (PE) was selected as the model MP because (i) it is the most prevalent MP type found in WWTP influents and effluents,[35] and (ii) it exhibits toxicity towards aquatic[36] and soil[37] organisms. The experimental runs were conducted at two different pH levels representing neutral (pH = 7) and alkaline (pH = 10) conditions, the latter being applied to enhance the decomposition of ozone leading to the generation of hydroxyl radicals and to promote the degradation of the studied antiviral drugs.

Chemicals and glassware

All volumetric glassware was rinsed first with acetonitrile and then with ultrapure water, which was sourced from a Sartorius Arium 611 VF water purification system. After being washed, other glassware items were placed in a Lenton ECF 12/6 muffle furnace and heated at 550 °C for 3.5 h to eliminate organic residues. For solid phase extraction (SPE) and liquid chromatography tandem mass spectrometry (LC-MS/MS) analyses, LC-MS grade (LiChrosolv®) solvents were utilized.

FAV and OSE were supplied in kind in powder form by Atabay Pharmaceuticals and Fine Chemicals Inc. (Turkey), while their stable isotope references, [^{13}C,^{15}N]-FAV and [$^{2}H_5$]-OSE hydrochloride salt, were imported from Alsachim company (France). Stock solutions were prepared at 5 mg L^{-1} for each reference isotope and at two different concentrations (i.e., 5 and 100 mg L^{-1}) for both FAV and OSE. All stock solutions were stored in a laboratory cold room at 4 °C, suitably diluted and injected into the investigated samples to achieve the desired concentrations for the experiments.

PE-based MPs at micro-size (200 μm) were purchased from Uçar Chemical company (Turkey) and used as received.

Preparation of synthetic municipal wastewater and biodegradation experiments

A mixture of meat extract and peptone (referred to as peptone mixture) was prepared to create synthetic municipal wastewater. The peptone mixture was chosen primarily because it is specified as the standard substrate in the ISO 8192 inhibition test procedure.[38] Although the peptone mixture lacks the particulate chemical oxygen demand (COD) fractions and therefore does not have the same particle size distribution, its biodegradation characteristics are very similar to domestic sewage, balancing readily biodegradable COD and slowly biodegradable COD fractions. Another significant advantage of using the peptone mixture as synthetic municipal wastewater is that its characteristics can remain consistent throughout the experimental period.

A laboratory-scale fill and draw reactor with a hydraulic retention time of 1 day, fed with the peptone mixture, was operated at steady state with a sludge age of 15 days. The reactor contained an initial peptone mixture concentration of about 500 mg COD L^{-1} to simulate real domestic wastewater. It had a net volume of 10 L and was seeded with activated sludge from an advanced WWTP. The reactor was periodically supplemented with specific amounts of nutrient solutions (10 mL of each per 1000 mg of COD) to eliminate nutrient limitations and buffer against pH fluctuations.[39] In order to confirm that the reactor was operated well throughout the biodegradability experiments, COD and mixed liquor volatile suspended solids (Standard Methods,[40] SM 2540-E) contents were recorded daily. The mixed liquor from the reactor was settled for 30 min, and the supernatant was filtered through 220 nm membrane filters before conducting ozonation and catalytic ozonation experiments.

Conventional characterization

To analyze the SMWE samples before and after ozonation and catalytic ozonation experiments, a conventional wastewater characterization study based on the measurements of COD, dissolved organic carbon (DOC), ultraviolet absorbance at 254 nm (UV_{254}), specific ultraviolet absorbance at 254 nm (SUVA), ammonia (NH_3-N), nitrite (NO_2^--N), total phosphorus (TP) and pH was applied. Analyses of COD (SM 5220-B), NH_3-N (SM 4500-NH_3-C), TP (SM 4500-P-D), and pH (SM 4500-H^+-B) were performed using the protocols described in Standard Methods.[40] The DOC was detected by a total organic carbon analyzer (TOC-V CPN, Shimadzu, Japan). The UV_{254} measurements were recorded with a Schimadzu UV-1800 spectrophotometer at a wavelength of 254 nm with a 1 cm quartz cell. SUVA (L (mg-m)$^{-1}$) was determined as the ratio of UV_{254} (cm^{-1}) over DOC concentration (mg L^{-1}) and multiplying by 100. Nitrite (NO_2^--N) concentrations were determined by ion chromatography using a Dionex ICS-3000 system (Sunnyvale, CA, USA) coupled with a conductivity detector and an analytical column AS11HC (Dionex IonPac). The pH measurement was carried out by a digital Orion model 920A pH-meter with an accuracy of ±0.01 unit (Orion Research Inc., Boston, MA, USA).

Ozonation and catalytic ozonation experiments

The experimental studies were conducted in a 500 mL laboratory semi-batch glass reactor, consisting of a cylindrical tube 2 cm high and 6 cm in internal diameter. An ozone generator (Arcbull Meo-20) with a capacity of 2500 mg O_3 h^{-1} was connected to an oxygen concentrator (Healthtime OC-5). Ozone gas was introduced into the reactor from the bottom via a sintered glass plate diffuser at a flow rate of 0 to 1 L min^{-1}. SMWE samples of 300 mL volume were subjected to ozonation and catalytic ozonation at room temperature. A schematic of the experimental set-up is shown in Fig. 1.

Excess ozone gas leaving the reactor was collected in two gas washing bottles filled with 2% w/v potassium iodide (KI) solution. Two additional gas washing bottles containing 2% w/w KI solution were placed after the gas inlet line to measure and calibrate the ozone flow rate accurately. Ozone concentration at the reactor's inlet and outlet was determined using iodometric titration as per Standard Methods 2350-E.[40] The off-gas ozone concentration remained near zero due to over 95% ozone utilization, making the applied and transferred ozone doses approximately equal. The dissolved ozone concentration during the

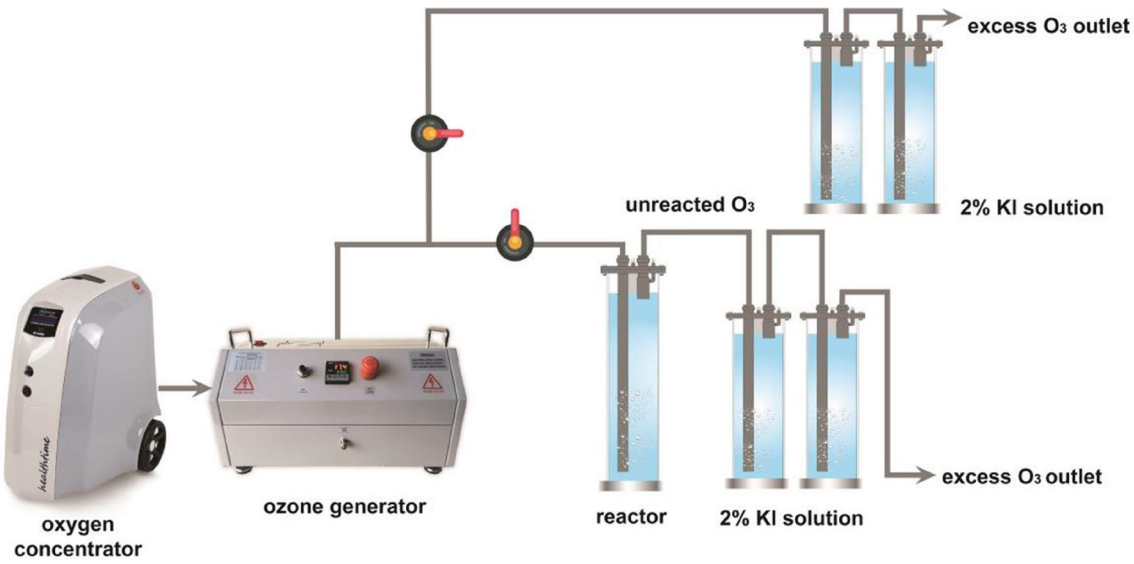

Figure 1. Schematic diagram of the experimental set-up.

reaction, measured by the indigo colorimetric method,[40] was below 1% of the applied dose and was thus omitted from the ozone consumption calculation. With around 20 mg L^{-1} of DOC in SMWE samples and only a few µg L^{-1} of FAV and OSE, most of the applied ozone and produced •OH reacted with the bulk DOC rather than the antiviral drugs. Therefore, antiviral removal was related to both the applied and specific ozone dose (mg O_3 (mg DOC)$^{-1}$), the latter allowing for comparisons between ozonation processes at WWTPs with varying DOC levels. Due to the low NO_2^--N level in the investigated samples (≤0.07 mg N L^{-1}), the specific ozone dose was calculated by dividing the ozone consumption by the DOC value at the reactor inlet.

In the catalytic ozonation experiments, ZnFe LDH powder was added to the reaction medium at a concentration of 0.1 g L^{-1} just before ozone was introduced. The ZnFe LDH was prepared using the co-precipitation method with a M^{2+}/M^{3+} molar ratio of 3.[41] For this purpose, 3 mmol of $ZnCl_2$ and 1 mmol of $FeCl_3.6H_2O$ were dissolved in ultra-pure water purged with N_2 gas. The solution pH was gradually raised to 8 by slowly adding 2 M NaOH solution while stirring vigorously. After aging the mixture for 24 h under an N_2 atmosphere, the catalyst was centrifuged and washed multiple times with deionized water. The ZnFe LDH was then dried in an oven at 50 °C for 8 h, followed by grinding and sieving.

Detection of antivirals

A concentration step was not necessary for Sample 1 since it was spiked with a relatively high content (50 µg L^{-1}) of FAV and OSE. However, due to the low spiked concentration (500 ng L^{-1}) of target analytes in Sample 2, they were isolated by SPE using Oasis HLB SPE cartridges (6 mL, 200 mg, Waters, Milford, MA, USA). Prior to the extraction, the cartridges were preconditioned with 5 mL methanol and 5 mL ultrapure water. Samples were filtered from cartridges at a flow rate of 3–5 mL min^{-1} using an automatic SPE vacuum manifold (VacMaster, Biotage, NC, USA) and washed with 6 mL of ultrapure water with an adjusted pH of 3. The cartridges were dried under vacuum for 1 h, after which the analytes retained on the cartridges were eluted by using 5 mL methanol and 5 mL acetonitrile. Finally, the organic solvent was evaporated to dryness under nitrogen at a pressure of 10 bar using a Caliper TurboVap II (Caliper Life Sciences, Hopkinton, MA, USA) and the extracts were reconstituted in 1 mL of a methanol-ultrapure water mixture (30:70, v/v). For the quantitation of the investigated antivirals by LC-MS/MS, the resulting solution was filtered through a 220-nm membrane filter and transferred into 2-mL vials.

The LC-MS/MS measurements were performed with a Thermo Scientific Fortis Model Mass Spectrometer connected to a Thermo Scientific Ultimate 3000 Model Ultra Performance Liquid Chromatograph, operating in tandem. Because of its resistance to high temperatures and pH levels, a Waters XBridge BEH C18 (50 mm × 4.6 mm × 3.5 µm) column was used for the chromatographic separation of FAV and OSE. The injection volume was set to 5 µL as higher volumes led to poor separation of the chromatographic peaks. Mobile phase A consisted of 0.1% formic acid in distilled water, while mobile phase B was 0.1% formic acid in pure methanol. The flow rate was maintained at 0.6 mL min^{-1} for 10 min.

Toxicity experiments

Changes in acute toxicity after ozonation and catalytic ozonation of Sample 1 under selected operating conditions were monitored using *Vibrio fischeri*. The acute toxicity towards the marine bacterium *V. fischeri* was assessed with a BioToxTM assay kit (*V. fischeri* code 1243–157; Aboatox Oy, Finland) following the ISO 11348-3 test protocol.[42] The percentage inhibition of bioluminescence was determined by mixing 0.5 mL of raw or treated (ozonated and catalytic ozonated) Sample 1 with 0.5 mL of luminescent bacterial suspension. All tests were conducted at 15 °C using thermostats, and light emission was measured after a 15-min incubation on an Aboatox C110 BioTox luminometer (Aboatox Oy, Finland). The toxicity of the raw and treated effluents was expressed as the percentage of bioluminescence inhibition compared to an uncontaminated reference. A positive control (2% NaCl, w/v) was included in each test. One-way analysis of variance (ANOVA) was used to identify which data sets significantly differed from the control at the $P < 0.05$ level.

The toxicity test with *Enchytraeus crypticus* was conducted according to OECD Guideline 220,[43] but using an exposure duration of 3 weeks as recommended by Castro-Ferreira *et al.*[44] Natural standard LUFA 2.2 soil (Lufa Speyer, Germany) with approximately 1.6% organic carbon and a pH_{CaCl2} of 5.5 was used as a control to ensure the health of the test organisms and the validity of the bioassays. Ten adult animals were randomly placed in each 100 mL glass jar containing 20 g dry weight equivalent of moist test soil and fed with a few grains of oat flakes. The test lasted for 21 days at 20 ± 1 °C with a 12/12 h light/dark photoperiod, using four replicates per test concentration and control. Food and water were replenished twice a week based on weight loss. After 3 weeks, all samples were fixed with 10 mL of 96% ethanol, transferred to a plastic container, and stained with a 1% Bengal rose solution in ethanol. The boxes were briefly shaken and incubated overnight in a refrigerator at 4 °C for optimal staining. The pink-stained animals were separated from soil particles by sieving over 160 µm, then transferred into white trays and counted under a magnifying glass.

RESULTS AND DISCUSSION

Conventional characterization

The physicochemical characteristics of SMWEs used to simulate secondary effluents from municipal WWTPs are presented in Table 1. The experimental data on COD and DOC contents listed in the table were comparable to those measured in both real and synthetic effluents.[45,46] The concentrations of main inorganic nutrients (NH_3-N, NO_2^--N, and TP) fell within the range of values

Table 1. Physicochemical characteristics of SMWE samples

Sample no	COD (mg L^{-1})	DOC (mg L^{-1})	UV_{254} (cm^{-1})	SUVA (L (mg-m)$^{-1}$)	NH_3-N (mg N L^{-1})	NO_2^--N (mg N L^{-1})	TP (mg P L^{-1})	pH
Sample 1	50	18.80	0.410	2.18	0.8	0.07	2.0	7.03
Sample 2	45	16.61	0.377	2.27	0.7	0.06	1.8	6.92

reported previously for real municipal wastewater effluents.[45,47] The analysis of characterization results obtained for the studied samples revealed that the biological treatment decreased the COD concentration from 500 to a level of 45–50 mg L^{-1}, achieving a removal efficiency of approximately 90%.

Effect of ozonation and catalytic ozonation on FAV and OSE

Data on the removal of antivirals derived from ozonation and catalytic ozonation processes on SMWE samples are shown in Figs. 2 and 3. As depicted in Fig. 2, ozonation of Sample 1 at pH 7 yielded a removal rate of approximately 84 ± 1% for FAV and 64 ± 1% for OSE, regardless of the specific ozone dose. Raising the pH from 7 to 10 resulted in only a slight improvement in FAV removal, with an increase of about 2 ± 1%, while OSE exhibited an additional reduction of 21 ± 2%. The presence of MPs adversely affected the removal of antivirals, leading to a decrease in the degradation of FAV by almost 30% at both pH levels. The addition of MPs caused a decline in the OSE removal efficiency by 35% and 12% at pH 7 and 10, respectively. In contrast, catalytic ozonation provided different results for antiviral removal compared with ozonation alone. In catalytic ozonation experiments conducted without MPs, a removal rate of 53 ± 2% for FAV was achieved at specific ozone doses of 0.2 and 0.6 mg O_3 (mg DOC)$^{-1}$, independent of the pH value. However, increasing the specific ozone dose to 1 mg O_3 (mg DOC)$^{-1}$ at both pH conditions demonstrated an additional 18% increase in FAV degradation. Catalytic ozonation in the presence of MPs resulted in an enhancement of the abatement efficiency for both antivirals, regardless of the specific ozone dose. For OSE, removal rates reached 28 ± 2% at pH 7 and 63 ± 2% at pH 10, indicating that raising the pH led to a 36 ± 2% improvement in OSE reduction. In the absence of MPs, the use of the catalyst at pH 7 and 10 decreased the FAV degradation by 32 ± 2% and 34 ± 5%, respectively, when focusing on the first two specific ozone doses; while the removal of OSE dropped by 37 ± 3% and 22 ± 1%, independent of the specific ozone dose applied.

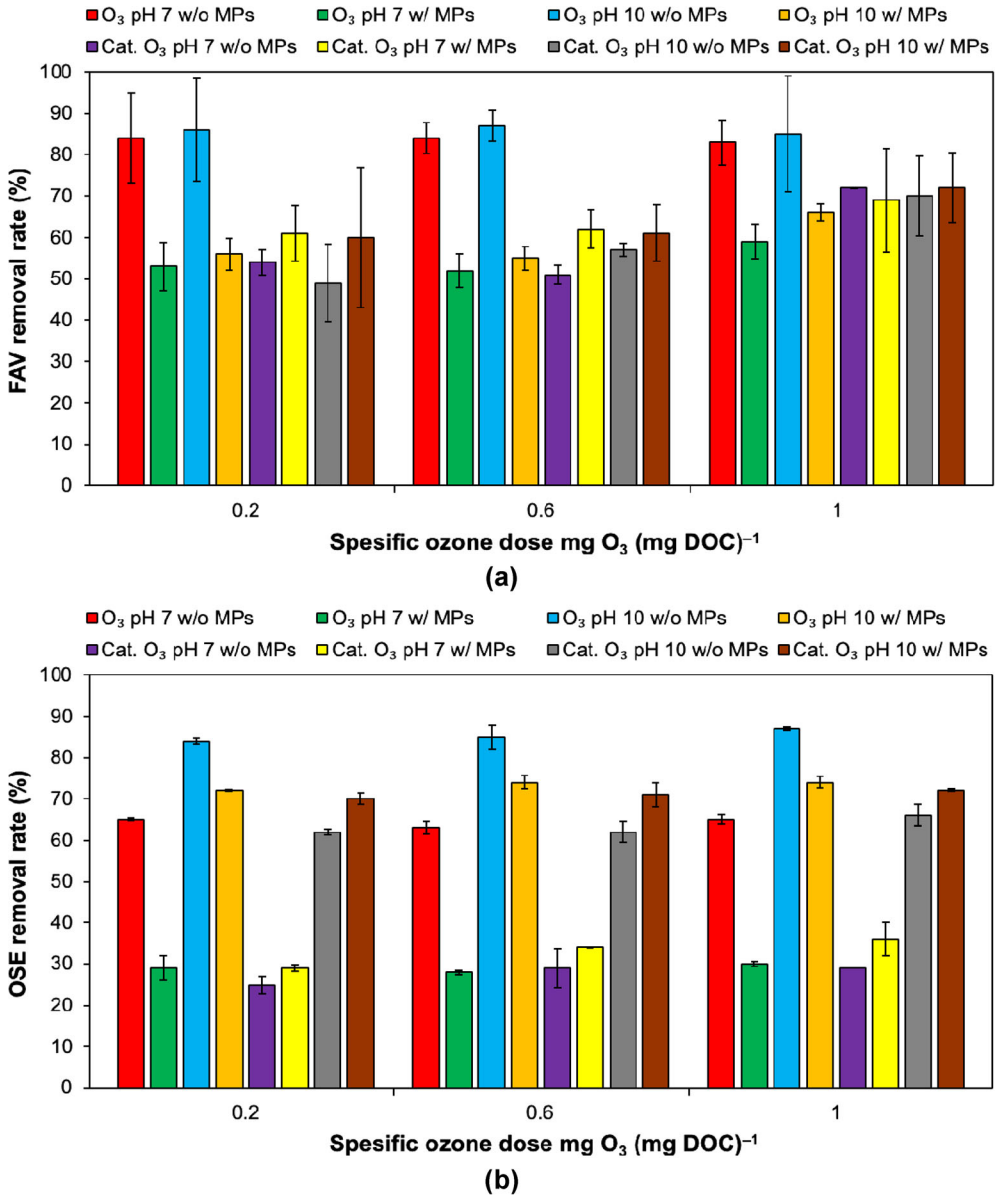

Figure 2. Antiviral removal efficiencies after ozonation and catalytic ozonation of Sample 1 with (w/) and without (w/o) MPs: (a) FAV; (b) OSE.

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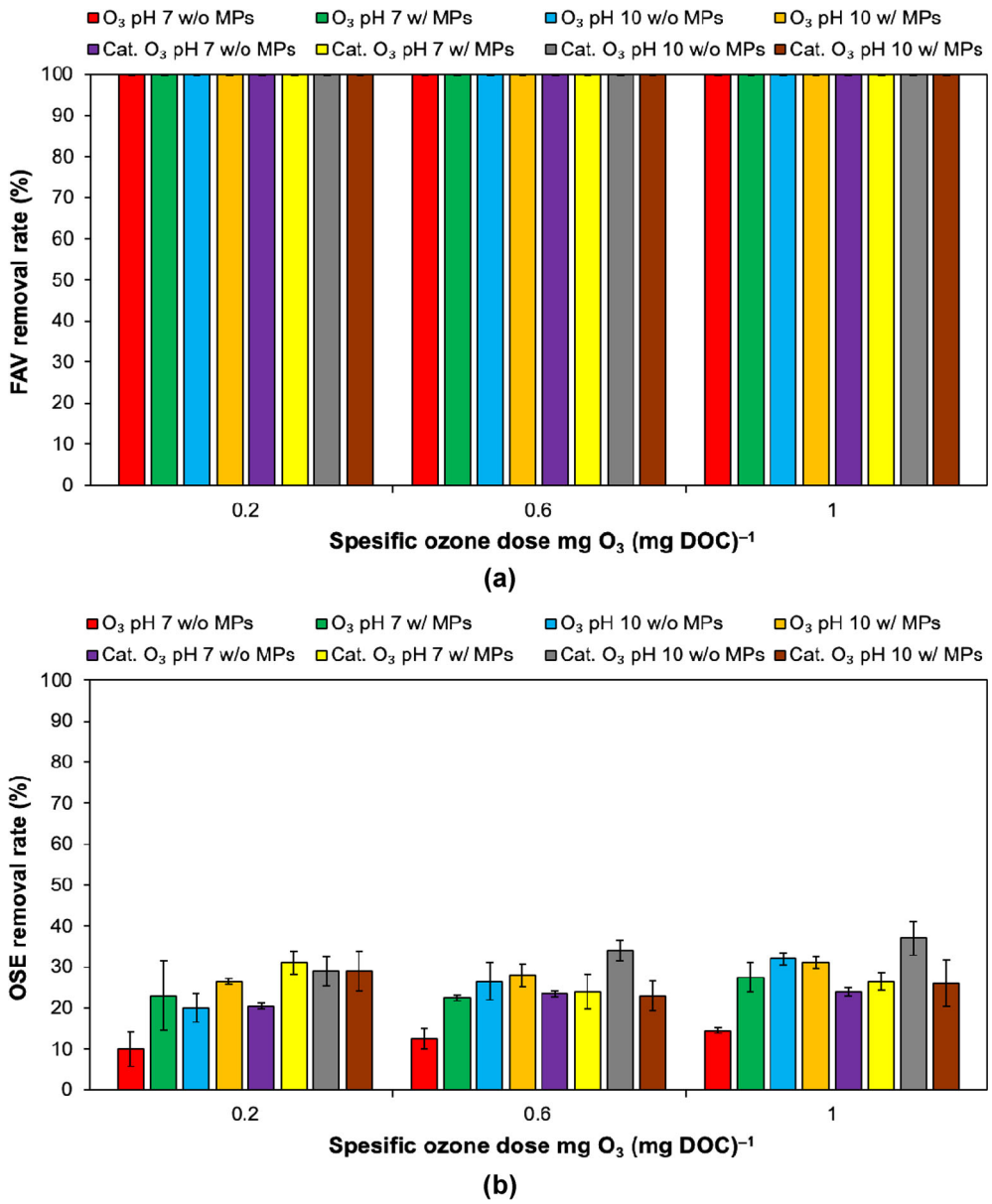

Figure 3. Antiviral removal efficiencies after ozonation and catalytic ozonation of Sample 2 with (w/) and without (w/o) MPs: (a) FAV; (b) OSE.

For the case of Sample 2, the experimental findings presented in Fig. 3(a) revealed that the degradation of FAV, with an initial concentration of 500 ng L^{-1}, was not affected by operational conditions such as the specific ozone dose, pH level, or the presence of MPs; both ozonation and catalytic ozonation processes achieved a removal efficiency of up to 100% since FAV was measured below the limit of quantification (LOQ) after each experimental trial. In the course of ozonation, adjusting the pH from 7 to 10 increased the reduction rate of OSE from 12 ± 2 to 26 ± 6% (Fig. 3(b)). During the catalytic ozonation process, raising the pH from 7 to 10 improved the degradation of OSE from 23 ± 2% to 33 ± 4%, indicating that both catalyst and alkaline environment positively impacted the OSE reduction. The data from catalytic ozonation experiments involving 12 runs with MPs divulged that they did not cause a significant change in the average removal rate of OSE when all process conditions were evaluated together.

In general, many studies concur that the degradation efficiency of a recalcitrant organic compound tends to decline as its initial concentration increases, which can be attributed to the fact that both the availability of oxidizing species and the generation efficiency of hydroxyl radicals diminish when the target compound is present at higher concentrations.[48,49] However, some researchers have found that the removal efficiency of compounds such as Acid Red 88 dye,[50] ciprofloxacin,[51] trimethoprim,[52] and tetracycline[53] is directly proportional to the initial concentration of the substance being investigated, similar to the different trend observed during ozonation of OSE in this study. The increase in degradation rates at higher initial concentrations could be explained by the generation of intermediates that accelerated the reaction.[52,54]

For Sample 1, unexpectedly, single ozonation outperformed catalytic ozonation. This outcome is likely due to the limited availability of radicals in the medium and an insufficient number of

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active sites on the catalyst, which are inadequate to effectively react with the relatively high contents (50 μg L^{-1}) of FAV and OSE. During catalytic ozonation of Sample 1, the catalyst surface may become saturated with the excess amount of antivirals, hindering further interaction between the catalyst and reactants and restricting the generation of more radicals.[55] Another possible reason for the reduced degradation of antivirals at higher concentrations is the increased formation of by-products, which may intensify the competition for radicals and active sites. Moreover, these compounds could have a stronger affinity for oxidative species, potentially making the related reactions more favorable.[56]

Effect of ozonation and catalytic ozonation on acute toxicity

The evolution of the acute toxicity of Sample 1 during ozonation and catalytic ozonation processes was monitored using the ISO 11348-3 test protocol[42] based on the inhibition of bioluminescence of marine bacteria *V. fischeri* after 15 min of exposure. Following the results presented and discussed in the preceding sub-section, although increasing the pH from 7 to 10 during treatment technologies in the absence of MPs did not make a difference in FAV degradation, an improvement of 21 ± 2% and 36 ± 2% was recorded in the removal of OSE for ozonation and catalytic ozonation conducted under alkaline pH conditions, respectively. Therefore, acute toxicity measurements were carried out on tertiary treated effluents ozonated at the elevated pH value of 10 with the highest specific ozone dose of 1 mg O_3 (mg DOC)$^{-1}$. Since the municipal secondary effluents are likely to have a complex nature and extremely variable composition of effluent organic matter (EfOM), the toxic strength of Sample 1 was first examined without spiking the antiviral drugs. As shown in Fig. 4, 56% of bioluminescence inhibition was observed for Sample 1 that was not spiked with FAV and OSE. Compared to Sample 1 containing no antivirals, a lower percentage of bioluminescence inhibition of *V. fischeri* (49%) was obtained after ozonation, indicating that the tested technique led to the formation of less toxic intermediates than the original compounds. When Sample 1 was spiked separately with FAV and OSE at 50 μg L^{-1}, the bioluminescence inhibition increased to 68 and 66%, respectively, reflecting FAV-spiked sample posed slightly more toxicity than OSE-spiked sample. Evaluation of the individual and combined ecotoxicity of the investigated antivirals using the *V. fischeri* under acute exposure conditions revealed that the adverse effect of the mixture (73%) was higher than those of the single drugs. The trends of *V. fischeri* inhibition observed during ozonation of samples spiked with either FAV or OSE were different from each other. For FAV, ozonation of Sample 1 at the initial FAV concentration of 50 μg L^{-1} exhibited an almost identical response to that of non-ozonated counterpart, reaching a bioluminescence inhibition ratio of 70%. Given the efficient removal of the parent compound by ozonation (Fig. 2(a)), the similarity in toxicity was attributed to the formation of toxic TPs during FAV degradation. The luminescence inhibition was significantly enhanced after ozonation of OSE (85%), indicating the possible formation of some highly toxic TPs for the studied bacteria. For Sample 1 containing 50 μg L^{-1} of each antiviral, the ecotoxicological assays demonstrated that ozonation resulted in almost no change in the bioluminescence inhibition (69%). Thus, the use of ozone led to the formation of TPs of FAV and OSE, the collective impact of which was found to be nearly equal to that of their parent compounds.

In the case of Sample 1 containing no antivirals, outcomes from *V. fischeri* divulged that the toxicity was not significantly affected by the amount of MPs (0.1 mg L^{-1}) used during the ozonation process, corresponding to a bioluminescence inhibition of 49%. Considering Sample 1 spiked with antivirals at 50 μg L^{-1}, ozonation in the absence and presence of MPs induced similar inhibition effects on *V. fischeri*'s luminescence (69 and 68%, respectively), verifying the negligible contribution of MPs to the acute toxicity. The presence of the catalyst during ozonation of Sample 1 containing both antivirals and MPs decreased the bioluminescence inhibition from 68 to 64%, which could be attributed to the fact that the toxicity of TPs generated by ozonation was likely higher than that by catalytic ozonation.

The impact of wastewater ozonation on toxicity presents conflicting observations and interpretations, complicating scientific understanding and practical application.[57] While some studies reported that ozonation reduced the bioluminescence inhibition

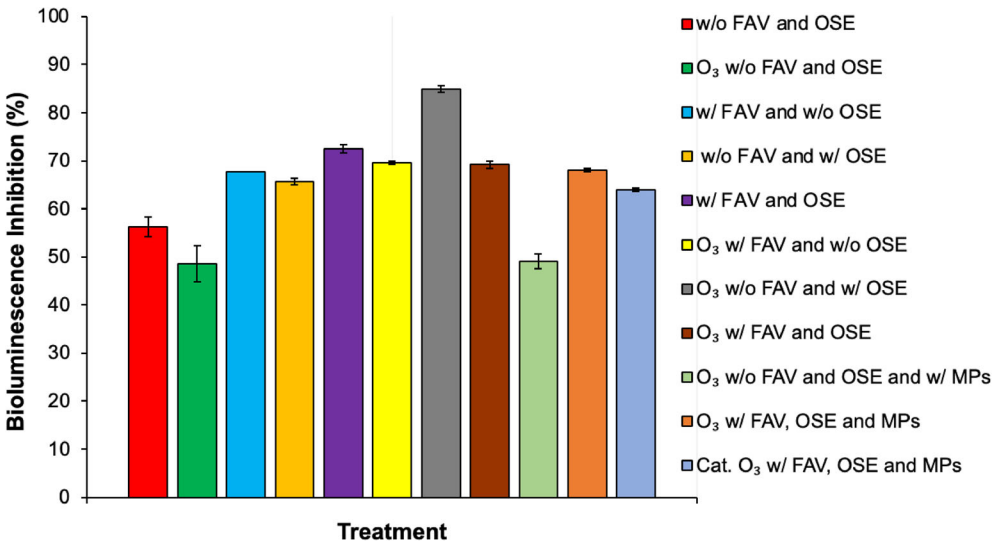

Figure 4. Inhibition on the luminescence of marine bacteria *V. fischeri* after exposure to Sample 1 with (w/) and without (w/o) FAV, OSE and MPs under ozonation and catalytic ozonation.

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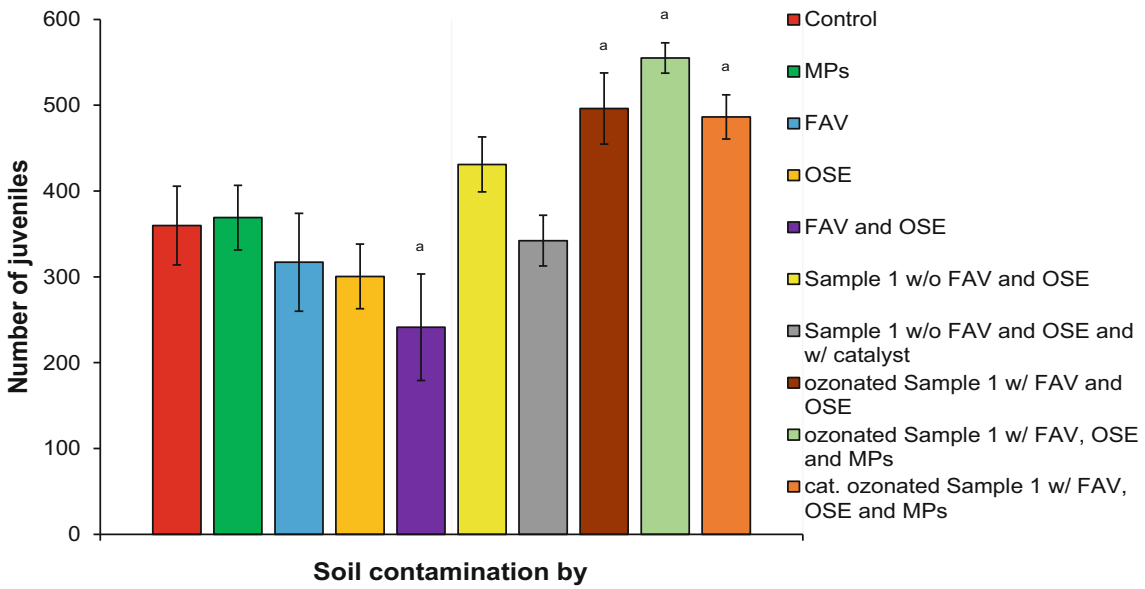
of *V. fischeri*,[58,59] others found the opposite, noting an increase in the acute toxicity towards *V. fischeri* after ozonation.[19,60] The effect of ozonation on bioluminescence inhibition is closely related to the removal of parent compounds and the formation of their TPs.[61] The amounts and types of TPs with different toxicological profiles may affect the observed trend, complicating the direct comparison of different studies. The variation in the toxicity-related impacts arises from the reaction kinetics, which dictate whether the process is primarily driven by ozone, hydroxyl radicals, or a combination of both, as well as the structure and properties of the micropollutants involved.[62]

Remarkably, there are very few studies in the literature on the ecotoxicological effects of FAV and OSE used against COVID-19. Escher *et al.*[63] used the quantitative structure–activity relationship (QSAR) approach to assess the mixture effects of oseltamivir ethylester (OE) and its metabolites on marine bacterium *V. fischeri* and pointed out that OE and oseltamivir acid (OA) acted as baseline toxicants in the bioluminescence inhibition test. In a previous research, Kuroda *et al.*[5] provided a model-based evaluation on the occurrence, fate and ecotoxicological effects of therapeutic agents associated with COVID-19 treatment during pandemic events. They suggested that among 11 therapeutic agents and their 13 metabolites, FAV, OSE, and OC posed a relatively low ecotoxicological risk for aquatic organisms in receiving waters, whereas an appreciably high ecotoxicological risk was observed for T705M1, the major inactive metabolite of FAV. In another study conducted by van Beek,[64] where the acute toxicity towards *V. fischeri* of treated and untreated OP samples were monitored, the bioluminescence inhibition screening tests revealed that both ozonated and non-ozonated OP samples in buffered and real wastewater matrices did not increase toxicity. Besides, the significant increase in the bioluminescence inhibition during ozonation of OSE achieved in this study was apparently in line with the observations of some previous research on the ozonation of certain drugs such as oxytetracycline,[65] ampicillin,[66] ketoprofen[67] and tamoxifen,[68] where toxicity tests by *V. fischeri* proved that the TPs appeared to be more toxic than the parent compounds. Additionally, the minimal impact of MP exposure on acute toxicity observed in this study was confirmed by several previous studies. Booth *et al.*[69] examined the effects of a wide range of environmentally relevant and elevated concentrations of poly(methylmethacrylate)-based plastic and fluorescent plastic nanoparticles, concluding that nano-sized plastics had no ecotoxicological effects on the bacteria *V. fischeri*. Similarly, Gagné[70] found no toxic impacts of microbeads of PE on quorum sensing in *Aliivibrio fisheri* at environmental concentrations. In another study, Gambardella *et al.*[71] assessed the acute toxicity of PE-based MPs of various sizes (1–500 µm) on *V. fischeri* and discovered that these MPs were not toxic to the unicellular marine organisms, even at concentrations higher than those found in the environment. Moreover, the non-toxic nature of the catalyst towards *V. fischeri* observed in this study was also reported by Koba-Ucun *et al.*,[72] who found that among the Zn—Fe LDHs samples prepared within 3 h and tested at the concentration range of 0.05–2 g L^{-1}, only those above 0.2 g L^{-1} exhibited an inhibitory effect on bioluminescence intensity after 15 min of exposure.

Effect of ozonation and catalytic ozonation on the reproduction of *E. crypticus*

In the first step of the reproduction toxicity tests with *E. crypticus*, a stock solution of FAV and OSE at 100 mg L^{-1} concentration was prepared in ultra-pure water, and the required volume of stock solution was added to 20 g of dry soil to achieve a nominal concentration of 10 mg antiviral (kg dry soil)$^{-1}$. The MP contents may differ significantly across studies due to research focus (0.02–1.5%,[73] 0.1–10%,[74] 0.005–5%[75]). MPs at a concentration range of 0.2 to 1.2% (w/w) in the soil surface are also considered to be environmentally relevant,[76] so the amount of MPs in dry soil was set at 0.5% to evaluate the potential adverse effects of PE on the earthworms. The results of the reproduction test with *E. crypticus* after 21 days of exposure to soil contaminated with MPs and antivirals, both single and combined exposures,



[a] Significant change in the number of juveniles compared to the control group.

Figure 5. Reproduction of *E. crypticus* after exposure to Sample 1 with (w/) and without (w/o) FAV, OSE, and MPs under ozonation and catalytic ozonation.

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are shown Fig. 5. As compared with the control group, exposure of the earthworms to 0.5% MPs for 21 days did not pose a significant change in the reproduction. There was no statistical difference between the control and the single drug-exposed samples as neither FAV nor OSE induced an effect on the number of juveniles produced. Unlike the findings from the single exposures, the co-presence of FAV and OSE significantly reduced the number of earthworm juveniles ($P < 0.05$), revealing that the combined exposure led to more severe stress compared to FAV and OSE, individually. Following the preliminary experiments, similar to the acute toxicity measurements, the ecotoxicity of both antivirals and MPs to soil fauna was performed on Sample 1 treated at the elevated pH level of 10 and at the highest specific ozone dose of 1 mg O_3 (mg DOC)$^{-1}$. During ecotoxicological experiments conducted on tertiary effluents, 20 g of dry soil was wetted with 4 mL of Sample 1 from each of the treatment conditions. As shown in the figure, 21 days of exposure to Sample 1 that was not spiked with FAV and OSE did not result in any significant change in the number of juveniles compared to the control. For Sample 1 containing no antivirals, the comparison of reproduction tests performed in the absence and presence of catalyst demonstrated that the number of juveniles produced by *E. crypticus* was not significantly different, which suggested the environmentally friendly and non-toxic nature of the catalyst. In the case of Sample 1 containing 50 μg L^{-1} of each analyte, ozonation in the absence and presence of MPs provided significant increases ($P < 0.05$) in the number of juveniles at the end of the test period, divulging that TPs of antiviral drugs resulting from ozonation treatment appeared to be less toxic to *E. crypticus* than the parent compounds. As conventional ozonation in the absence and presence of MPs exhibited a comparable increase in the number of *E. crypticus* juveniles, it could be inferred that the reproduction of earthworms was not noticeably affected by the exposure to PE. Similarly, catalytic ozonation of Sample 1 containing both antivirals and MPs significantly increased the reproduction of earthworms by 35% compared with the control group, confirming the non-toxic behavior of the catalyst towards *E. crypticus*.

Even though very few studies have been conducted to evaluate the ecotoxicological effects of FAV and OSE on aquatic organisms,[5,63,64] to the best of the authors' knowledge, previous research that has attempted to address the potential impacts of micropollutants on soil ecotoxicity did not include these antivirals. Nevertheless, the results from the single exposures with FAV and OSE obtained in this study were in agreement with the outcomes of previous pharmaceutical exposure studies such as the one reported by Ma *et al.*,[77] who stated that the exposure of oxytetracycline at an environmentally relevant concentration of 10 mg antiviral (kg dry soil)$^{-1}$ did not cause a change in the number of juveniles produced per worm over 21 days. Likewise, Jin *et al.*[78] declared that the reproduction rate of *E. crypticus* was not significantly different from that of the control after exposure to fluoroquinolone antibiotics (enrofloxacin, norfloxacin, and levofloxacin) at the concentration of 10 mg kg^{-1}. In another research by Mendes *et al.*,[79] where *E. crypticus* was exposed through contaminated soil with nanopolystyrene, alone and in combination with diphenhydramine, silver nitrate and vanadium nanoparticles, it was found that diphenhydramine at 10 mg kg^{-1} induced no effects in the reproduction of *E. crypticus* after 28 days. Similar to the interaction between FAV and OSE observed in the present study, resulting in a significant decrease in the number of *E. crypticus* juveniles, Zhou *et al.*[80] concluded that the toxicity of the mixture of cypermethrin and chlorpyrifos was significantly higher than either of these pesticides individually, leading to a significant reduction in the reproduction rate of earthworms. According to recent research carried out by Bart *et al.*,[81] it was deduced that the co-occurrence of azoxystrobin and prochloraz enhanced the toxicity to *E. crypticus* since prochloraz, like other azole fungicides, appeared to act as a synergist. Furthermore, the insignificant effect of PE exposure on *E. crypticus* reproduction noticed in the current study was also ascertained by various earlier studies. Rodríguez-Seijo *et al.*[82] recorded no significant differences in the average number of juveniles produced by earthworms after 56 days of exposure to the different MP concentrations. Amorim and Scott-Fordsmand[83] observed no deleterious effects of nanoparticles of PE on *E. crypticus* reproduction. More recently, Yang *et al.*[84] exposed the earthworms to artificially added PE at a concentration ranging from 0.05 to 20 g kg^{-1} soil for 60 days and found that the reproduction of earthworms was not noticeably affected by the exposure to MPs for 20 or 40 days. Considering the complexity of both the soil characteristics[85] and the structural composition of the catalysts,[86] it is difficult to predict the toxicity of catalysts to soil invertebrates since in soil, over extended periods of ageing, nanomaterials might undergo several processes, including aggregation/agglomeration, dissolution, and reaction with other molecules, which could impact their interaction with soil organisms.[87,88] Yet, the non-toxic nature of the catalyst investigated in this work was also witnessed in studies focusing on copper,[89] nickel oxide,[90] and magnesium oxide[91] nanoparticles where no significant differences were detected in the number of produced juveniles between *E. crypticus* exposed to nanoparticles and the control groups.

CONCLUSIONS

This study evaluated the effectiveness of ozonation and catalytic ozonation as tertiary treatment methods for the removal of FAV and OSE from SMWE samples, both in the presence and absence of MPs. A comparative assessment of the ecotoxicity of effluents treated by ozonation and catalytic ozonation was also performed to anticipate the potential ecotoxicological impacts of implementing these tertiary treatment technologies in municipal WWTPs. Based on the experimental data presented and discussed in the preceding sections, the following may be expressed as the concluding remarks of this research:

At high concentration level of 50 μg L^{-1} (Sample 1), ozonation at neutral pH range and at the lowest specific ozone dose of 0.2 mg O_3 (mg DOC)$^{-1}$ yielded a FAV removal of 84%, while the presence of catalyst or MPs alone decreased the removal efficiency by 30%. The degradation of FAV in Sample 2 with an initial concentration of 500 ng L^{-1} was not influenced by operational conditions, including specific ozone dose, pH value, and the presence of MPs, achieving a 100% elimination in both ozonation and catalytic ozonation processes.

During ozonation of OSE at an initial concentration of 50 μg L^{-1} (Sample 1), adjusting the pH to 10 improved the elimination level from 64 to 85%, regardless of the specific ozone dose applied. For all specific ozone doses, the addition of MPs to the ozonation process or the implementation of catalytic ozonation at neutral pH reduced the OSE removal efficiency by 30–40%. Ozonation and catalytic ozonation experiments conducted on Sample 2 containing low concentration of OSE (500 ng L^{-1}) showed a relatively pH-independent removal usually in the range of 20–40%, disclosing that both MPs and catalyst had practically no impact on the extent of OSE removal.

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Acute toxicity tests using *V. fischeri* bacteria carried out on Sample 1 treated at pH 10 with the highest specific ozone dose of 1 mg O_3 (mg DOC)$^{-1}$ indicated that the TPs of OSE were more toxic than the parent chemical, while ozonation of FAV almost retained the same toxicity towards the studied bacteria as that of the original compound. Ozonation of Sample 1, containing both antivirals, resulted in the formation of TPs of FAV and OSE, with their combined effect nearly equal to that of the parent antivirals. Luminescent bacteria toxicity testing revealed the non-toxic nature of the catalyst, whereas the MPs induced a negligible toxicity effect on *V. fischeri*.

Reproduction toxicity tests with *E. crypticus* performed on Sample 1 treated at the elevated pH level of 10 and at the highest specific ozone dose of 1 mg O_3 (mg DOC)$^{-1}$ revealed that, in contrast to the results from single exposures, the combined presence of FAV and OSE significantly reduced the number of earthworm juveniles. TPs of antiviral drugs formed during ozonation appeared to be less toxic to *E. crypticus* compared to the parent compounds, while the reproduction of earthworms was not significantly influenced by the exposure to either MPs or catalyst.

The experimental findings suggested that ozonation and catalytic ozonation are promising methods for upgrading existing WWTPs. These techniques not only helped to decrease the release of antivirals from domestic effluents, but also considerably improved the quality of treated wastewater for use in agricultural irrigation. Though the obtained results are promising, further research is necessary to scale up the examined processes from the laboratory to field conditions and to validate the applied approach in pilot and full-scale systems. Future studies should expand on the current research by providing additional data on assessing the degradation of FAV and OSE in real secondary wastewater effluents, identifying TPs and potential reaction pathways, exploring the sustainability and recyclability of the catalyst, investigating changes in the surface morphology of the spent catalyst after treatment, conducting ecotoxicity tests on various organisms using programs like Ecological Structure Activity Relationships (ECOSAR), and evaluating the economic feasibility of the proposed water reuse scheme.

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DATA AVAILABILITY STATEMENT

Data available upon reasonable request from corresponding author.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1 Wang R, Luo J, Li C, Chen J and Zhu N, Antiviral drugs in wastewater are on the rise as emerging contaminants: a comprehensive review of spatiotemporal characteristics, removal technologies and environmental risks. *J Hazard Mater* **457**:131694 (2023).
2 Funke J, Prasse C and Ternes TA, Identification of transformation products of antiviral drugs formed during biological wastewater treatment and their occurrence in the urban water cycle. *Water Res* **98**:75–83 (2016).
3 Dai H, Wang C, Yu W and Han J, Tracing COVID-19 drugs in the environment: are we focusing on the right environmental compartment? *Environ Pollut* **339**:122732 (2023).
4 Nannou C, Ofrydopoulou A, Evgenidou E, Heath D, Heath E and Lambropoulou D, Antiviral drugs in aquatic environment and wastewater treatment plants: a review on occurrence, fate, removal and ecotoxicity. *Sci Total Environ* **699**:134322 (2020).
5 Kuroda K, Li C, Dhangar K and Kumar M, Predicted occurrence, ecotoxicological risk and environmentally acquired resistance of antiviral drugs associated with COVID-19 in environmental waters. *Sci Total Environ* **776**:145740 (2021).
6 Morales-Paredes CA, Rodríguez-Díaz JM and Boluda-Botella N, Pharmaceutical compounds used in the COVID-19 pandemic: a review of their presence in water and treatment techniques for their elimination. *Sci Total Environ* **814**:152691 (2022).
7 Nannou C, Ofrydopoulou A, Evgenidou E, Heath D, Heath E and Lambropoulou D, Analytical strategies for the determination of antiviral drugs in the aquatic environment. *Trends Environ Anal Chem* **24**: e00071 (2019).
8 Fernández LP, Brasca R, Attademo AM, Peltzer PM, Lajmanovich RC and Culzoni MJ, Bioaccumulation and glutathione S-transferase activity on *Rhinella arenarum* tadpoles after short-term exposure to antiretrovirals. *Chemosphere* **246**:125830 (2020).
9 Nippes RP, Macruz PD, da Silva GN and Scaliante MHNO, A critical review on environmental presence of pharmaceutical drugs tested for the covid-19 treatment. *Process Saf Environ Prot* **152**:568–582 (2021).
10 Cappelli F, Longoni O, Rigato J, Rusconi M, Sala A, Fochi I *et al.*, Suspect screening of wastewaters to trace anti-COVID-19 drugs: potential adverse effects on aquatic environment. *Sci Total Environ* **824**: 153756 (2022).
11 Wen J, Duan L, Wang B, Dong Q, Liu Y, Huang J *et al.*, Stability and WBE biomarkers possibility of 17 antiviral drugs in sewage and gravity sewers. *Water Res* **238**:120023 (2023).
12 Azuma T, Nakada N, Yamashita N and Tanaka H, Synchronous dynamics of observed and predicted values of anti-influenza drugs in environmental waters during a seasonal influenza outbreak. *Environ Sci Technol* **46**:12873–12881 (2012).
13 Takanami R, Ozaki H, Giri RR, Taniguchi S and Hayashi S, Antiviral drugs zanamivir and oseltamivir found in wastewater and surface water in Osaka, Japan. *J Water Environ Technol* **10**:57–68 (2012).
14 Dogruel S, Cetinkaya Atesci Z, Aydin E and Pehlivanoglu-Mantas E, Ozonation in advanced treatment of secondary municipal wastewater effluents for the removal of micropollutants. *Environ Sci Pollut Res* **27**:45460–45475 (2020).
15 Rizzo L, Gernjak W, Krzeminski P, Malato S, McArdell CS, Perez JAS *et al.*, Best available technologies and treatment trains to address current challenges in urban wastewater reuse for irrigation of crops in EU countries. *Sci Total Environ* **710**:136312 (2020).
16 Giannakis S, Vives FAG, Grandjean D, Magnet A, De Alencastro LF and Pulgarin C, Effect of advanced oxidation processes on the micropollutants and the effluent organic matter contained in municipal wastewater previously treated by three different secondary methods. *Water Res* **84**:295–306 (2015).
17 Garrido-Cardenas JA, Esteban-García B, Agüera A, Sánchez-Pérez JA and Manzano-Agugliaro F, Wastewater treatment by advanced oxidation process and their worldwide research trends. *Int J Environ Res Public Health* **17**:170 (2020).
18 Rizzo L, Malato S, Antakyali D, Beretsou VG, Đolić MB, Gernjak W *et al.*, Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban wastewater. *Sci Total Environ* **655**:986–1008 (2019).
19 Chys M, Demeestere K, Ingabire AS, Dries J, van Langenhove H and van Hulle SWH, Enhanced treatment of secondary municipal wastewater effluent: comparing (biological) filtration and ozonation in view of micropollutant removal, unselective effluent toxicity, and the potential for real-time control. *Water Sci Technol* **76**:236–246 (2017).
20 Singh S, Seth R, Tabe S and Yang P, Oxidation of emerging contaminants during pilot-scale ozonation of secondary treated municipal effluent. *Ozone Sci Eng* **37**:323–329 (2015).
21 Blackbeard J, Lloyd J, Magyar M, Mieog J, Linden KG and Lester Y, Demonstrating organic contaminant removal in an ozone-based water

reuse process at full scale. *Environ Sci Water Res Technol* **2**:213–222 (2016).
22 Ma D, Yi H, Lai C, Liu X, Huo X, An Z *et al*., Critical review of advanced oxidation processes in organic wastewater treatment. *Chemosphere* **275**:130104 (2021).
23 Issaka E, Amu-Darko JNO, Yakubu S, Fapohunda FO, Ali N and Bilal M, Advanced catalytic ozonation for degradation of pharmaceutical pollutants – a review. *Chemosphere* **289**:133208 (2022).
24 Mohapatra L and Parida K, A review on the recent progress, challenges and perspective of layered double hydroxides as promising photocatalysts. *J Mater Chem A* **4**:10744–10766 (2016).
25 Jin Y, Li L, Yu L, Li L, Zhang S and Huang Y, Quantitative analysis of the structure of organic acids and their degradation rates during ozonation catalyzed with ZnAl layered double hydroxide. *Ozone Sci Eng* **45**:202–212 (2023).
26 Vuppaladadiyam SSV, Vuppaladadiyam AK, Sahoo A, Urgunde A, Murugavelh S, Šrámek V *et al*., Waste to energy: trending key challenges and current technologies in waste plastic management. *Sci Total Environ* **913**:169436 (2023).
27 Xu X, Jian Y, Xue Y, Hou Q and Wang L, Microplastics in the wastewater treatment plants (WWTPs): occurrence and removal. *Chemosphere* **235**:1089–1096 (2019).
28 Adeel M, Maniakova G and Rizzo L, Tertiary/quaternary treatment of urban wastewater by UV/H_2O_2 or ozonation: microplastics may affect removal of *E. coli* and contaminants of emerging concern. *Sci Total Environ* **907**:167940 (2024).
29 Enfrin M, Dumée LF and Lee J, Nano/microplastics in water and wastewater treatment processes – origin, impact and potential solutions. *Water Res* **161**:621–638 (2019).
30 Yu F, Yang C, Zhu Z, Bai X and Ma J, Adsorption behavior of organic pollutants and metals on micro/nanoplastics in the aquatic environment. *Sci Total Environ* **694**:133643 (2019).
31 Fan X, Gan R, Liu J, Xie Y, Xu D, Xiang Y *et al*., Adsorption and desorption behaviors of antibiotics by tire wear particles and polyethylene microplastics with or without aging processes. *Sci Total Environ* **771**:145451 (2021).
32 Munoz M, Ortiz D, Nieto-Sandoval J, de Pedro ZM and Casas JA, Adsorption of micropollutants onto realistic microplastics: role of microplastic nature, size, age, and NOM fouling. *Chemosphere* **283**:131085 (2021).
33 El Hassani K, Kalnina D, Turks M, Beakou BH and Anouar A, Enhanced degradation of an azo dye by catalytic ozonation over Ni-containing layered double hydroxide nanocatalyst. *Sep Purif Technol* **210**:764–774 (2019).
34 Hu R, Li JY, Yu Q, Yang SQ, Ci X, Qu B *et al*., Catalytic ozonation of reverse osmosis concentrate from coking wastewater reuse by surface oxidation over Mn-Ce/γ-Al_2O_3: effluent organic matter transformation and its catalytic mechanism. *J Hazard Mater* **471**:134363 (2024).
35 Yaseen A, Assad I, Sofi MS, Hashmi MZ and Bhat SU, A global review of microplastics in wastewater treatment plants: understanding their occurrence, fate and impact. *Environ Res* **212**:113258 (2022).
36 Martín C, Fajardo C, Costa G, Sánchez-Fortún S, San Andrés MD, González F *et al*., Bioassays to assess the ecotoxicological impact of polyethylene microplastics and two organic pollutants, simazine and ibuprofen. *Chemosphere* **274**:129704 (2021).
37 Sobhani Z, Panneerselvan L, Fang C, Naidu R and Megharaj M, Chronic and transgenerational effects of polyethylene microplastics at environmentally relevant concentrations in earthworms. *Environ Technol Innov* **25**:102226 (2022).
38 International Organization for Standardization (ISO), *Water Quality – Test for Inhibition of Oxygen Consumption by Activated Sludge for Carbonaceous and Ammonium Oxidation*. Ref No. ISO 8192–2007, 2nd edn. Genova (2007).
39 O'Connor JT, *Environmental Engineering Unit Operations and Unit Processes Laboratory Manual*. Association of Environmental Engineering Professors, University of Texas, Austin (1972).
40 American Public Health Association, American Water Works Association, Water Environment Federation, in *Standard Methods for the Examination of Water and Wastewater*, 23rd edn, ed. by Eaton AD, Rice EW and Baird RB. American Public Health Association, Washington, DC (2017).
41 Keyikoglu R, Khataee A and Yoon Y, Layered double hydroxides for removing and recovering phosphate: recent advances and future directions. *Adv Colloid Interface Sci* **300**:102598 (2022).
42 International Organization for Standardization (ISO), Water Quality – Determination of the Inhibitory Effect of Water Samples on the Light Emission of Vibrio Fischeri (Luminescent Bacteria Test) – Part 3: Method Using Freeze-dried Bacteria. Ref No. ISO 11348-3–2007, 1st edn. Genova (2007).
43 Organization for Economic Co-operation and Development (OECD), OECD Guidelines for the Testing of Chemicals No. 220: Enchytraeid Reproduction Test. Paris (2004).
44 Castro-Ferreira MP, Roelofs D, van Gestel CA, Verweij RA, Soares AM and Amorim MJ, Enchytraeus crypticus as model species in soil ecotoxicology. *Chemosphere* **87**:1222–1227 (2012).
45 Chen Z, Li M and Wen Q, Comprehensive evaluation of three sets of advanced wastewater treatment trains for treating secondary effluent: organic micro-pollutants and bio-toxicity. *Chemosphere* **189**:426–434 (2017).
46 Yin Z, Zhu J, Wang Z, Liu Y, Yang Z and Yang W, Novel Fe/N co-doping biochar based electro-Fenton catalytic membrane enabling enhanced tetracycline removal and self-cleaning performance. *J Clean Prod* **402**:136731 (2023).
47 Bellucci M, Marazzi F, Naddeo LS, Piergiacomo F, Beneduce L, Ficara E *et al*., Disinfection and nutrient removal in laboratory-scale photobioreactors for wastewater tertiary treatment. *J Chem Technol Biotechnol* **95**:959–966 (2020).
48 Barik AJ and Gogate PR, Degradation of 4-chloro 2-aminophenol using combined strategies based on ultrasound, photolysis and ozone. *Ultrason Sonochem* **28**:90–99 (2016).
49 Mohod AV, Teixeira ACSC, Bagal MV, Gogate PR and Giudici R, Degradation of organic pollutants from wastewater using hydrodynamic cavitation: a review. *J Environ Chem Eng* **11**:109773 (2023).
50 Saharan VK, Pandit AB, Satish Kumar PS and Anandan S, Hydrodynamic cavitation as an advanced oxidation technique for the degradation of acid red 88 dye. *Ind Eng Chem Res* **51**:1981–1989 (2012).
51 Abdulrahman SA, Shnain ZY, Ibrahim SS and Majdi HS, Photocatalytic degradation of ciprofloxacin by UV light using n-doped TiO_2 in suspension and coated forms. *Catalysts* **12**:1663 (2022).
52 Alsaidi M, Azeez FA, Al-Hajji LA and Ismail AA, Impact of reaction parameters for photodegradation pharmaceuticals in wastewater over gold/titania photocatalyst synthesized by pyrolysis of NH_2-MIL-125 (Ti). *J Environ Manage* **314**:115047 (2022).
53 Zhang Y, Wang L, Lu L, Liu M, Yuan Z, Yang L *et al*., Highly efficient decontamination of tetracycline and pathogen by a natural product-derived emodin/HAp photocatalyst. *Chemosphere* **305**:135401 (2022).
54 Pi Y, Schumacher J and Jekel M, Decomposition of aqueous ozone in the presence of aromatic organic solutes. *Water Res* **39**:83–88 (2005).
55 Gomes J, Lincho J, Mazierski P, Miodyńska M, Zaleska-Medynska A and Martins RC, Unexpected effect of ozone on the Paraben's mixture degradation using TiO_2 supported nanotubes. *Sci Total Environ* **743**:140831 (2020).
56 Fernandes E, Drosopoulou S, Mazierski P, Miodyńska M, Gołaszewska D, Zaleska-Medynska A *et al*., Carbon nitride photoactivation evaluation and degradation of a mixture of parabens by ozone assistance. *J Water Process Eng* **49**:103018 (2022).
57 Wu QY, Yang LL, Du Y, Liang ZF, Wang WL, Song ZM *et al*., Toxicity of ozonated wastewater to HepG2 cells: taking full account of nonvolatile, volatile, and inorganic byproducts. *Environ Sci Technol* **55**:10597–10607 (2021).
58 Reungoat J, Escher BI, Macova M, Argaud FX, Gernjak W and Keller J, Ozonation and biological activated carbon filtration of wastewater treatment plant effluents. *Water Res* **46**:863–872 (2012).
59 Nasuhoglu D, Isazadeh S, Westlund P, Neamatallah S and Yargeau V, Chemical, microbial and toxicological assessment of wastewater treatment plant effluents during disinfection by ozonation. *Chem Eng J* **346**:466–476 (2018).
60 Oktem YA, Yuzer B, Aydin MI, Okten HE, Meric S and Selcuk H, Chloride or sulfate? Consequences for ozonation of textile wastewater. *J Environ Manage* **247**:749–755 (2019).
61 Lashuk B and Yargeau V, A review of ecotoxicity reduction in contaminated waters by heterogeneous photocatalytic ozonation. *Sci Total Environ* **787**:147645 (2021).
62 Lee Y and von Gunten U, Advances in predicting organic contaminant abatement during ozonation of municipal wastewater effluent: reaction kinetics, transformation products, and changes of biological effects. *Environ Sci Water Res Technol* **2**:421–442 (2016).

63 Escher BI, Bramaz N, Lienert J, Neuwoehner J and Straub JO, Mixture toxicity of the antiviral drug Tamiflu® (oseltamivir ethylester) and its active metabolite oseltamivir acid. *Aquat Toxicol* **96**:194–202 (2010).

64 van Beek SJ, *Treatment of Antiviral Drugs in Wastewater Using Advanced Oxidation Processes – Ozonation of Oseltamivir Phosphate*. MSc Thesis. University of Alberta, Alberta (2021).

65 Li K, Yediler A, Yang M, Schulte-Hostede S and Wong MH, Ozonation of oxytetracycline and toxicological assessment of its oxidation by-products. *Chemosphere* **72**:473–478 (2008).

66 Jung YJ, Kim WG, Yoon Y, Hwang TM and Kang JW, pH effect on ozonation of ampicillin: kinetic study and toxicity assessment. *Ozone Sci Eng* **34**:156–162 (2012).

67 Illés E, Szabó E, Takács E, Wojnárovits L, Dombi A and Gajda-Schrantz K, Ketoprofen removal by O_3 and O_3/UV processes: kinetics, transformation products and ecotoxicity. *Sci Total Environ* **472**:178–184 (2014).

68 Ferrando-Climent L, Gonzalez-Olmos R, Anfruns A, Aymerich I, Corominas L, Barceló D *et al*., Elimination study of the chemotherapy drug tamoxifen by different advanced oxidation processes: transformation products and toxicity assessment. *Chemosphere* **168**:284–292 (2017).

69 Booth AM, Hansen BH, Frenzel M, Johnsen H and Altin D, Uptake and toxicity of methylmethacrylate-based nanoplastic particles in aquatic organisms. *Environ Toxicol Chem* **35**:1641–1649 (2016).

70 Gagné F, Toxicity and disruption of quorum sensing in *Aliivibrio fisheri* by environmental chemicals: impacts of selected contaminants and microplastics. *J Xenobiot* **7**:7101 (2017).

71 Gambardella C, Piazza V, Albentosa M, Bebianno MJ, Cardoso C, Faimali M *et al*., Microplastics do not affect standard ecotoxicological endpoints in marine unicellular organisms. *Mar Pollut Bull* **143**:140–143 (2019).

72 Koba-Ucun O, Ölmez Hanci T, Arslan-Alaton I, Arefi-Oskoui S, Khataee A, Kobya M *et al*., Toxicity of Zn-Fe layered double hydroxide to different organisms in the aquatic environment. *Molecules* **26**:395 (2021).

73 Selonen S, Dolar A, Kokalj AJ, Skalar T, Dolcet LP, Hurley R *et al*., Exploring the impacts of plastics in soil – the effects of polyester textile fibers on soil invertebrates. *Sci Total Environ* **700**:134451 (2020).

74 Li M, Wu D, Wu D, Guo H and Han S, Influence of polyethylene-microplastic on environmental behaviors of metals in soil. *Environ Sci Pollut Res* **28**:28329–28336 (2021).

75 Šmídová K, Selonen S, van Gestel CA, Fleissig P and Hofman J, Microplastics originated from agricultural mulching films affect enchytraeid multigeneration reproduction and soil properties. *J Hazard Mater* **479**:135592 (2024).

76 Huerta Lwanga E, Gertsen H, Gooren H, Peters P, Salanki T, van der Ploeg M *et al*., Microplastics in the terrestrial ecosystem: implications for *Lumbricus terrestris* (Oligochaeta, Lumbricidae). *Environ Sci Technol* **50**:2685–2691 (2016).

77 Ma J, Zhu D, Sheng GD, O'Connor P and Zhu YG, Soil oxytetracycline exposure alters the microbial community and enhances the abundance of antibiotic resistance genes in the gut of *Enchytraeus crypticus*. *Sci Total Environ* **673**:357–366 (2019).

78 Jin MK, Zhang Q, Zhao WL, Li ZH, Qian HF, Yang XR *et al*., Fluoroquinolone antibiotics disturb the defense system, gut microbiome, and antibiotic resistance genes of *Enchytraeus crypticus*. *J Hazard Mater* **424**:127509 (2022).

79 Mendes LA, Barreto A, Santos J, Amorim MJ and Maria VL, Co-exposure of nanopolystyrene and other environmental contaminants – their toxic effects on the survival and reproduction of *Enchytraeus crypticus*. *Toxics* **10**:193 (2022).

80 Zhou S, Duan C, Michelle WHG, Yang F and Wang X, Individual and combined toxic effects of cypermethrin and chlorpyrifos on earthworm. *J Environ Sci* **23**:676–680 (2011).

81 Bart S, Short S, Jager T, Eagles EJ, Robinson A, Badder C *et al*., How to analyse and account for interactions in mixture toxicity with toxicokinetic-toxicodynamic models. *Sci Total Environ* **843**:157048 (2022).

82 Rodríguez-Seijo A, Lourenço J, Rocha-Santos TAP, da Costa J, Duarte AC, Vala H *et al*., Histopathological and molecular effects of microplastics in *Eisenia andrei* Bouché. *Environ Pollut* **220**:495–503 (2017).

83 Amorim MJ and Scott-Fordsmand JJ, Plastic pollution – a case study with *Enchytraeus crypticus* – from micro-to nanoplastics. *Environ Pollut* **271**:116363 (2021).

84 Yang X, Zhang X, Shu X, Gong J, Yang J, Li B *et al*., The effects of polyethylene microplastics on the growth, reproduction, metabolic enzymes, and metabolomics of earthworms *Eisenia fetida*. *Ecotoxicol Environ Saf* **263**:115390 (2023).

85 Caballero-Guzman A and Nowack B, A critical review of engineered nanomaterial release data: are current data useful for material flow modeling? *Environ Pollut* **213**:502–517 (2016).

86 Gatica JM, García-Cabeza AL, Yeste MP, Marín-Barrios R, González-Leal JM, Blanco G *et al*., Carbon integral honeycomb monoliths as support of copper catalysts in the Kharasch-Sosnovsky oxidation of cyclohexene. *Chem Eng J* **290**:174–184 (2016).

87 García-Gómez C, García S, Obrador A, Almendros P, González D and Fernández MD, Effect of ageing of bare and coated nanoparticles of zinc oxide applied to soil on the Zn behaviour and toxicity to fish cells due to transfer from soil to water bodies. *Sci Total Environ* **706**:135713 (2020).

88 Jośko I, Kusiak M, Xing B and Oleszczuk P, Combined effect of nano-CuO and nano-ZnO in plant-related system: from bioavailability in soil to transcriptional regulation of metal homeostasis in barley. *J Hazard Mater* **416**:126230 (2021).

89 Unrine JM, Tsyusko OV, Hunyadi SE, Judy JD and Bertsch PM, Effects of particle size on chemical speciation and bioavailability of copper to earthworms (*Eisenia fetida*) exposed to copper nanoparticles. *J Environ Qual* **39**:1942–1953 (2010).

90 Adeel M, Ma C, Ullah S, Rizwan M, Hao Y, Chen C *et al*., Exposure to nickel oxide nanoparticles insinuates physiological, ultrastructural and oxidative damage: a life cycle study on *Eisenia fetida*. *Environ Pollut* **254**:113032 (2019).

91 Ogliari AJ, Borges WG, Silva LL, de Mello JMM, Baretta D, Fiori MA *et al*., Magnesium oxide nanoparticles and their ecotoxicological effect on edaphic organisms in tropical soil. *J Appl Toxicol* **42**:553–569 (2022).

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