



Full-scale multipurpose ozonation in urban wastewater with strong textile input: removal of colour, micropollutants, pathogens and antimicrobial resistance indicators[☆]

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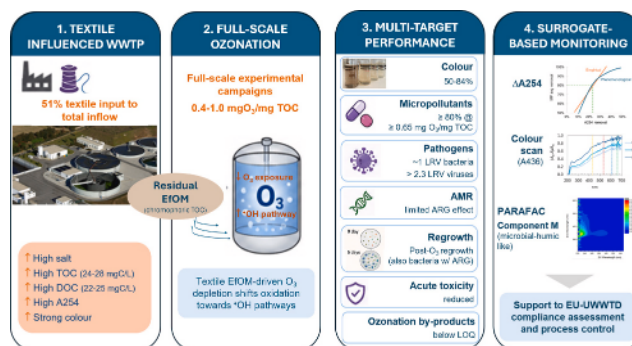
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HIGHLIGHTS

- Textile EfOM increased ozone demand and shifted oxidation to $\cdot\text{OH}$ pathways.
- 50–84% colour removal and reduced toxicity at 0.4–1.0 mg O_3/mg TOC
- $\geq 80\%$ EU-UWWTD micropollutant removal at ≥ 0.65 mg O_3/mg TOC
- Compounds depending on both $\text{O}_3/\cdot\text{OH}$ pathways showed the highest removal sensitivity.
- Only ~ 1 LRV bacterial & AMR gene reduction; regrowth promoted at > 0.8 mg O_3/mg TOC

GRAPHICAL ABSTRACT



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ABSTRACT

Understanding multipurpose ozonation performance under realistic and variable wastewater conditions is critical for investment decisions under the revised EU Urban Wastewater Treatment Directive (EU-UWWTD). As most studies focus on single treatment objectives or homogeneous municipal effluents, integrated evaluations are particularly needed for industry-influenced wastewaters, where ozone reactivity and process efficiency can differ markedly. This study aims to provide a holistic assessment of full-scale ozonation by simultaneously investigating the responses of chemical and biological contaminants, as well as microbial community dynamics,

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Monitoring indicators
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to variable ozone doses applied primarily for textile colour control. To our knowledge, this is one of the first integrated full-scale studies of ozonation in a strongly textile-influenced urban WWTP (51% of total inflow). Ozonation performance was assessed through three full-scale experimental campaigns, each evaluating three ozone doses in the range of 0.4–1.0 mg O₃/mg TOC. The study evaluated colour removal, micropollutant abatement, pathogen and antimicrobial resistance (AMR) indicators reduction, by-product formation, acute toxicity and surrogate parameters for process monitoring. Ozonation had little effect on bulk organic parameters but achieved 50–84% colour removal and near-complete elimination of terrestrial humic-like fluorescence. High organic matter content and colour constrained ozone exposure, shifting micropollutant abatement towards predominantly •OH-driven oxidation. Nevertheless, ozonation alone achieved ≥80% removal of the monitored micropollutants at ozone doses ≥0.65 mg O₃/mg C, indicating its major contribution towards EU-UWWTD compliance targets. Somatic coliphages and bacteria log-reductions ranged from 0.9 to >2.3 and from 0.2 to 1.2, respectively. Acute toxicity was reduced, whereas AMR gene abundance showed limited sensitivity to ozone dose. Post-treatment microbial regrowth was observed, particularly at higher ozone doses, including bacterial groups carrying resistance genes, suggesting a selection-like process. Operational surrogate indicators (A254, A436, colour-scan and PARAFAC components) were identified/validated to support process monitoring and EU-UWWTD implementation in complex wastewater matrices.

1. Introduction

Water scarcity, environmental and public health concerns, together with increasingly demanding legislation, are placing growing pressure on urban wastewater treatment plants (WWTPs). In the European Union, the revised Urban Wastewater Treatment Directive [1] introduces quaternary treatment requirements for selected WWTPs, targeting a minimum average micropollutant (MP) removal of 80%. The directive also strengthens requirements related to nutrient removal, energy neutrality, antimicrobial resistance (AMR) monitoring and water reuse, increasing the need for treatment strategies capable of simultaneously addressing chemical and microbial risks. In parallel, the EU-One Health Action Plan [2] identifies wastewater systems as relevant reservoirs and dissemination pathways of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (AMR genes or ARGs).

Within this regulatory context, ozonation is increasingly considered for WWTP upgrading and is already implemented in several European WWTPs for micropollutant control [3,4]. Ozonation has also been extensively used in drinking water treatment for pre-oxidation and disinfection [5], as well as for industrial wastewater decolourisation [6,7].

Unlike many ozone-based advanced oxidation processes (AOP), which rely on hydroxyl radical (•OH) formation, usually using catalysts, a combination of oxidants and/or higher energy inputs [8,9], ozonation combines selective oxidation by molecular ozone (direct pathway) with in situ formation of hydroxyl radicals due to ozone reaction with organic matter (indirect pathway) [10]. In theory, this combination enables the simultaneous control of colour, micropollutants and microbial targets [10]. Both molecular ozone and •OH contribute to the degradation of micropollutants, effluent organic matter (EfOM), and textile dyes [11–14]. However, microbial inactivation is generally attributed primarily to molecular ozone [13,15,16], whereas the role of •OH remains less well understood [17]. Contradictory findings have been reported regarding the role of •OH in microbial inactivation, with some studies reporting lower [13,15,18] and others higher or accelerated pathogen inactivation [16,19,20] under conditions promoting exposure to •OH radical.

The relative contribution of direct and indirect ozonation pathways depends strongly on wastewater characteristics, particularly the EfOM and alkalinity, which governs ozone consumption, radical generation and scavenging reactions [17,21,22]. Consequently, wastewater composition can markedly influence ozone demand and effective ozone exposure, thereby affecting micropollutant abatement, disinfection performance, as well as undesired by-products formation and toxicity [3,9,23,24]. Bromate and nitrosamines (e.g., NDMA) are among the main by-products of concern, particularly in wastewaters with elevated bromide or nitrogen-containing precursors [3,25,26]. Ozonation may also modify wastewater toxicity, with variable responses reported

depending on matrix composition and bioassay endpoints [25,27–29].

Therefore, and regardless of extensive research, important knowledge gaps remain. Namely, most full-scale ozonation studies have focused on a limited set of treatment objectives, particularly micropollutant removal and ozonation by-products [14,23,30–33], whereas fewer have addressed disinfection, including AMR reduction [24,34–38], and toxicity [39–42]. Consequently, integrated evaluations simultaneously assessing chemical contaminants, microbial risks, by-products and operational indicators remain scarce. Furthermore, most full-scale studies concern conventional municipal wastewater with dissolved organic carbon (DOC) concentrations typically below 10 mg C/L, whereas industrially influenced wastewaters remain poorly represented. Only a limited number of studies have partially investigated ozonation in systems receiving substantial textile industrial inputs [43–45], despite the potentially strong influence of colour and EfOM on ozone demand and treatment performance.

As for disinfection, reported ozonation performance varies considerably under full-scale conditions and has been shown to be substantially lower compared with laboratory-cultured indicator microorganisms' values and the predictions of linear kinetic models [13,24]. For example, values ranging from approximately 1 to >5 LRV (log reduction value) have been reported for *Escherichia coli* at specific ozone doses between 0.3 and 1.1 mg O₃/mg DOC [13,16,24,46–49]. Regarding AMR, 0.8–3 LRV have been reported for cultivable ARB, whereas ARG responses are generally lower and more variable, probably influenced by factors such as wastewater characteristics and microbial community composition [35,46,49,50]. Moreover, post-ozonation microbial regrowth has been observed in several studies, although inconsistent responses have also been reported, highlighting the need to assess reactivation potential under realistic operating conditions [35,46,51–53].

Frequent monitoring of micropollutants, pathogens and AMR indicators is costly and time incompatible with operational decision-making. Consequently, surrogate indicators based on UV absorbance and fluorescence-based parameters have been proposed to support process control [17,54–57]. However, their robustness under variable and industrially influenced wastewater conditions remains insufficiently documented.

Unlike most previous full-scale studies focused on isolated treatment objectives, this work provides a holistic assessment of ozonation by simultaneously evaluating ozone and •OH exposure, EfOM and colour removal, micropollutant abatement, by-product formation, toxicity, pathogen and AMR indicators reduction and post-treatment regrowth, as well as surrogate indicators. To the best of the authors' knowledge, this is one of the first comprehensive full-scale evaluations of multi-purpose ozonation in an urban WWTP strongly impacted by textile wastewater (51% influent contribution), thereby addressing several knowledge gaps relevant for EU-UWWTD implementation.

2. Materials and methods

2.1. Ozonation campaigns in Lordelo WWTP

Full-scale ozonation campaigns (Cps) were carried out at the Lordelo (LOR) WWTP (Guimarães, Portugal), operated by TRATAVE at the time of the study. With a treatment capacity of 28,063 m³/d (187 087 population equivalent; average flowrate of 19,000 m³/d in dry weather), this plant has a high input of textile effluents (51% annual average of total flowrate). The treatment line (Fig. S1, Supplementary Information (SI) I) comprises activated sludge with oxygen injection, sand filters and tertiary ozonation (CFV20-Dégremont Technologies, pure oxygen fed; 20 kg/h maximum ozone production, corresponding to 30 g O₃/m³ for the average flow). The WWTP has two ozone contact towers (usually only one line is working), each with three chambers in series with a total volume of 400 m³ (Fig. S1), where ozone is injected through ceramic diffusers.

Three experimental campaigns were carried out between September and October 2024, each testing three ozone doses. The generated ozone dose ranged from 14 to 30 g O₃/m³. Assuming an ozone transfer efficiency of 85% (project data, confirmed by oxygen supplier) the corresponding transferred ozone dose was estimated as 11.6 to 24.2 g O₃/m³, equivalent to EfOM-normalised transferred ozone doses of 0.4–1.0 mg O₃/mg TOC, where TOC refers to total organic carbon. An independent volumetric ozone mass transfer coefficient (KLa) was not determined, as no separate clean-water/acidic-pH ozone absorption test was performed. Therefore, ozonation performance was expressed in terms of generated ozone dose, estimated transferred ozone dose, TOC-normalised transferred ozone dose, and molecular ozone/•OH exposures estimated with the phenomenological model briefly described in Section 2.3.

In each campaign, a 5-hour composite sample was collected from the filtered secondary effluent feeding the ozonation reactor (LOR-E-FIL.M, Fig. S1 in SI I) and three grab samples were taken from the ozone reactor effluent (LOR-E-EFL), each corresponding to a different ozone dose. A 1.5 h stabilization period, a lag time of at least 3 times the hydraulic retention time on the ozone chamber and predefined after a preliminary test, was applied before sampling the ozonated water for each dose.

2.2. Analytical parameters and methods

Water samples were monitored for routinely monitored and additional physicochemical parameters (Table S1 in SI I) including alkalinity, 5-day biochemical oxygen demand (BOD₅), chemical oxygen demand (COD), chloride, electrical conductivity, nutrients (ammonia, nitrite, nitrate, total nitrogen and total phosphorus), pH, temperature, total suspended solids, and turbidity. EfOM was characterised by TOC and DOC, UV absorbance at 254 nm and 436 nm (A254 and A436), UV-transmittance at 254 nm (T254) and colour scanning. Analyses also included chemical contaminants of emerging concern (CECs), pathogen indicators, AMR indicators, acute toxicity, and oxidation by-products (bromate, NDMA).

Briefly, photometric methods were used for chemical oxygen demand and nutrients quantification; gravimetric methods for solids; nephelometric method for turbidity; high combustion (external commercial lab) or UV/persulfate oxidation with infrared detection method for TOC and DOC quantification; spectrophotometric methods for A254 and A436, T254, and colour scanning (UV-Vis absorbance spectrum at 400–700 nm range). Multiparametric equipment and probes for in situ measurement of electrical conductivity (EC), pH and temperature were used. Acute toxicity (*Vibrio fischeri* bioluminescence inhibition assay - Microtox), bromide and bromate (ion chromatography) and NDMA (GC-MS/MS) were determined by an external lab.

To better characterise EfOM composition and investigate both the transformation of organic matter during ozonation and the potential influence of EfOM characteristics on ozone demand and treatment

performance, an extended EfOM characterisation was conducted. This included biodegradable organic matter (BDOC), organic matter rapid fractionation (EfOM RF), UV-Vis absorbance spectra (200–700 nm) and fluorescence excitation-emission matrix (FEEM) spectra (excitation wavelengths of 220–500 nm and fluorescence emissions of 220–600 nm, further analysed by PARAFAC). EfOM RF is a resin-based fractionation method that separates dissolved organic matter according to its hydrophobicity and charge characteristics into very hydrophobic, slightly hydrophobic, charged hydrophilic, and neutral hydrophilic fractions. Portable UV-Vis absorbance probes were additionally used for in situ measurements. Further analytical details are provided in text S1 (SI II).

Thirty-one chemical CECs - pharmaceuticals and metabolites, and anticorrosive agents, including the 12 micropollutant indicators listed in EU-UWWTD (Table S2, SI I) - were analysed by UHPLC-MS/MS using solid phase extraction (Strata-X SPE cartridges, Phenomenex, USA) and filtered sample extracts (0.22 µm PTFE syringe filters, labfil, China) before the chromatographic analysis. Instrumental analysis was conducted using a Nexera UHPLC-MS/MS (LCMS-8030, Shimadzu Corporation, Kyoto, Japan) liquid chromatograph with two solvent delivery pumps, a column oven, an autosampler, a degasser, a system controller, a triple-quadrupole mass spectrometer operated in electrospray ionization (ESI) mode. LabSolutions software, version 5.80 (Shimadzu Corporation, Kyoto, Japan), was used for instrument control and data analysis. The analytical methods of Paça et al. [58], further detailed in Viegas et al. [59], were used.

Disinfection efficacy was evaluated based on selected microbiological parameters, namely total coliforms, *E. coli* (as bacteria faecal contamination indicator), *C. perfringens* endospores (as protozoan indicator), and somatic coliphages (as viral indicators). Complementary to these standard indicators, total coliforms and *E. coli* and the respective cefotaxime (CTX)-resistant subpopulations were enumerated. In parallel, total bacteria (16S rRNA gene), class 1 integron integrase gene (*int1*) as a surrogate for ARG acquisition potential, and a 23S ribosomal RNA methyltransferase gene (*ermB*) as a surrogate for ARGs were analysed by quantitative polymerase chain reaction (qPCR). In addition, a set of 12 genes (*ISPPs*, *IS26*, *sul1*, *APH(6)-Id*, *aadA1*, *APH(3'')-Ia*, *bla_{OXA-58}*, *bla_{GES}*, *bla_{TEM}*, *bla_{VEB}*, *crAss64*, and the 16S rRNA) was quantified based on high-throughput quantitative PCR and the bacterial community composition was analysed based on 16S rRNA gene metabarcoding (Illumina sequencing, Novogene, Germany). Further details may be found in text S2 (SI II). To assess potential reactivation after ozonation, ozonated wastewater samples and pre-ozonation control samples were stored in the dark at room temperature (~ 20C) for one and five days, and further analysed as described above.

2.3. Data processing and statistical methods

Molecular ozone and •OH exposures were computed with an expeditious phenomenological model for chemical CEC abatement by ozone, considering a parallel second order reaction mechanism between the CEC and O₃ and •OH and considering a simplification of the pseudo first-order ozone decay in the water [59]. Statistical analyses of the correlations between contaminant removal (including chemical CECs, colour, pathogen indicators and genes) and various operating conditions and surrogate parameters of ozonation were performed using the PAST 4.03 software or IBM SPSS statistics v30.0.0.0. Briefly, one-way ANOVA (homogeneous variance with Levene's test) or Welch F test (unequal variance) was conducted for normal distributions, and Kruskal Wallis test was used for non-normal distributions (Shapiro-Wilk test, *p*-values < 0.05). Significance levels of 0.05 were applied. For the bacterial communities analyses the software STAMP was used [60].

Principal component analysis (PCA) was applied as an exploratory tool to compare CEC removal fingerprints across ozonation conditions and to support the grouping of compounds according to similar abatement behaviour. The input matrix X contained the 15 CECs as observations (rows) and the nine ozonation experiments as variables

(columns), corresponding to three intensive campaigns and three normalised ozone doses per campaign. Each matrix element represented the removal efficiency of a given CEC in each experiment. PCA was performed in SOLO (Eigenvector Research, Inc.) using singular value decomposition. Prior to modelling, variables were mean-centred and autoscaled to unit variance so that all ozonation experiments contributed equally to the analysis. The number of principal components (PCs) was selected by leave-one-out cross-validation, using the RMSECV of the reconstructed X matrix and retaining the smallest number of PCs that minimized RMSECV. Scores were interpreted as similarities among CECs, while loadings were used to assess the contribution of each ozonation experiment to the PCs. PCA was also used to assess bacterial community composition variations at the genus-level, possibly associated with ozonation and reactivation. In this analysis, samples and bacterial genera relative abundance were the observations and variables, respectively. The analysis permitted the evaluation of shifts in the bacterial community structure, due to the simultaneous change of multiple variables. To assess the consistency of the observed ordination patterns, complementary analyses were also performed based on distance matrices (PCoA) and non-parametric ordination methods (NMDS) using the PAST v5.3 software [61].

3. Results and discussion

3.1. Filtered secondary effluent quality

The quality of the filtered secondary effluent during the three ozonation campaigns is summarised in Table 1 and further detailed in Table S3 (SI I) and Text S3 (SI II). Overall, the filtered secondary effluent exhibited neutral pH, low suspended solids, and high levels of dissolved inorganic and organic matter, reflecting the strong influence of textile wastewater (~50% influent contribution). Elevated values of alkalinity, conductivity, colour, and UV absorbance (A254 and A436) and concentrations of chloride, bromide, ammonium, and organic carbon were observed compared to ranges reported in municipal secondary effluents [25,59,62–64].

Moreover, EfOM rapid fractionation indicated that dissolved organic matter was dominated by hydrophobic organic acids in campaigns 1 and 2 (Cp1 and Cp2) (64–83% of DOC), with very to slightly hydrophobic fractions (vHB and sHB) representing the largest share across campaigns. In contrast, Cp3 showed a relative increase in charged hydrophilic fraction (cHL) and a concomitant decrease in hydrophobic fractions (to 47% of DOC), suggesting a shift towards a more polar EfOM potentially affecting ozone reactivity and consumption behaviour. FEEM-PARAFAC analysis [65] further indicated that EfOM was predominantly humic-like in nature, with microbial humic-like (A_M) and terrestrial humic-like (A_C) components representing the main

fluorescent signals, followed by protein-like tryptophan-like (T) and highly conjugated humic-like components (M and C) (fluorescent components are detailed in Table S8 in SI II). While Cp 3 exhibited lower total fluorescence intensity, only minor changes were observed in the relative distribution of PARAFAC components across campaigns, suggesting that the main compositional features of EfOM remained largely unchanged.

Differences among campaigns were mainly observed in Cp 3, which, besides the EfOM differences already discussed, showed lower salt loads (alkalinity, conductivity, and chloride) but higher colour intensity (Fig. S2 in SI I confirms enhanced absorbance at 400–550 nm) and *E. coli* abundance. These differences coincide with variations in precipitation during the sampling period (8.7 mm d⁻¹ precipitation during Cp3 compared to 0 mm d⁻¹ in the other campaigns).

In addition to EfOM characteristics, chemical organic CECs and microbial indicators were also assessed. A total of 18 chemical CECs were consistently detected across campaigns (17–19 µg/L total concentration), with similar overall levels between campaigns ($p > 0.05$). Detailed concentrations may be found in Table S4, SI I. The ozonation influent contained total bacteria in the order of the 6.0–6.7 log gene copies/mL (Fig. S3, SI I). The abundance of the genes *int1* and *ermB* was, on average, 2.0 and 2.6 log gene copies/mL below, respectively. The counts of CTX-resistant coliforms and *E. coli* represented less than 1.4% of the total.

3.2. Ozone and •OH exposures and matrix effect

Molecular ozone and •OH exposure levels were computed as described in Section 2.3 and Viegas et al. [59] and presented as a function of the specific ozone dose (mg O₃/mg TOC) for the three full-scale campaigns at LOR WWTP (Fig. 1), together with literature data for comparison. Ozone exposure ranged from 4.0×10^{-6} M.s to 9.6×10^{-6} M.s (0.41–0.98 mg O₃/mg TOC), substantially lower than typical values reported for similar doses in municipal wastewater ($\approx 10^{-2}$ M.s). Comparable low exposures have been reported in wastewaters with high ozone demand, including high-DOC matrices [12] and mixed municipal-industrial matrices [29]. •OH exposure (8.3 – 22.5×10^{-11} M.s) was within the range reported for municipal wastewater [29,64].

The low ozone exposure indicates a high ozone demand in the filtered secondary effluent, associated with the presence of ozone-reactive constituents that do not necessarily contribute to •OH formation. The elevated A254 observed in this study (3 to 10 times higher than the values reported in [25,59,62,64]), supports this interpretation. Overall, ozone demand is linked to the elevated TOC and chromophoric textile-derived EfOM, which increase the competition for ozone and reduce oxidant availability for other contaminants.

The stability and efficiency of ozonation strongly depend on the water matrix, particularly pH, alkalinity, and the content and characteristics of organic matter [17,22]. Among the three campaigns, ozone exposure was lowest in Cp3, while •OH exposure remained comparable across all campaigns (Fig. 1). Since pH, DOC, and A254 were similar between campaigns (Table 1 and Table S3, SI I), differences in ozone exposure are attributed to variations in alkalinity, colour intensity, and EfOM composition.

In Cp1 and Cp2, higher alkalinity (as bicarbonate) likely enhanced ozone stability by limiting radical-driven ozone decomposition pathways. Bicarbonate acts as a •OH scavenger, reducing radical chain reactions that accelerate ozone decay [10,66]. This favours direct ozone reactions and results in higher ozone exposure.

Lower ozone exposure in Cp3 is consistent with higher colour intensity, which increases ozone consumption through rapid reaction with chromophoric compounds. In addition, EfOM rapid fractionation indicated a higher proportion of charged hydrophilic fractions and lower hydrophobic fractions in Cp3, suggesting a shift in organic matter polarity that may influence ozone consumption pathways.

Table 1

Filtered secondary effluent characteristics during the ozonation campaigns in LOR WWTP.

Parameter	Campaign 1 (Cp1)	Campaign 2 (Cp 2)	Campaign 3 (Cp 3)
pH	7.6	7.6	7.2
Temperature (°C)	27.3	26.7	26.4
Alkalinity (mg CaCO ₃ /L)	660	621	376
Conductivity (mS/cm)	4.2	3.7	2.8
Chloride (mg/L)	880	740	610
Bromide (mg/L)	0.5	0.4	0.4
DOC (mg/LC)	25.1	23.7	22.1
TOC (mg/LC)	25.9	27.9	23.7
COD (mgO ₂ /L)	93	78	78
A254 (cm ⁻¹)	1.0	1.1	0.97
A436 (cm ⁻¹)	0.099	0.118	0.133
Colour-scan	19	22	24
N-NH ₃ (mg/LN)	21	31	17

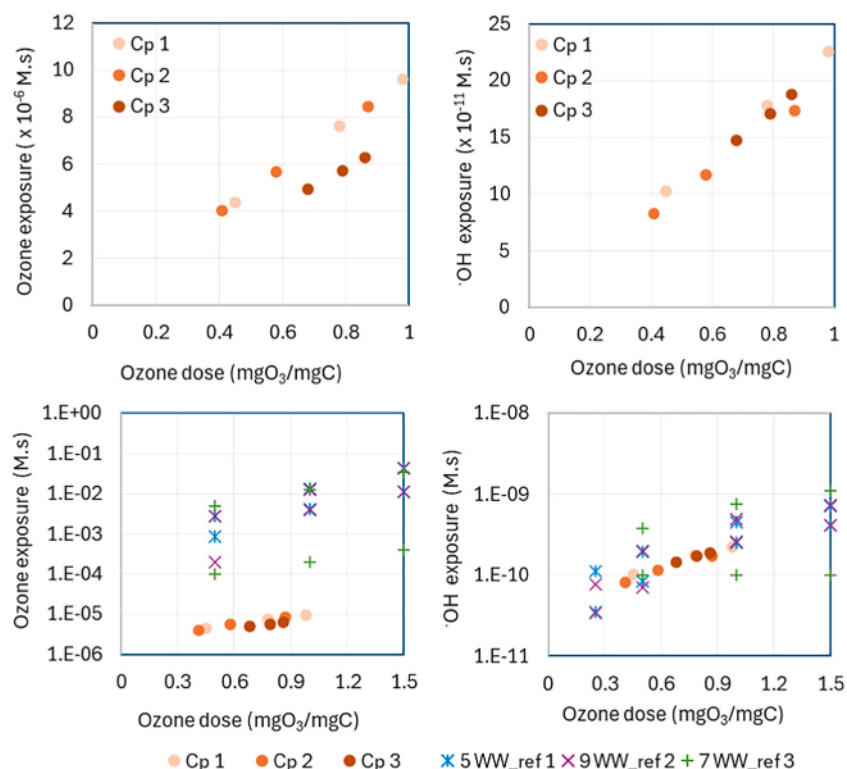
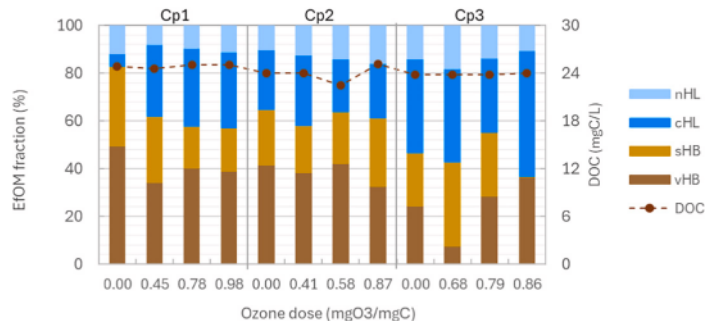
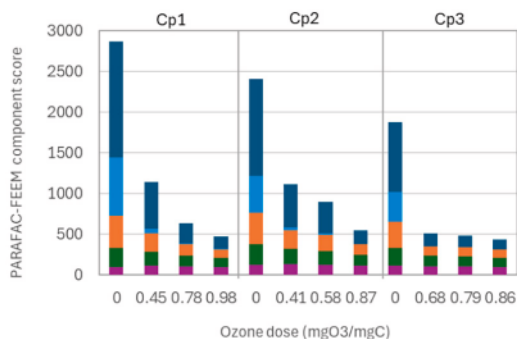


Fig. 1. Ozone (left) and $\cdot\text{OH}$ (right) exposure determined at various TOC-normalised ozone doses in the three ozonation campaigns in LOR WWTP (top) and comparison with wastewaters (WW) from other studies (bottom) (ref 1 – [64], ref. 2 – [12], ref. 3 – [29]).

a)



b)



c)

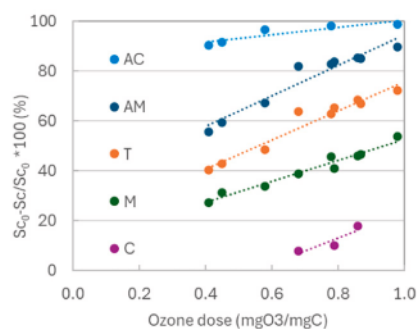


Fig. 2. Changes in EfOM composition after ozonation campaigns (Cp1, Cp2 and Cp3), showing a) hydrophobic (vHB, sHB) and hydrophilic (nHL, cHL) fractions, b) normalised PARAFAC scores of fluorescent components: terrestrial or soil humic-like (A_C), microbial humic-like (A_M), protein-like tryptophan like (T), highly conjugated microbial humic-like (M), and highly conjugated terrestrial or soil humic-like (C), and c) decreases in fluorescent components' scores as a function of TOC-normalised ozone dose.

Although $\cdot\text{OH}$ exposure appeared similar among campaigns, its theoretical availability should be lower in Cp1 and Cp2 due to scavenging by bicarbonate and chloride. Chloride, abundant in textile wastewater, has been reported to act as an $\cdot\text{OH}$ scavenger, forming less reactive chloride radical species that reduce oxidation [67].

3.3. Ozonation impact on organic matter and colour

Ozonation was not effective for the removal of DOC (Fig. 2a) or COD (17–22%, data not shown). Although no increase in biodegradability was observed based on BOD₅ and BDOC (data not shown), EfOM RF indicated an increase in the hydrophilic character of dissolved organic matter with increasing ozone dose, particularly in Cp1 (Fig. 2a), consistent with previous findings [68,69]. Hydrophobic organic acids (vHB and sHB) decreased 26 and 10 percentual points in Cp1 and Cp3, respectively, while neutral hydrophilic acids (nHL) remained largely stable and charged hydrophilic acids (cHL) increased 27 and 13 percentual points in Cp1 and Cp3, respectively.

Across the five fluorescent EfOM components, normalised PARAFAC scores decreased systematically with increasing ozone dose (Fig. 2b). The strongest reduction was observed for humic-like components associated with terrestrial or soil-derived organic matter (A_C), which decreased by 90–100%, consistent with their high content of electron-rich aromatic and activated phenolic moieties (e.g. [45]). Microbial humic-like (A_M); and protein-like, tryptophan-like (T) components showed intermediate reductions (56–90% and 40–72%, respectively) at ozone doses above 0.45 mg O₃/mg TOC (Fig. 2c). Highly conjugated humic-like components (M and C) were less reactive, with reductions of 27–54% and 8–18%, respectively (Fig. 2c).

UV–Vis absorbance evolution confirmed a wavelength-dependent response (Fig. S4 in SI I and Fig. 3a–c). The strongest relative absorbance decline was observed in the visible range (400–660 nm), associated with chromophoric structures responsible for effluent colour. These compounds typically contain extended conjugated unsaturated bonds, such as azo linkages ($-\text{N}=\text{N}-$), $\text{C}=\text{C}$, and $\text{C}=\text{O}$ conjugations, which are highly reactive towards ozone [11,70]. In contrast, absorbance at 254 nm showed substantially lower removal ($\leq 36\%$), indicating limited degradation of aromatic structures within the investigated ozone dose range.

Absorbance changes in the UV region (220–300 nm) were associated with oxidation of aromatic and phenolic moieties. Nitrate, which strongly absorbs at 200–230 nm, remained low (< 0.5 – 2.5 mg N/L) before and after ozonation, suggesting that nitrate did not significantly contribute to the observed spectral changes. Although differential absorbance at 254 nm increased with ozone dose, overall removal remained limited (Fig. 4), confirming partial transformation rather than complete oxidation of aromatic structures. Ozonation was shown to produce smaller aromatic oxidation by-products that continue to absorb

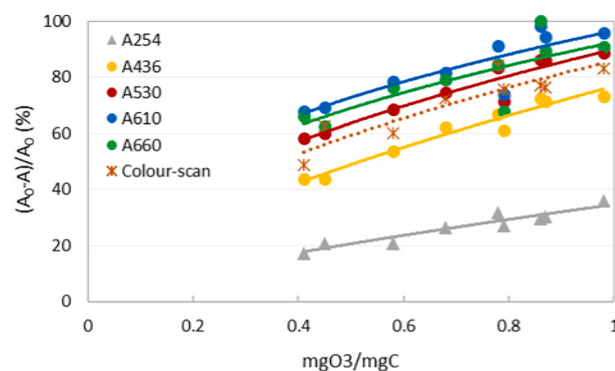


Fig. 4. Absorbance and colour-scan attenuation as a function of TOC-normalised ozone dose: A254 representing aromatic organic matter; A436 representing yellow colour, typical of domestic wastewater; A530 representing red colour; A610 representing blue colour; and A660 representing green colour. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in the UV region [45,71].

Colour-scan results confirmed an effective decrease in effluent colour, with overall colour removal ranging from 50% to 84% for ozone doses between 0.4 and 1.0 mg O₃/mg TOC (Fig. 4). Colour removal followed a consistent wavelength-dependence, with blue $\approx$ green $>$ red $>$ yellow. Blue and green fractions were almost completely removed (68–98% and 63–100%, respectively), red fraction showed intermediate removal (58–89%), whereas yellow fraction showed the lowest removal (44–73%), consistent with overlap between chromophores and humic-like absorption bands. The absence of a plateau in colour removal across wavelengths indicates that ozone demand was not fully satisfied within the applied dose range. This is consistent with the persistence of UV-absorbing compounds and confirms that reactive EfOM fractions act as major ozone sinks.

Overall, EfOM transformation patterns indicate that ozone preferentially reacts with chromophoric and highly electron-rich organic fractions, which dominate ozone consumption and are expected to limit oxidant availability for target contaminants, including micropollutants and microbial indicators.

3.4. Elimination of micropollutants and other chemical CECs

Chemical CEC removal increased with increasing specific ozone dose, rising from 6 substances removed by $\geq 80\%$ at the lowest dose tested (0.41 mg O₃/mg TOC) to 17 substances (out of 18 quantified) at the highest dose (0.98 mg O₃/mg TOC) (Fig. S5 and Table S5, SI I). Influent and effluent concentrations of chemical CECs are presented in

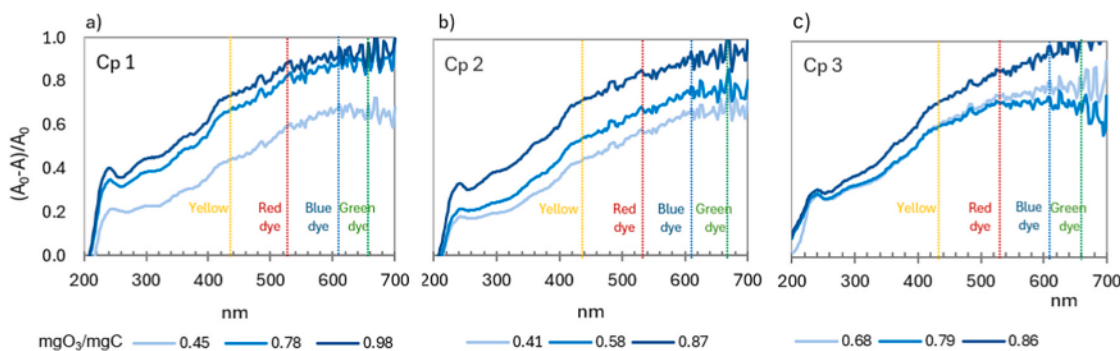


Fig. 3. Dose-resolved UV–Vis absorbance removal spectra ($(A_0 - A)/A_0$) during ozonation campaigns in LOR WWTP (a, b, c for campaigns 1, 2, 3, respectively) with ozone doses of 0.41–0.98 mg O₃/mg TOC. Representative wavelengths associated with observed pigment colours (central values of the visible spectrum) are indicated in coloured vertical lines for comparison. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table S4 (SI I).

CEC removal during ozonation is strongly influenced by compound reactivity towards ozone (k_{O_3}) and hydroxyl radicals (k_{OH}). Accordingly, several classification schemes have categorised compounds based on these second-order rate constants, differing whether k_{OH} is considered and in the cut-off values used to define the reactivity categories [3,10,12–14,64]. In this study, compounds were classified into five reactivity groups following Lee et al. [12] (Table S2, SI I): I. High ozone reactivity ($k_{O_3} \geq 10^5 \text{ M}^{-1} \text{ s}^{-1}$); II. Intermediate ozone reactivity ($10 \leq k_{O_3} < 10^5 \text{ M}^{-1} \text{ s}^{-1}$); III. Low ozone reactivity but high $^{\bullet}\text{OH}$ reactivity ($k_{O_3} < 10 \text{ M}^{-1} \text{ s}^{-1}$; $k_{OH} \geq 5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$); IV. Low ozone reactivity but intermediate $^{\bullet}\text{OH}$ reactivity ($k_{O_3} < 10 \text{ M}^{-1} \text{ s}^{-1}$; $10^9 \leq k_{OH} < 5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$); V. Low ozone and low $^{\bullet}\text{OH}$ reactivities ($k_{O_3} < 10 \text{ M}^{-1} \text{ s}^{-1}$; $k_{OH} < 10^9 \text{ M}^{-1} \text{ s}^{-1}$). No representatives of groups IV and V were analysed.

Groups removals as a function of ozone and $^{\bullet}\text{OH}$ exposures and ozone doses in each campaign are presented in Fig. 5 (values in Table S5 SI I).

Chemical CECs in group I, including carbamazepine and diclofenac (EU-UWWTD micropollutant indicators), sulfamethoxazole, and naproxen exhibited very high and stable removal efficiencies across all tested ozone exposures (77% to >99% for carbamazepine and > 91% for the remaining compounds, Fig. 5). Their high ozone reactivity is well documented and has been attributed to the presence of electron-rich moieties, such as olefins (carbamazepine), activated aromatics (diclofenac), and anilines (sulfamethoxazole) [3,13,25]. Trazodone and the metabolites *O*-desmethylvenlafaxine and 10,11-epoxycarbamazepine, for which k_{O_3} and k_{OH} values are unavailable, also consistently exhibited very high removals (> 94%, Fig. 5), showing a behaviour comparable to group I compounds.

In contrast, the remaining CECs, including most EU-UWWTD micropollutant indicators, showed variable removals among campaigns and ozone doses, indicating greater sensitivity to operating conditions and water quality. Group II compounds, comprising the EU-UWWTD-category 1-indicators hydrochlorothiazide, venlafaxine, amisulpride and citalopram, together with fluoxetine and the EU-UWWTD-category 2-indicators 4/5-methyl-1H-benzotriazole, candesartan, benzotriazole and irbesartan, achieved approximately 40–70% removal at low-to-intermediate ozone doses (0.4–0.6 mg O_3 /mg TOC), increasing to 60% – >90% at doses $\geq 0.7 \text{ mg } \text{O}_3$ /mg TOC and generally increasing with both ozone and $^{\bullet}\text{OH}$ exposures (Fig. 5).

Group III compounds (ibuprofen and ketoprofen) showed similar removals across the investigated dose range (approximately 40–70% at 0.4–0.6 mg O_3 /mg TOC and 70–90% at $\geq 0.7 \text{ mg } \text{O}_3$ /mg TOC, Fig. 5). Ibuprofen is known to be ozone-resistant but highly reactive with $^{\bullet}\text{OH}$, while benzotriazole has been reported as ozone-resistant but moderately removed ($\approx 60\%$) through oxidation by non-selective $^{\bullet}\text{OH}$ radicals [25]. Hydroxyibuprofen, for each no group classification was available,

followed a similar removal pattern to ibuprofen but with consistently lower efficiencies across all doses.

Overall, groups II and III exhibited comparable removal behaviour, particularly for compounds with $k_{O_3} \leq 6 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$ and comparable k_{OH} . This observation is consistent with Macsek et al. [63], who reported no significant differences between group III compounds and the lower range of group II compounds, particularly for those with $k_{O_3} \leq 2.2 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$.

These trends are further supported by the PCA of CEC removal values (Fig. 6). In this analysis, CECs were treated as observations and the nine ozonation experiments as variables. Consequently, compounds located close to each other in the scores plot exhibit similar removal fingerprints across campaigns and ozone doses. PC1 explained 82.9% of the total variance and separated compounds with consistently high removals from those showing lower and more variable abatement across campaigns and ozone doses. PC2 explained a further 8.9% of the variance and captured secondary variability related with operating conditions and matrix effects.

Based on the distribution of PC1 scores and the natural gaps observed in the scores plot, three PCA-derived treatability groups were identified (Fig. 6). Group I ($\text{PC1} \geq 2$) included naproxen (NPX), carbamazepine (CBZ), sulfamethoxazole (SMX), and diclofenac (DCF); Group II ($-1 \leq \text{PC1} < 2$) comprised fluoxetine (FLX), venlafaxine (VEN), amisulpride (AMSP), and citalopram (CITA); and Group III ($\text{PC1} \leq -1$) encompassed

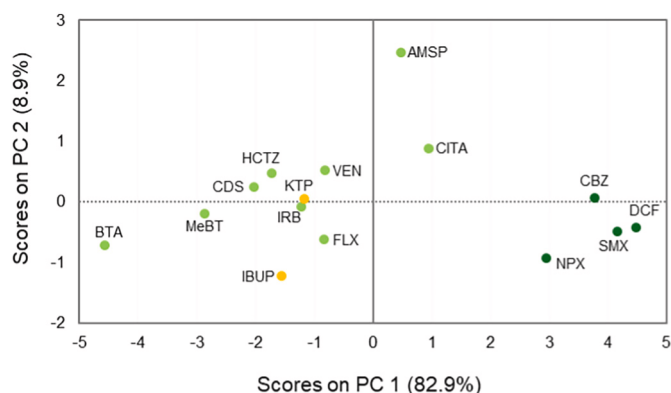


Fig. 6. PCA of CEC removal values during the three ozonation campaigns. Scores plot of PC1 (82.9% of variance) versus PC2 (8.9% of variance), illustrating the separation of compounds according to removal behaviour and oxidation-related response patterns. Compounds with high and consistent removals cluster at positive PC1 values, while compounds with lower and more variable removals cluster towards negative PC1 values. PC2 captures secondary variability associated with ozone dose and campaign-specific matrix effects.

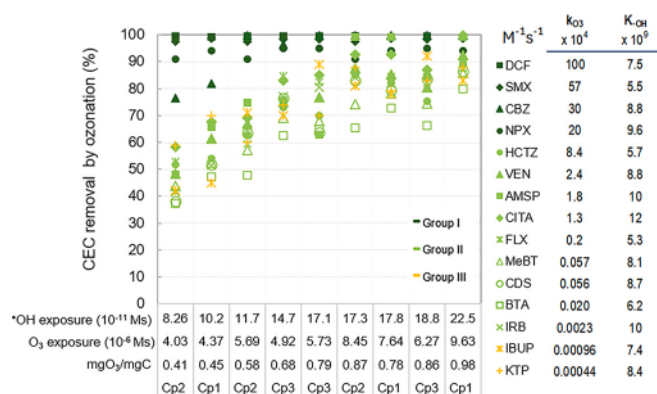


Fig. 5. CEC removal by ozonation as a function of ozone and $^{\bullet}\text{OH}$ exposure in each campaign and ozone dose tested (mg O_3 /mg TOC). Second-order rate constants for ozone and $^{\bullet}\text{OH}$ are also presented. Dark green symbols represent group I compounds, light green symbols group II compounds, and yellow symbols group III compounds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ketoprofen (KTP), irbesartan (IRB), ibuprofen (IBUP), hydrochlorothiazide (HCTZ), candesartan (CDS), 4/5-methyl-1H-benzotriazole (MeBT), and benzotriazole (BTA). The separation along PC2, with amisulpride and citalopram showing more positive scores and benzotriazole, fluoxetine, and ibuprofen showing more negative scores, may be associated with differences in $\bullet\text{OH}$ reactivity. The former compounds exhibit comparatively higher $k_{\bullet\text{OH}}$, whereas the latter show lower $k_{\bullet\text{OH}}$. Group II and III in PCA include most EU-UWWTD micropollutant indicators and were associated with mixed or predominantly non-selective oxidation pathways.

To investigate the relative contributions of ozone and $\bullet\text{OH}$ oxidation pathways, the apparent second-order rate constant (k_{app}) was computed for each CEC as the sum of ozone and $\bullet\text{OH}$ contributions ($k_{\text{app}} = k_{\text{O}_3} + k_{\bullet\text{OH}} \text{Rct}$), using the modelled Rct values from Viegas et al. [59], and plotted as a function of k_{O_3} (Fig. 7). The parameter k_{app} represents the combined oxidative capacity of the system towards each compound. A flat slope indicates $\bullet\text{OH}$ -dominated oxidation, a slightly increasing slope reflects mixed $\text{O}_3/\bullet\text{OH}$ contributions, whereas a steep slope indicates ozone-dominated oxidation. Using the Elovitz and von Gunten [72] Rct approach, the relative contribution of $\bullet\text{OH}$ was quantified as $f_{\bullet\text{OH}} = (k_{\bullet\text{OH}} \text{Rct}) / (k_{\bullet\text{OH}} \text{Rct} + k_{\text{O}_3})$.

The calculated k_{app} values indicated that, under the limited ozone exposure conditions investigated, compounds with very high ozone reactivity ($k_{\text{O}_3} \geq 2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$) were preferentially removed via direct ozone mechanism. Compounds with intermediate ozone reactivity ($2 \times 10^3 < k_{\text{O}_3} < 2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$) were oxidized through combined pathways, with a dominant role of $\bullet\text{OH}$, whereas compounds with $k_{\text{O}_3} \leq 6.0 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$ were removed exclusively via $\bullet\text{OH}$ -mediated oxidation.

Comparison of campaigns at similar ozone doses further showed that the slightly higher $\bullet\text{OH}$ exposure and lower ozone exposure observed during Cp3, resulting from the matrix effects discussed in Section 3.2 (Fig. 1), did not significantly affect the removal of group I and III compounds; ibuprofen removal even showed a slight increase. In contrast, compounds at the upper end of group II exhibited reduced removal efficiencies. This pattern reflects the distinct oxidation pathways governing these groups. Compounds predominantly degraded via ozone-selective or $\bullet\text{OH}$ -mediated oxidation were largely unaffected, whereas those requiring a more balanced contribution of both oxidants were disadvantaged.

Overall, the observed variability in CEC removal across compounds and campaigns reflects the highly ozone-demanding textile-impacted matrix discussed in Sections 3.2 and 3.3. Chromophoric and other ozone-reactive EfOM constituents act as major ozone sinks, reducing

ozone exposure and, consequently, the efficiency of selective ozone oxidation compared with conventional municipal secondary effluents. As a result, compounds requiring a balanced contribution of both O_3 and $\bullet\text{OH}$ oxidation pathways were the most sensitive to matrix variations among campaigns.

3.5. Ozonation inactivation potential for pathogens and AMR indicators

The LRVs of *E. coli*, *Clostridium perfringens* endospores and somatic coliphages as a function of ozone dose, ozone exposure (i.e., Ct, the residual disinfectant concentration x contact time) and $\bullet\text{OH}$ exposure are presented in Fig. 8a. Overall, the observed inactivation followed the order coliphages > *E. coli* > *C. perfringens* endospores, with LRV ranging from 0.9 to >2.3, 0.2–1.2 and < 0.5, respectively. These values are within the minimum reported in wastewater ozonation studies, where typical reductions vary between 0.5 and 4 LRV for *E. coli*, 1 to >6 LRV for coliphages and < 1 LRV for endospores at comparable ozone doses [13,16,24,48,49]. Similar magnitudes (ca. 2 LRV of somatic coliphage) have also been reported for conventional disinfection processes applied to secondary effluents.

Virus inactivation was consistently higher than bacterial inactivation, while endospores showed the lowest reduction values, which was also observed in the WRRF [64] study. This pattern reflects structural differences and oxidative stress response, with bacterial cells additionally protected by extracellular polymeric substances, whereas virions are more directly exposed to oxidative attack [17].

When comparing LRVs of *E. coli*, *Clostridium perfringens* endospores and somatic coliphages at similar ozone doses, no apparent major differences were observed across campaigns (Fig. 8a). *E. coli* inactivation increased with increasing ozone exposure but showed some scatter and no clear linear dependence (Fig. S6b, SI I), indicating that Ct alone does not fully describe disinfection performance in this matrix. This limitation of the Ct approach, widely used in drinking water treatment, to wastewater systems has been previously reported and explained by the rapid ozone decay in complex matrices [24,73,74] and bi-phasic cell membrane damage kinetics [38]. In contrast, $\bullet\text{OH}$ exposure exhibited a moderate linear correlation with *E. coli* LRV ($R^2 = 0.56$, Fig. S6c, SI I), suggesting that hydroxyl radical-mediated reactions contributed more significantly to bacterial inactivation under the studied wastewater (Fig. 8a). For somatic coliphages (Fig. S6d-f, SI I), inactivation correlated with both ozone and radical exposures, indicating a combined oxidation mechanism.

For coliforms, *E. coli* and the respective CTX-resistant subpopulations higher LRVs were observed at ozone doses above 0.6 mg O_3/mg TOC

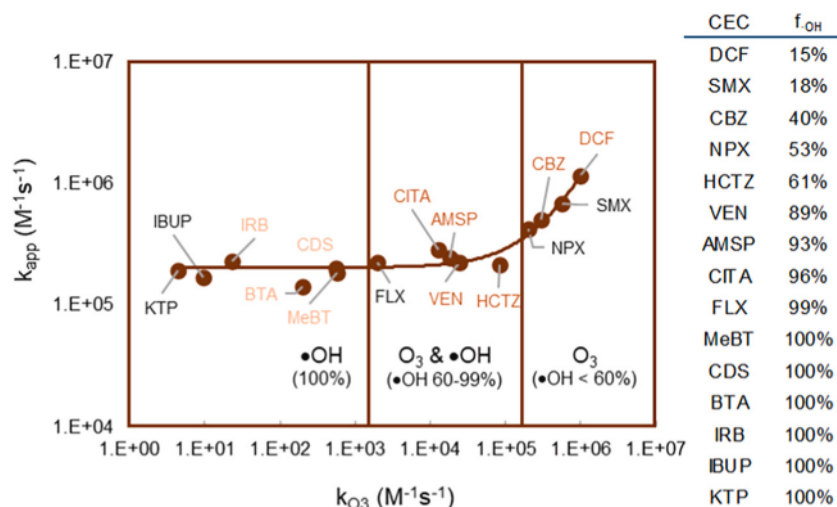


Fig. 7. Apparent second-order rate constant (k_{app}) as a function of the ozone second-order rate constant with ozone (k_{O_3}) for the LOR WWTP campaigns and the corresponding dominant oxidation mechanism, expressed as the fraction of CEC degraded by $\bullet\text{OH}$ ($f_{\bullet\text{OH}}$).

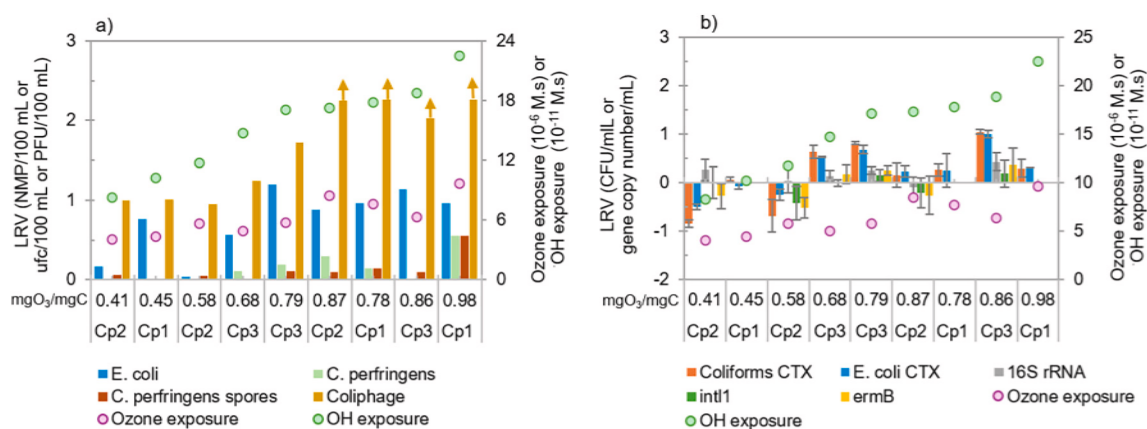


Fig. 8. Log-reduction values during ozonation campaigns for (a) *E. coli*, *C. perfringens* endospores, and coliphages, and (b) cefotaxime (CTX)-resistant coliforms and *E. coli*, as well as 16S rRNA, *int1*, and *ermB* genes as a function of TOC-normalised ozone dose. Ozone exposure (Ct of 0.003–0.008 mg·min/L) and [•]OH exposure are shown on the secondary y-axis. Data are organized by increasing [•]OH exposure; arrows indicate LRVs exceeding the plotted values (corresponding to < LOQ results).

(Fig. 8b). However, this dose-dependent effect was not reflected in DNA-based quantification of total bacterial abundance (16S rRNA gene) or of the genes *int1* and *ermB*, which showed smaller reductions. This discrepancy was also evidenced in Foroughi et al. [46] comprehensive literature review.

Regrowth experiments (T1 and T5, Fig. S7 in SI I) showed no

significant differences between ozonated and control samples, irrespective of the ozone doses used, indicating largely stochastic regrowth patterns. This suggests the transient inactivation effect of ozone on some bacterial groups, as previously noted [51,75].

In a more detailed analysis, a broader set of genes, including genes associated to mobile genetic elements (ISPPs, IS26), AMR (*sul1*, *APH(6)*-

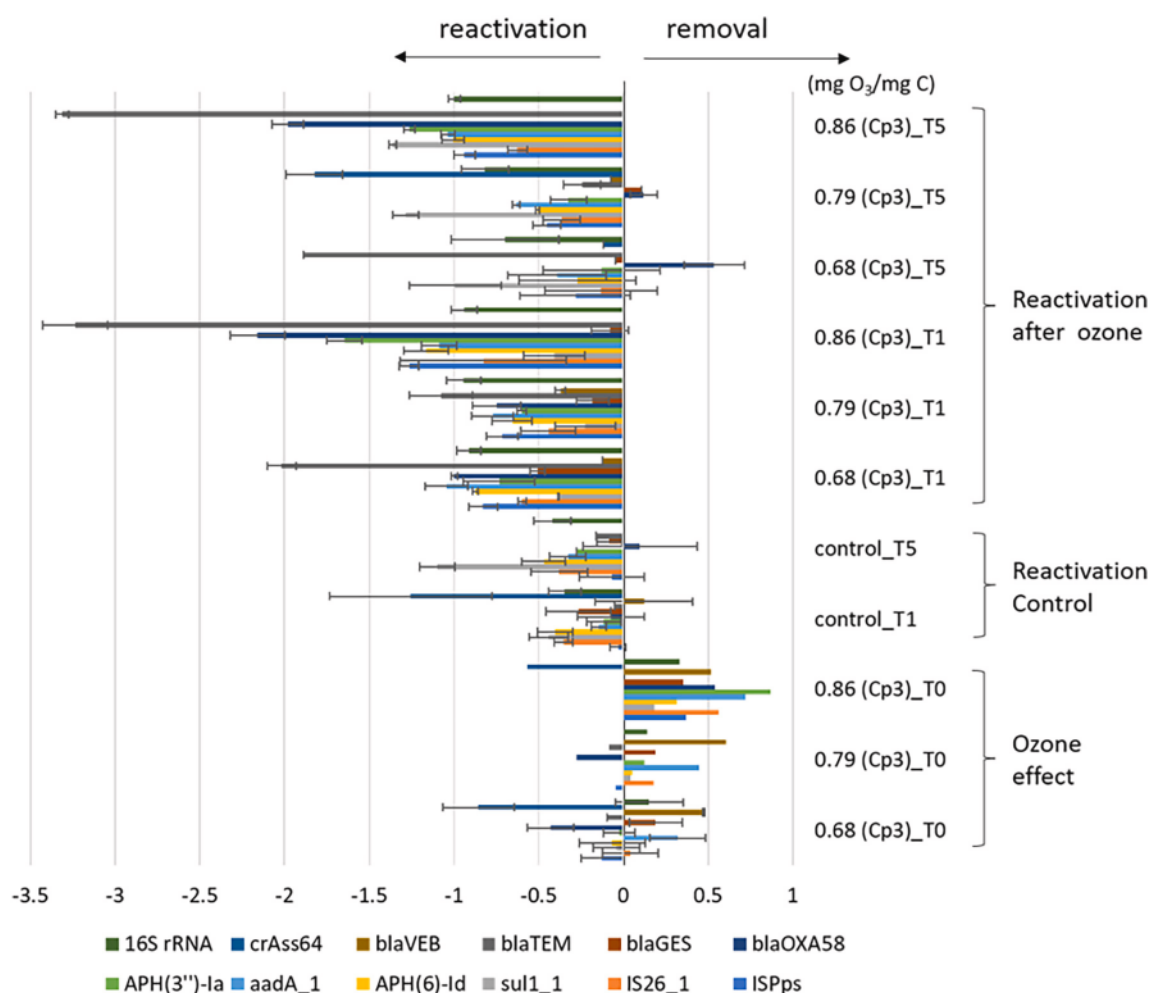


Fig. 9. Log reduction values (LRVs) for the abundance (gene copy/volume) of group of genes associated with mobile genetic elements and antibiotic resistance for ozonated samples (three ozone doses in campaign 3, 0.68–0.86 mg O₃/mg TOC) and non-ozonated control samples incubated for 1 day (T1) and 5 days (T5). Samples T0 were not incubated.

Id, *aadA1*, *APH(3'')-Ia*, *bla_{OXA-58}*, *bla_{GES}*, *bla_{TEM}*, *bla_{VEB}*), faecal contamination (crAss64) and total bacterial load (16S rRNA), was quantified by high-throughput quantitative PCR (Fig. S8, SI I). Among the most abundant of these genes were the mobile genetic elements ISPPs and IS26, and the sulphonamide resistance gene *sul1*, which is commonly associated with class 1 integrons. The results suggested that higher ozone doses led to higher reduction of gene abundance (average LRV of 0.4 log gene copies/mL for the highest dose versus −0.06 log gene copies/mL for the lowest dose). However, regrowth assays showed that total bacterial loads increased in both control and ozonated samples (approximately 0.5–1 log-unit gene copies/mL), although more in the latter (Fig. 9). The genes showing the highest increase after regrowth were the beta-lactamase *bla_{TEM}*, the sulphonamides resistance gene *sul1*, and the aminoglycosides resistance gene *APH(3'')-Ia*. These results suggest that bacterial populations holding these genes may be those capable of recovering damages and/or growing faster during the storage period. In most cases, regrowth was more pronounced at higher ozone doses than at lower doses, suggesting that higher ozone doses may exert a stronger selective pressure on the microbial community.

Because ozonation affects distinct bacterial populations differently [76], bacterial communities were analysed based on the 16S rRNA gene sequencing (Fig. S9, SI I) to assess the dynamics of the community after ozone exposure and storage effect. Ozonation promoted a non-significant ($p > 0.05$) decrease in bacterial diversity, with the Shannon diversity index declining from 4.06 to 3.51 (average values) but triggered significant ($p < 0.05$) decrease on community evenness (from 0.09 to 0.06), meaning that some bacterial species became more dominant (Table S6 SI I). Community composition shifted immediately after treatment, but differences between ozonated and control samples diminished after 5 days, suggesting the transient inactivation effect of ozone as observed for cultivable bacteria.

Overall, no clear relationship was observed between ozone dose and bacterial community composition (Fig. 10). Interestingly, samples exposed to the highest ozone dose in Cp3 clustered closely with control samples in the PCA, indicating limited community-level alteration, despite comparable inactivation of cultivable bacteria. This is consistent with Cp3 exhibiting the lowest ozone exposure due to matrix changes, as discussed in Section 3.2 (Figs. 10 and S10 SI).

Nevertheless, ozone triggered an immediate shift in the bacterial community structure across all doses (Figs. 10 and S11 SI), characterised by an increased relative abundance of genera such as *Acidovorax*, *Romboutsia*, *Clostridium*, *Acinetobacter*, *Flavobacterium*, and *Hydrogenophaga* gaining a competitive advantage after treatment ($p < 0.01$). These shifts, however, were not persistent, as community profiles of ozonated samples gradually converged towards control samples after 5 days of storage (T5), suggesting partial recovery over time. Storage promoted the overgrowth of genera such as *Flavobacterium* and

Novosphingobium in both ozonated and control samples (Fig. S11 SI). However, ozonated samples maintained a distinct signature with significantly higher relative abundance of *Acidovorax*, *Cloacibacterium*, *Kerstersia*, AKCV (*Burkholderiaceae*), *Rhodobacter* and *Bosea*, while several initially dominant genera (e.g. PAC000016 and GQ396872) decreased significantly after treatment (Fig. S11 SI). Shifts in the bacterial community composition due to disinfection, including ozonation, have been reported [50,77,78] either after disinfection or storage, and have been mainly characterised by the overgrowth of *Pseudomonadota*. In this study, a diverse group of taxa was observed to overgrow, possibly due to the intense textile industry input that may shape the microbial community at the influent.

Although most of the detected genera have no clinical relevance and are not expected to pose a risk to humans, a more detailed analysis of putative human pathogens revealed a significantly higher relative abundance of *Acidovorax*, *Flavobacterium*, and *Acinetobacter* in ozonated wastewater both immediately after treatment and after storage (Fig. S12 SI). These results highlight the potential selective effect that ozonation may exert on certain undesirable genera, indicating that further studies are needed to define optimal treatment conditions that minimise this selection.

Overall, ozonation resulted in limited inactivation of bacteria and ARGs. The results indicate that bacterial inactivation in this system was mainly governed by hydroxyl radical-mediated reactions. The high organic load and radical scavenging capacity of the effluent altered oxidant partitioning, reducing the relative contribution of selective ozone reactions and favouring non-selective radical pathways, which constrained overall disinfection efficiency.

3.6. Oxidation by-products and toxicity

During the LOR WWTP ozonation campaigns, NDMA remained below the quantification limit (~ 100 ng/L) across all ozone doses tested, which aligns with the WHO drinking water guideline of 100 ng/L [79]. Previous studies have shown that NDMA formation is not directly correlated with the specific ozone dose but is primarily governed by effluent composition and the presence of specific precursors [3,24], which were not investigated in this study.

Bromide concentrations in the secondary effluent (400 to 500 $\mu\text{g/L}$) exceeded the 50 $\mu\text{g/L}$ guideline referenced for ozonation in wastewater treatment [3], likely reflecting the substantial contribution of textile wastewater. Despite this, bromate formation remained below 20 $\mu\text{g/L}$ in all campaigns. Although this limit exceeds the current WHO drinking water guideline of 10 $\mu\text{g/L}$ (the contracted LOQ of 10 $\mu\text{g/L}$ was not achieved), it is consistent with previous WHO limits and is not expected to be critical in this context as the effluent is not intended for potable reuse and concentrations remain below 50 $\mu\text{g/L}$, an environmental-

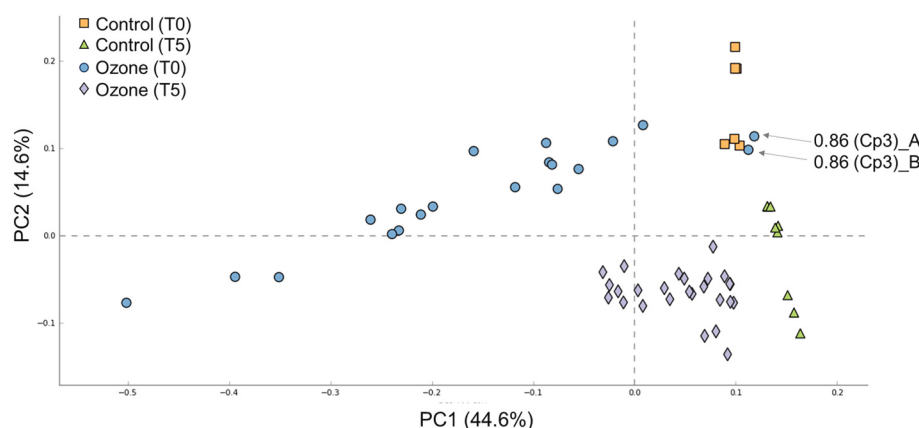


Fig. 10. PCA of the bacterial community composition, at the genus level, for control (non-ozonated), and ozonated samples before (T0) and after 5 days storage (T5). Each point represents an individual sample, based on genus-level relative abundance profiles.

quality-standard proposed for surface waters [3]. The absence of significant bromate formation is consistent with literature showing weak or inconsistent relationships between bromide concentration, ozone dose, and bromate yield in complex matrices [23], and reflects the dominant role of matrix composition and ozone decay pathways. Under conditions where ozone decomposition is rapid and radical chemistry prevails, bromate formation is typically suppressed, even at elevated bromide levels [9].

Acute toxicity, assessed using *Vibrio fischeri* bioluminescence inhibition, increased slightly across the influent samples (19–25% inhibition across campaigns), Fig. 11, corresponding to no toxicity (< 20% inhibition) to slight to moderate toxicity (20–50% inhibition) according to ISO 113-48-3:2007/Amd 1:2018. Ozonation consistently reduced acute toxicity in all campaigns, with nearly all treated samples falling within the “no toxicity” range (1.4–19.7% inhibition). Only one condition at lowest ozone dose in Cp1 showed negligible improvement relative to the influent.

Overall, toxicity removal did not scale linearly with O_3 or $\bullet OH$ exposure, suggesting a non-linear and matrix-dependent detoxification behaviour. A minimum oxidant exposure seems necessary to achieve effective toxicity reduction, whereas higher exposures did not provide proportional additional benefits.

3.7. Surrogates for assessing ozone performance and inferring EU-UWWTD compliance

Statistical analyses (PAST 4.03) were conducted to explore correlations between contaminant removal (chemical CECs, colour, and pathogen indicators) and operational or surrogate parameters of ozonation. The objective was to evaluate the applicability of established indicators to this highly challenging textile-influenced wastewater matrix and to identify more reliable alternatives for assessing ozonation performance (Table S7, SI I). Because the investigated wastewater exhibited unusually high colour and EfOM content compared with conventional municipal secondary effluents, the derived surrogate-performance relationships are expected to be matrix-specific and should therefore be extrapolated to other wastewater matrices with caution.

The organic matter-normalised ozone dose and the A254-normalised ozone dose showed the strongest and most consistent and significant correlations with water quality parameters, including micropollutants listed under the EU-UWWTD. TOC was selected as the basis for ozone dose normalisation because it provided slightly stronger correlations with treatment performance indicators than DOC. However, DOC- and TOC-normalised ozone doses were very similar for the studied wastewater, reflecting the minor difference between DOC and TOC concentrations in the secondary effluent (Table S3, SI I).

These relationships enabled the derivation of a minimum ozone requirement for compliance with the 80% removal target. Among the six best-removed indicator substances (carbamazepine, diclofenac, citalopram, and amisulpride from category 1, and irbesartan and candesartan from category 2), a minimum dose of 0.65 mg O_3 /mg TOC or 16 mg O_3 /cm⁻¹ A254 was required to achieve an average removal $\geq 80\%$ by ozonation alone (i.e. not considering the overall removal across the WWTP as intended by the EU-UWWTD) (Fig. 12). This range is consistent with previous studies reporting ozone requirements of approximately 0.55–1.0 mg O_3 /mg DOC for achieving similar chemical CEC removals [3,12,13,25,63,64], despite the substantially higher colour and EfOM content of the studied wastewater (approximately four-fold higher TOC and five-to ten-fold higher A254).

At this dose (0.65 mg O_3 /mg TOC), a 68% reduction in colour (colour-scan) was achieved (Fig. S13 in SI I), meeting the discharge requirements of the studied WWTP (non-visible colour at 1:10 or 1:20 dilution, depending on the season). In contrast, A254 decreased by only 24%, a transformation level typically associated with substantially lower ozone doses (~0.2–0.3 mg O_3 /mg DOC, [44]). For disinfection, ozone doses above 0.6 mg O_3 /mg TOC increased the LRVs of cultivable coliforms and *E. coli*, including CTX-resistant subpopulations. However, the maximum reduction observed at 0.65 mg O_3 /mg C remained close to 1 LRV, which is insufficient for achieving the water quality needed many water reuse applications. Moreover, no significant ARG removal is expected at this dose according to the results obtained in this study. Similar limitations associated with elevated organic matter and chromophoric constituents have been reported in textile-influenced wastewaters [43,44]. Consequently, upstream significant improvements in secondary effluent quality, such as enhanced nitrification via higher sludge retention times, membrane filtration (MBR/UF) or pre-coagulation, are required to improve ozonation efficiency.

Measured energy consumption, provided by the utility, ranged from 0.07 to 0.21 kWh/m³ across ozone doses of 0.41–0.98 mg O_3 /mg TOC. These values place the system within the lower energy range reported for AOPs and are consistent with comparative assessments identifying ozonation-based processes among the most energy-efficient AOPs [9].

Strong and significant ($p < 0.05$) correlations were observed between the removal of A254, A436, and PARAFAC fluorescence components and the removal of CECs, including EU-UWWTD-listed micropollutants (Fig. 13 and Table S7 SI I). Two complementary approaches were evaluated: (i) an empirical approach, consisting of a linear regression of micropollutant removal against the removal of each proxy; and (ii) a phenomenological approach previously proposed by Viegas et al. [59], which expresses micropollutant removal using Eq. (1):

$$Er = 1 - (1 - rem) \frac{k_{O_3} + k_{OH} \cdot Rct}{k} \quad (1)$$

where rem is the removal of the proxy, Rct is the ratio $\bullet OH$ -exposure to O_3 -exposure, and k is a function of the ozone-decay constant and the proxy's ozone-decay constant. Similar to the empirical approach, Eq. (1) contains two fitted parameters, namely Rct (water-specific) and k (water- and proxy-specific).

Among the five fluorescent components identified, component M (highly conjugated microbial humic-like fluorescence) was the best descriptor for micropollutant removal, followed by A_M (microbial humic-like fluorescence; $R^2 = 0.90$) and T (protein-like tryptophan like fluorescence; $R^2 = 0.89$). Following site-specific calibration, both empirical and phenomenological approaches can be used to indirectly estimate micropollutant removal and support assessment of compliance with the EU-UWWTD. The phenomenological approach can additionally be applied to estimate the removal of any individual micropollutant, provided its ozonation rate constants (k_{O_3} and $k_{\bullet OH}$) are known.

ΔA_{254} and A254 removal were also strong predictors of colour-scan attenuation ($R^2 = 0.81$ and 0.91 , respectively; $p < 0.05$) and somatic coliphage inactivation ($R^2 = 0.85$ and 0.82 , respectively; $p < 0.05$). PARAFAC component M showed comparable predictive capacity for

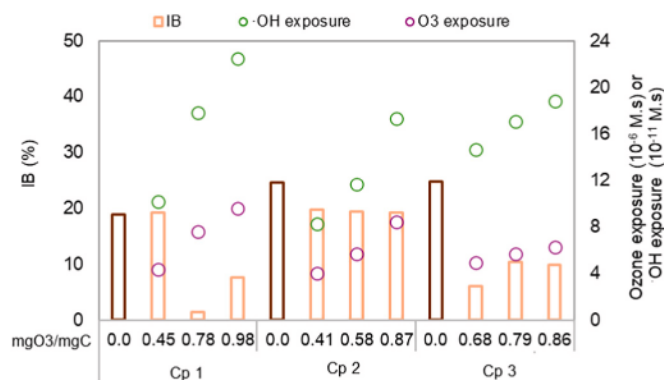


Fig. 11. *Vibrio fischeri* bioluminescence inhibition ratio ($IB = (I_0 - I) / I_0 \times 100$, where I_0 is light intensity of blank and I is light intensity of sample) in ozonation influents and ozonation effluents for each dose tested (mg O_3 /mg TOC) in the three campaigns in LOR WWTP.

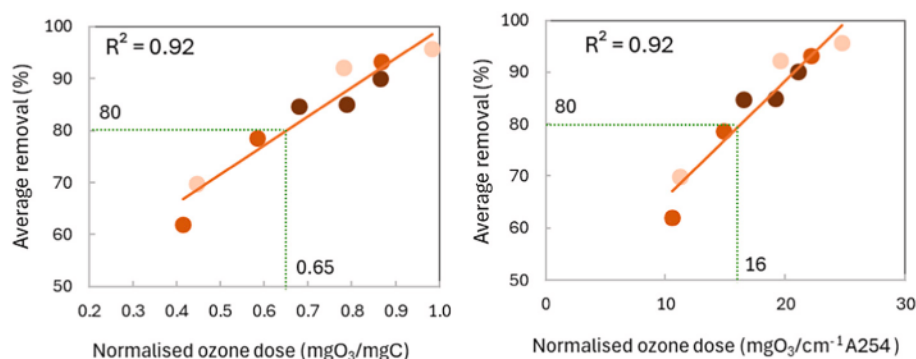


Fig. 12. Correlations obtained between the 6-best removed micropollutants from those listed in the revised EU-UWWTD and TOC-normalised ozone dose (left) or A254-normalised ozone dose (right). Different symbol colours represent each campaign in LOR WWTP. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

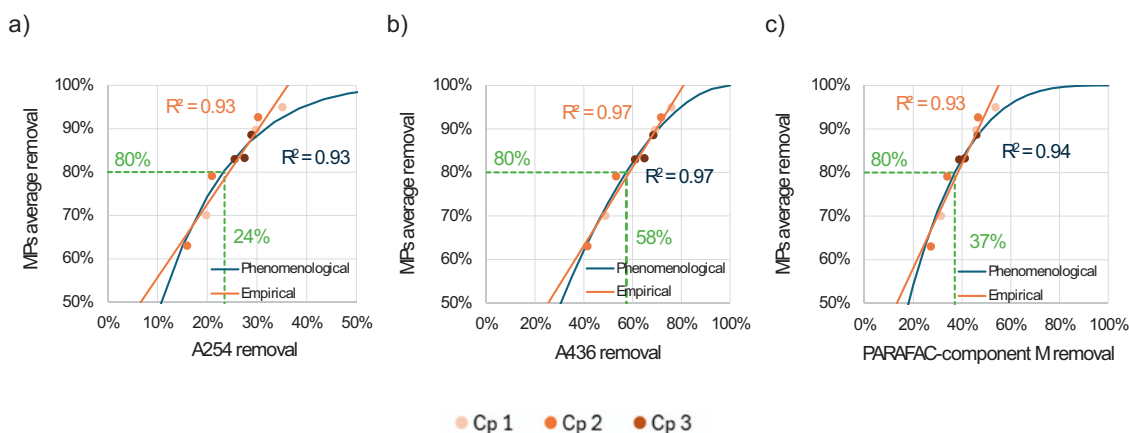


Fig. 13. Correlations between the 6-best removed micropollutants listed in the EU-UWWTD and the surrogate parameters A254 removal (a), A436 removal (b) and PARAFAC-FEEM component M removal (c), using the empirical and phenomenological approaches (different symbol colour for each campaign in LOR WWTP).

viral indicators (phenomenological approach, $R^2 = 0.85$), indicating that both absorbance- and fluorescence-based surrogates can provide useful information on treatment performance beyond micropollutant abatement.

Components M, A_M , and T also showed strong correlations with colour-scan attenuation ($R^2 = 0.84$ – 0.87) and specific colour removal, namely yellow (A436), red (A530), and blue (A610) ($R^2 = 0.84$ – 0.93). Correlations further improved when Cp3 was excluded ($R^2 = 0.94$ – 0.99).

For *E. coli*, correlations were weaker but remained significant for A254-based parameters ($R^2 = 0.41$ and 0.54 ; $p < 0.05$) and for PARAFAC components T and A_M (phenomenological approach, $R^2 = 0.55$ and 0.53 , respectively). Among the evaluated surrogates, colour-scan removal exhibited the strongest association with bacterial inactivation ($R^2 = 0.65$; $p < 0.05$; Table S7, SI I), although its predictive capacity remained moderate.

Overall, the results indicate that changes in A254, colour, and selected PARAFAC fluorescence components constitute useful surrogate indicators for monitoring ozonation performance and inferring micropollutant removal in textile-influenced secondary effluents, whereas their applicability for predicting bacterial inactivation is more limited.

4. Conclusions

This study demonstrates the feasibility and limitations of full-scale ozonation as a multi-target advanced treatment for textile-influenced municipal wastewater.

Ozone preferentially reacted with chromophoric and highly electron-

rich EfOM, resulting in near-complete removal of terrestrial humic-like fluorescence and 50–84% colour removal at ozone doses of 0.4–1 mg O_3 /mg TOC. The main novel finding is that, under the high organic load of this textile-impacted matrix, EfOM-driven ozone consumption substantially reduced ozone exposure and shifted oxidation towards hydroxyl radical-mediated pathways. Consequently, only compounds with very high ozone reactivity ($k_{O_3} > 2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$) were primarily removed via direct ozone oxidation, whereas compounds requiring a balanced contribution of O_3 and $\bullet OH$ were the most sensitive to matrix variability across campaigns.

Ozonation alone achieved the EU-UWWTD target of $\geq 80\%$ micropollutant removal at approximately 0.65 mg O_3 /mg TOC despite the high textile contribution (51%) to the influent. In contrast, microbiological performance was more limited, potentially constraining several water reuse applications. Virus inactivation exceeded bacterial inactivation, while ARG abundance was only marginally affected, with no significant reduction in total bacterial abundance (16S rRNA) or key ARG (int11, ermB). Post-treatment storage demonstrated transient bacterial inactivation, with regrowth and partial recovery of the microbial community, including bacteria carrying resistance genes, particularly at higher ozone doses, indicating a trade-off between initial disinfection and post-treatment microbiological stability. Despite high influent bromide concentrations (400–500 $\mu\text{g/L}$), bromate remained below 20 $\mu\text{g/L}$ (LOQ), consistent with an $\bullet OH$ -dominated pathway that limited bromate formation. Acute toxicity was consistently reduced, although with diminishing returns at higher ozone doses.

Operational surrogate parameters were successfully validated for this complex wastewater. Changes in A254, colour, and selected

PARAFAC fluorescence components accurately predicted micropollutant removal and viral inactivation, supporting process monitoring and indirect EU-UWWTD compliance assessment after site-specific calibration. The phenomenological approach further enables prediction of micropollutant removal from ozone and $\bullet\text{OH}$ reactivity constants.

Overall, this study shows that textile-derived EfOM fundamentally governs ozone demand, shifts oxidation pathways and determines overall treatment efficiency. Across all investigated endpoints, the results highlight the critical role of upstream treatment in controlling residual organic carbon and colour prior to ozonation for micropollutant removal and disinfection. The results also reinforce the need for site-specific validation when transferring surrogate-based compliance and process design approaches from domestic to industrially influenced wastewater systems.

CRedit authorship contribution statement

Margarida Campinas: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft. **Ivone Vaz-Moreira:** Data curation, Formal analysis, Visualization, Writing – original draft. **Rui M.C. Viegas:** Formal analysis, Funding acquisition, Methodology, Visualization, Writing – original draft. **Elsa Mesquita:** Formal analysis, Investigation, Visualization, Writing – original draft. **Diana Ribeiro:** Data curation, Formal analysis. **Afonso Ruivo:** Formal analysis. **Joana Soares:** Formal analysis. **Paula Paíga:** Formal analysis, Methodology, Validation. **Cristina Delerue-Matos:** Formal analysis, Methodology, Resources. **Gabriela Faria:** Data curation, Investigation, Methodology, Resources. **Catarina Silva:** Data curation, Funding acquisition, Investigation, Methodology, Visualization, Project administration. **Claudio Costa:** Investigation, Project administration, Methodology, Resources. **Célia M. Manaia:** Conceptualization, Formal analysis, Methodology, Visualization, Supervision, Writing – original draft. **Maria João Rosa:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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