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Strategies for mitigating chlorinated disinfection byproducts in wastewater treatment plants

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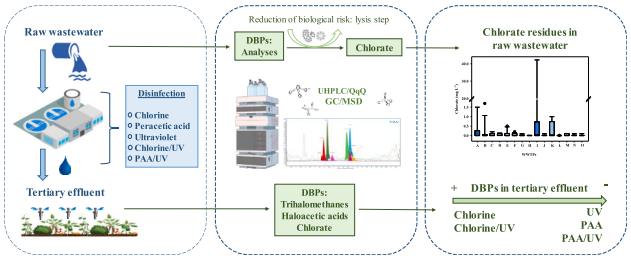
HIGHLIGHTS

- Disinfection byproducts (DBPs) in 15 full-scale wastewater plants were assessed.
- Inactivation step before DBP analyses reduced trihalomethanes and haloacetic acids.
- Chlorate residues were already present in the raw wastewater.
- Chlorine (or with UV) promoted DBPs while peracetic acid and/or UV did not.
- Mitigation of DBPs was possible for reclaimed water intended for irrigation.

GRAPHICAL ABSTRACT



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ABSTRACT

A case study of 15 wastewater treatment plants (WWTPs) at a full-scale was assessed for the risks of disinfection byproduct (DBP) formation, mainly the regulated trihalomethanes (THMs) and haloacetic acids (HAAs) and chlorate as an inorganic byproduct regulated recently in the EU. Raw wastewater from large, medium/small urban areas were treated with single or combined disinfection processes (i.e., chlorine, peracetic acid (PAA) and ultraviolet (UV) radiation). Sampling was executed once a month over seven months for the medium/small WWTPs and twice a month for the large ones. Due to the potential risk of SARS-CoV-2 contaminated wastewater, several inactivation methods were examined before the DBP analysis. Due to the inactivation step, the stability of THM4 and HAA9 suffered reductions, monitoring their presence only in the effluents after the disinfection treatments. In contrast, chlorate levels remained unchanged after the inactivation treatment; thus both raw wastewater and effluents were examined for their occurrence before disinfection treatments. Results showed that chlorate residues in the raw wastewater varied greatly from undetected levels to as high as 42.2 mg L^{-1}. As the continuous monitoring of DBPs was performed, a positive correlation with chlorine or chlorine/UV was found. Changes in the physicochemical parameters indicated that the quality of the raw wastewater varied considerably depending on the WWTPs, and it influenced byproduct formation. In all WWTPs, chlorine alone or combined with UV significantly increased the presence of THMs, HAAs, and chlorate levels in the treated effluents. When the same WWTPs changed to PAA or PAA/UV, DBPs were diminished completely. This study highlights the risk of chlorate residues in raw wastewater during the pandemic. It also showed how the chemical risks of DBP

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formation could be reduced by changing the chlorinated disinfection technologies to PAA or PAA/UV, particularly if reclaimed water is intended for agricultural irrigation to minimize DBP residues.

1. Introduction

The management of wastewater treatment plants (WWTPs) has become crucial to generate high quality reclaimed water that can solve water shortage problems of surface and groundwater resources. One relevant use of reclaimed water is agriculture, promoting the saving of water for irrigation and decreasing the impact of wastewater discharge on the environment (Alcalde-Sanz and Gawlik, 2017; Donnaz, 2020; EC, 2020a).

Wastewater collected primarily by municipalities includes used water from domestic and industrial sources that undergo different standardized and conventional processes in WWTPs (ISO 16075–2:2020). Before applying the reclaimed wastewater in agricultural irrigation, microbiological safety must be assured as the primary requirement by selecting appropriate disinfection agents (EU, 2017; Cui et al., 2020; EC, 2020a; Truchado et al., 2021a). Disinfection treatments are usually the final stage in the wastewater treatment process to inactivate pathogens (ISO 16075–2:2020). For the safe reuse of treated urban wastewater in agricultural irrigation, the application of disinfection processes through optimal doses is determined based on the minimum water quality requirements (i.e., controlling the concentration of *Escherichia coli*) (EC, 2020a). Disinfection technologies mainly include chemical disinfectants such as chlorine, the most common disinfection agent used in WWTPs (USEPA, 2012; Cui et al., 2020). As a potent oxidizer, chlorine reacts with natural organic matter generating harmful chlorinated disinfection byproducts (DBPs) of concern due to potential adverse effects on aquatic environment and human health (Richardson and Ternes, 2011, 2018; Ding et al., 2013; Gong and Zhang, 2015, 2016; Sun et al., 2019; Zhong et al., 2019; Tak et al., 2020).

Trihalomethanes (THMs) and haloacetic acids (HAAs) are generated in substantially larger quantities than other organic halogenated DBPs (Zhang et al., 2000). Chloroform (trichloromethane) and three bromine-containing trihalomethanes (collectively THM4) plus five HAAs (HAA5) have been used as indicators for regulated DBPs (Tak et al., 2020), although more than 700 DBPs have been identified, being some more toxic than the regulated ones (Luo et al., 2020; Richardson and Plewa, 2020). In drinking water, THM4 and HAA5 are regulated with maximum contaminant levels of 80 and 100 μg L^{-1} for THM4 in the US and EU, respectively, whereas for HAA5 the maximum level is 60 μg L^{-1} in the US and EU (USEPA, 1998; EC, 2020b). On the other hand, chlorate can be accumulated in chlorine-treated wastewater effluents as a degradation byproduct (Uzun et al., 2019; Zhong et al., 2019). Chlorine, in the form of concentrated sodium hypochlorite (NaClO) breaks down to hypochlorite ion (OCl^-) and the degradation of OCl^- results in the formation of chlorate (OCl_3^-) via a chlorite intermediate stage (OCl_2^-) (AHDB, 2016). Recently, the maximum residue levels for chlorate have been regulated in fresh produce (EC, 2020c) and also in drinking water when a disinfection method that generates chlorate is used (0.7 mg L^{-1}) (EC, 2020b). Chlorinated irrigation water is the main factor contributing to chlorate residues in the crop (Dannehl et al., 2016; López-Gálvez et al., 2018a). If chlorine-treated wastewater is used for irrigation, the maximum residue levels (MRLs) regulated can be exceeded due to the continuous uptake of chlorate by the crop during the growth (EC, 2020c). Garrido et al. (2020) demonstrated that despite the moderate concentrations of chlorate in reclaimed wastewater (0.2 mg L^{-1}), lettuce irrigated with this water presented high levels of chlorates (0.6 mg kg^{-1}) due to the accumulation during the growing cycle.

Chlorine has been extensively used to disinfect wastewater before discharge for microbial inactivation. Chlorinated DBPs are formed in freshwater effluents while brominated DBPs are also formed in saline effluents which may have negative effects to the organisms present in the receiving water body (Yang and Zhang, 2013, 2014; Liu and Zhang, 2014; Yang et al., 2015). Researchers are focused on searching for disinfection technologies that prevent DBPs formation in reclaimed water and accumulation in the irrigated product and the adverse impacts on public health (EFSA, 2015; López-Gálvez et al., 2018b; Bernstein et al., 2021; Sharma et al., 2021). Ultraviolet (UV) radiation applied individually has been shown as a safe and efficient wastewater disinfection treatment that avoids the formation of DBPs (Lazarova et al., 1999; Millan-Sango et al., 2017; Collivignarelli et al., 2021). However, this physical technology has some limitations, such as the penetration capacity and lack of a residual disinfectant level. In the past years, UV combined with chlorine has attracted more attention as this combination allows minimizing the residual chlorine while maintaining residual protection (Hua et al., 2021). Wang et al. (2019) reported that chlorine's photolysis leads to the production of chlorate through the dimerization of ClO^- and the subsequent reactions. As a suitable alternative to chlorine, peracetic acid (PAA) combined or not with UV has been a feasible solution for municipal wastewater due to a broad spectrum of antimicrobial activity and the negligible formation of DBPs (Kitis, 2004; Dell'Erba et al., 2007; Domínguez Henao et al., 2018; Ragazzo et al., 2020; Zhang et al., 2020). However, some disadvantages of PAA should be also considered, as it is a colorless liquid with a strong, pungent acrid odor not as effective in the degradation of chemical pollutants and as antimicrobial agent as chlorine (Luongo et al., 2020; Truchado et al., 2021b).

The presence of the SARS-CoV-2 in wastewater has been reported, and concerns about the potential of wastewater mediated transmission have been described (Randazzo et al., 2020; Kataki et al., 2021). The management of wastewater samples has become even riskier not only in the WWTPs but also in the lab (Silverman and Boehm, 2020). Even though only the molecular material of the virus has been detected (Randazzo et al., 2020), safety measures must be taken. USEPA (2015) proposed some precautions when collecting wastewater samples for field screening and laboratory analysis. Wastewater samples must be handled under safety requirements to prevent contamination through aerosols for the operators and technicians. However, there are no recommendations to handle wastewater samples for DBP analyses. The impact of inactivation protocols on the stability of DBPs is unknown.

On the other hand, due to excess use of chlorine derived compounds throughout the pandemic, wastewater may already contain DBP residues (Li et al., 2021). In these situations, the DBP basal levels in wastewater should be considered as DBPs are of concern particularly in reclaimed water (EC, 2020a). There is little information about the presence of DBPs in the effluents of WWTPs that may affect the selection of disinfection technologies for treated wastewater for agricultural irrigation. The objectives of this study were to: (i) examine if a microbial inactivation protocol causes interferences for the analysis of DBPs (ii) establish if chlorate residues were present before disinfection technologies, (iii) evaluate the impact of individual and combined disinfection treatments including chlorine, PAA, and UV on the regulated DBPs, THMs and HAAs, and chlorate as inorganic byproduct. Fifteen WWTPs were examined over seven months during the COVID-19 pandemic.

2. Materials and methods

2.1. Wastewater treatment plants and sampling procedures

Samples were collected from 15 WWTPs in several municipal locations (Murcia, Spain); four of them corresponded to large urban areas (B, E, F, and L) and 11 to medium/small localities (A, C, D, G, H, I, J, K, M, N, O). The general information of the WWTPs, the sampling date, and

the tertiary treatment applied is included Table S1. From June to December 2020, the medium/small WWTPs were sampled once, while the large WWTPs were sampled twice a month for the same periodTwo samples of 250 mL were taken each sampling time, one from the raw wastewater and another from the tertiary effluent. Wastewater was primarily treated through aeration grit, including separation of suspended solids and particles, desanding-grease removal, and a primary setting tank of different dimensions. A secondary treatment consisted of biological aerobic or anaerobic treatment in a secondary settling tank, including coagulation/flocculation and complementary lamellar clarification. Further, disinfection processes of either chlorine, PAA, and UV or the combined chlorine/UV and PAA/UV, as tertiary treatments were carried out except for WWTP-M that an electroactive biofilter (EB) was used. This treatment consisted of an electroactive bacteria combined with electroconductive material. The disinfection treatments applied were adjusted based on the minimum effective doses required for microbiological quality (data not shown). For WWTPs that used chlorine, sodium hypochlorite with 10–20% active chlorine (NewChem, SL, Alicante, Spain) was added to maintain a residual concentration of free chlorine (FC) that varied between 0.1 and 3.0 mg L^{-1}. In the case of PAA, a commercial solution of 15% PAA+ 16% acetic acid +24% hydrogen peroxide was used (Brenntag, Essen, Germany) reaching concentrations between 4.0 and 5.0 mg L^{-1} (Table S2). Combined treatments were processed by adding aqueous chlorine solution or PAA into the tank followed by a closed pipe or open channel UV disinfection system under the various conditions described in Table S1. Samples for DBP analyses were aliquoted in amber glass bottles with no headspace. The collected samples were transported to the lab (CEBAS-CSIC, Murcia, Spain) within 2 h from the sampling and prepared for the DBP analyses. THMs were immediately analyzed, while for HAA and chlorate analyses, samples were stored under refrigeration (4 °C) and analyzed within 24 h from the sampling.

2.2. Inactivation of samples before DBP analyses

To avoid any potential microbiological risk of SARS-CoV-2 when manipulating samples, an inactivation protocol that did not interfere with the analysis of DBPs was needed. The impact of the inactivation method was studied by comparing the DBPs analyzed in the non-inactivated versus the inactivated samples. For the safety handling procedure, instead of using raw wastewater with potential contamination risks, effluent samples corresponded to five WWTPs were examined as they were already subjected to different disinfection treatments (chlorine, PAA, UV, chlorine/UV, PAA/UV).

The inactivation methods of phenol and guanidine isothiocyanate described for SARS-CoV-2 were tested, mimicking safety protocols for biological risks in the biosafety laboratory (Darnell et al., 2004; Bain et al., 2020). The protocol consisted of a centrifugation step at 2500×*g* for 10 min and the filtration of the supernatant through a 0.45 μm polyethersulfone (PES) filter. Then, the lysis buffer AVL (Qiagen, Hilden, Germany) was added to the filtrated sample to ensure nucleic acids lysis. Once the samples were settled down for 1–5 min, the analyses of DBPs were carried out in the instrumental laboratory.

Additionally, another inactivation methodology based on paraformaldehyde (PFA) adapted to wastewater samples was also studied (Kumar et al., 2015). First, a centrifugation step at 2500×*g* for 10 min and the filtration of the supernatant through a 0.45 μm filter were performed. Subsequently, samples were diluted in PFA (stock solution 20% PFA, Electron Microscopy Science, Hatfield, England) to reach a final concentration of 4% PFA. Samples were left for 30 min before analysis. Dilution factors were taken into account in both protocols for DBP quantification.

2.3. Physicochemical characteristics of wastewater

The physicochemical parameters examined in the raw wastewater samples and tertiary effluent samples included pH, electrical conductivity (EC, mS cm^{-1}), and chemical oxygen demand (COD, mg L^{-1}). These analyses were carried out in each WWTP by the exploitation WWTP company (Acciona S.A., Spain). COD was measured by the standard photometric method (APHA, 2012) using the Spectroquant NOVA 60 photometer. For measuring FC and PAA in the effluent samples, a chronoamperometry test Kemio™ disinfection (Palintest, Gateshead, UK) was used. Detailed information on the physicochemical characteristics of the raw wastewater samples and tertiary effluents of each WWTP and sampling time is shown in Table S2.

2.4. THM analysis

The four main THMs detected were: (1) trichloromethane (TCM or chloroform, $CHCl_3$), (2) bromodichloromethane (BDCM, $CHCl_2Br$), (3) chlorodibromomethane (CDBM, $CHClBr_2$) and (4) tribromomethane (TBM, $CHBr_3$). The total sum corresponded to THM4. THMs analyses were carried out in all the tertiary effluent samples as previously described (Gómez-López et al., 2013), except WWTP-M as the microbial safety was not validated. Before the analysis, aliquots of 5 mL of effluent samples were spiked with 1-bromo-3-chloropropane, (Sigma-Aldrich, Merck, Darmstadt, Germany) as an internal standard at a final concentration of 20 μg L^{-1}. The analysis was performed on a Gas Chromatograph/Mass Selective Detector (GC/MSD) with Triple-Axis High Energy Diode Electron Multiplier Detector (Agilent 5975C series inert MSD). Chromatographic separation of THMs was carried out with an HP-5MS 30 m × 0.25 mm (i.d.) capillary column (Agilent Technologies, Santa Clara, CA). Injections were performed in splitless, except in those with THMs concentration above 200 μg L^{-1}, where a split of 1:100 was applied. The Full Scan data acquired was used to verify the individual THMs by using the spectral search with NIST 2008 standard GCMS library. The SIM data from the standards were used to plot the calibration curves for the quantitative method. For this quantification, a mix of THMs was used for the calibration curves (certified reference material EPA 501/601 THMs calibration mix, Supelco, Merck, Darmstadt, Germany). The results were expressed in μg L^{-1} and the limits of quantification (LoQ) were: 0.05 μg L^{-1} for TCM, 0.1 μg L^{-1} for BDCM and CDBM, and 0.5 μg L^{-1} for TBM LoQ were calculated as the lowest concentration validated with acceptable accuracy (trueness and precision).

2.5. HAA analysis

The analysis of HAAs included the five regulated HAAs in drinking water (HAA5) and the four unregulated HAAs, to a total nine (HAA9) as: (1) dichloroacetic acid (DCAA), (2) trichloroacetic acid (TCAA), (3) monochloroacetic acid (MCAA), (4) monobromoacetic acid (MBAA), (5) dibromoacetic acid (DBAA), (6) tribromoacetic acid (TBAA), (7) bromochloroacetic acid (BCAA), (8) bromodichloroacetic acid (BDCAA) and (9) chlorodibromoacetic acid (CDBAA). The analysis was carried out according to the methodology described by do Lago and Daniel (2019), with some modifications (Marín et al., 2020). All effluent samples were analyzed except those from the WWTP-M, as explained before for THMs.

An ultra-high-pressure liquid chromatograph (UHPLC) (Agilent 1290 infinity, Agilent Technologies, Santa Clara, CA) coupled to triple quadrupole mass spectrometer (Agilent 6460, Agilent Technologies, Santa Clara, CA) and equipped with Jet Stream electrospray ionization (ESI) source performing in negative ion mode was used. Chromatographic separation was carried out with an InfinityLab Poroshell HPH-C18 column of 3 × 150 mm, 2.7 μm particle size (Agilent Technologies, Santa Clara, CA). For the quantification of HAA9, internal calibration curves were performed. A mix of HAA9 (certified reference material EPA 552.2 HAA mix, Supelco, Merck, Darmstadt, Germany) and as internal standards, isotope labeled HAAs were used: dichloroacetic acid-2-^{13}C (DCAA-^{13}C), trichloroacetic acid-2-^{13}C (TCAA-^{13}C), monochloroacetic acid-2-^{13}C (MCAA-^{13}C), monobroacetic acid-1-^{13}C (MBAA-^{13}C) (Dionex, Thermo Scientific, Sunnyvale, California, USA) and dibromoacetic acid-

1-^{13}C (DBAA-^{13}C) (Sigma-Aldrich, Merck, Darmstadt, Germany). Before the analysis, samples were filtrated through 0.22 μm pore size filters (Fisherbrand non-sterile PTFE syringe filter, Thermo Fisher Scientific, Massachusetts). Samples and standards were both spiked with internal standards at the final concentration of 100 μg L^{-1}. Results were expressed in μg L^{-1} and LoQ were: 1 μg L^{-1} for DCAA, TBAA, BCAA, 2 μg L^{-1} for TCAA, MBAA, BDCAA, CDBAA, 3 μg L^{-1} for MCAA and 5 μg L^{-1} for DBAA.

2.6. Chlorate analysis

The EU reference method for analyzing polar pesticides in plant foods was performed for chlorate analysis (Anastassiades et al., 2019), with minor variations for wastewater samples (Garrido et al., 2020). Samples from all raw wastewater and tertiary effluents were inactivated following the phenol and guanidine isothiocyanate protocol described in the inactivation method. After that, samples were filtrated through 0.22 μm pore size filters (Fisherbrand non-sterile PTFE syringe filter, Thermo Fisher Scientific, Massachusetts) and analyzed in the same UHPLC coupled to a mass spectrometer (QqQ) described for HAA analysis. Samples were diluted (methanol with formic acid 1%), depending on the initial chlorate content, and spiked with an isotope labeled internal standard ($Cl^{18}O_3^-$). The internal standard at the final concentration of 100 μg L^{-1} was spiked to make the calibration curves for chlorate quantification. The results were expressed in mg L^{-1} and the LoQ was 0.003 mg L^{-1}.

2.7. Statistical analysis

Data were analyzed using PASW Statistics 27 for Windows (SPSS Inc., Chicago, IL, USA). Shapiro-Wilk test was used for evaluating if the data set was modeled by a normal distribution and Levene's test for assessing the homogeneity of variance. After that, nonparametric tests were applied. Kruskal-Wallis test was carried out to compare disinfection technologies revealing significant differences ($p < 0.05$) while U-Mann Whitney test ($p < 0.05$) was performed to compare each disinfection technology with that of chlorine.

3. Results

3.1. Impact of the inactivation method on DBP analyses

Results showed that the tertiary effluent samples subjected to inactivation reduced the content of THM4 (Table 1). In particular, sample pretreatment based on centrifugation and filtration reduced THM4 by 36% as the mean value. The losses increased with the lysis buffer's subsequent inactivation process, reaching the mean value of THM4 reductions by 46%. However, the inactivation process that caused the most THM4 losses was PFA, with 85% losses as the mean value. As far as individual THMs, the loss was particularly relevant for chloroform as the main THM, which was reduced between 22 and 50% after centrifugation and filtration processes. Chloroform concentration was reduced between 29 and 62% after the inactivation using the lysis buffer. Additionally, the reductions reached between 62 and 100% after the inactivation with PFA (data not shown).

Regarding HAA9, the impact of the inactivation process did not follow a clear trend in the quantification of these compounds. In the WWTPs with high concentration of HAAs (WWTP-L), the concentration remained relatively stable after sample pretreatment and the subsequent inactivation processes. In the WWTPs with low concentration of HAAs (WWTP-K and WWTP-N), slight losses were observed, although they were not quantitatively significant. Only in the WWTPs where concentrations of HAAs were close to LoQ, the losses due to inactivation processes were evident (WWTP-B and WWTP-F). For this reason, it could not be ensured that after the inactivation of the samples reliable results could be obtained for the quantification of HAAs, especially for concentrations close to the LoQ (Table 1).

Table 1
Changes in the content of THM4 (μg L^{-1}), HAA9 (μg L^{-1}) and chlorate (mg L^{-1}) in the effluent samples of five WWTPs subjected or not to an inactivation process.

WWTPs	Disinfection system	Inactivation	THM4	HAA9	Chlorate
B	UV	No	0.8 ± 0.1	1.4 ± 0.6	**
		C + F	0.5 ± 0.1	*	**
		Lysis buffer	0.4 ± 0.1	*	**
		PFA	0.1 ± 0.0	*	**
F	PAA/UV	No	0.8 ± 0.1	1.1 ± 0.3	**
		C + F	0.4 ± 0.1	*	**
		Lysis buffer	0.3 ± 0.0	*	**
		PFA	*	*	**
K	PAA	No	2.2 ± 0.3	14.0 ± 0.2	**
		C + F	1.4 ± 0.2	13.0 ± 6.3	**
		Lysis buffer	0.9 ± 0.1	11.9 ± 3.0	**
		PFA	0.2 ± 0.1	8.6 ± 0.7	**
L	Chlorine/UV	No	2337.2 ± 103.8	761.8 ± 7.7	3.1 ± 0.1
		C + F	1806.7 ± 147.9	822.7 ± 16.4	3.1 ± 0.1
		Lysis buffer	1638.9 ± 57.4	778.9 ± 6.2	3.2 ± 0.0
		PFA	370.0 ± 45.8	780.8 ± 33.4	3.4 ± 0.1
N	Chlorine	No	56.5 ± 1.1	24.4 ± 2.1	1.6 ± 0.0
		C + F	38.1 ± 1.8	17.0 ± 1.3	1.6 ± 0.0
		Lysis buffer	39.4 ± 4.1	20.2 ± 1.2	1.6 ± 0.0
		PFA	19.6 ± 2.8	20.5 ± 1.4	1.8 ± 0.1

Values are the mean of three replicates ± standard deviation. *< LoQ (Limit of quantification). **< 0.05 mg L^{-1}, in the case of chlorate. No: no inactivation; C + F: centrifugation and filtration; PFA: paraformaldehyde.

Concerning chlorate, the levels in the inactivated effluents were similar to those detected in the no-inactivated ones, meaning that the inactivation protocols did not reduce the chlorate residues, probably because of its non-volatile nature (Table 1). The method of phenol and guanidine isothiocyanate described for SARS-CoV-2 (lysis buffer method) was chosen as the more accessible and faster methodology to carry out. Thus, the inactivation process was carried out in the raw wastewater before the analysis of chlorate as it did not cause interferences for quantification. On the contrary, the inactivation methods were not recommended for the raw wastewater samples before the analysis of THM4 and HAA9 due to their poor stability.

3.2. Presence of chlorate before disinfection treatments

As an inorganic byproduct, chlorate was analyzed in the raw wastewater samples of the 15 WWTPs after the inactivation protocol for safe handling. Surprisingly, chlorate levels were present in all WWTPs except in WWTP-H and WWTP-L that showed low levels some samplings (Fig. 1). The presence of chlorate in all raw wastewater was probably due to the elevated concentration of chlorinated disinfectants used during the pandemic to reduce the potential contamination risk with SARS-CoV-2. Although in most WWTPs, chlorate levels in the wastewater were below the maximum residue levels (MRL) set up for drinking water (0.7 mg L^{-1}), in other WWTPs, the levels exceeded this limit (e.g. WWTPs-A, B, I and K).

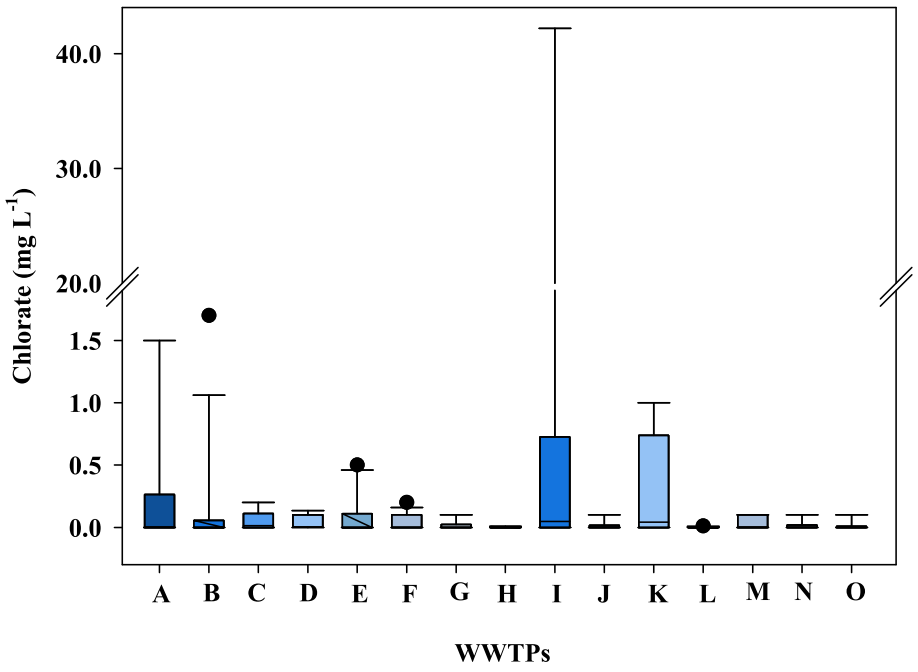

Fig. 1. Chlorate levels in the raw wastewater samples of 15 WWTPs. Horizontal line in the box represents the median value and the low and upper sides show percentiles 25 and 75, respectively. The whiskers from the box show percentiles 10 and 90 calculated according to Cleveland method. Symbols (●) represent outliers. See Table S1 for WWTP identification.

3.3. Monitoring DBPs in the effluents of WWTPs treated with different disinfection technologies

Fifteen WWTPs that utilized one of the three disinfection treatments (chlorine, PAA, UV) or their combinations (chlorine/UV and PAA/UV) were assessed for this study. Due to the different population size and the specific conditions of each WWTP, the production outputs, disinfectant dosages, and residual levels varied. Physicochemical data, such as pH, EC, COD, FC, dosage, and flow, in each WWTP, are included in the supplementary information (Table S2). In general, the tertiary effluents in each WWTP exhibited similar pH and EC values. The pH was relatively high in some WWTPs using chlorine regarding the recommended values (pH 6.0–7.0). EC was relatively constant and similar among WWTPs, except some WWTPs were punctually high (WWTP-B and WWTP-E). The organic matter content expressed as COD differed considerably between WWTPs with values as low as 102 mg L^{-1} or as high as 6253 mg L^{-1}. After the tertiary treatments, COD values were reduced tenfold approximately, compared with the raw wastewater samples, resulting in water quality with low organic matter content but slightly different depending on the WWTPs (Table S2).

Results obtained over the whole sampling period were grouped by disinfection technology to analyze the DBPs present in the tertiary effluents. The Shapiro-Wilk test showed that the data set did not follow a normal distribution because of the differences in the number of samples treated with the same disinfection technology. Then, the U-Mann-Whitney test was calculated, and compared each treatment with chlorine. For the content of THM4, significant differences were observed between chlorine and the other technologies except with chlorine/UV (Fig. 2). Curiously, the highest level of THM4 was detected when chlorine was combined with UV (chlorine/UV), with a median value of 300 μg L^{-1}. The high levels of THM4 could be due to the high COD of some wastewater samples and the need for higher chlorine doses. Punctually, in two of the samplings, effluents treated with PAA showed unusually high THM4 values (230 and 521 μg L^{-1}). However, as expected, the

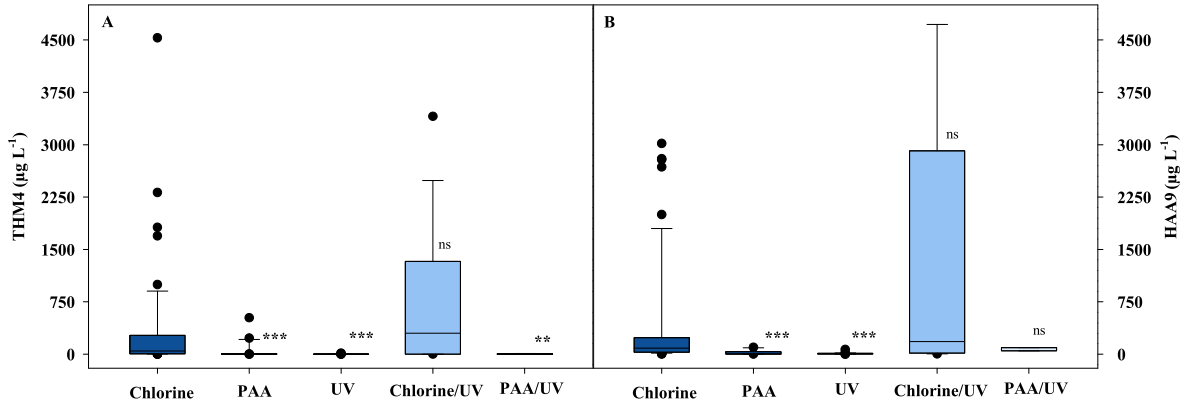

Fig. 2. Changes in THM4 (A) and HAA9 (B) in the effluent samples of WWTPs grouped by disinfection technologies. The horizontal line in the box represents the median value and the low and upper sides show percentiles 25 and 75, respectively. The whiskers from the box show percentiles 10 and 90 calculated according to Cleveland method. Symbols (●) represent outliers. The stars near symbols indicate significant differences at $P < 0.05$ when comparing chlorine with PAA, UV, chlorine/UV and PAA/UV, according to Mann-Withney *U* test: * ($p < 0.05$), **($p < 0.01$), ***($p < 0.001$), ns: not significant.

THM4 levels in the effluents treated either alone or in combination with PAA and UV were in general considerably low, in some cases even lower than the LoQ. It is noteworthy that the THM4 present in the highest concentration was TCM, followed by BDCM, CDBM, and TBM as the lowest (Fig. S1). TCM represented >85% of the total THM concentration in the chlorinated treatments.

The concentration of HAA9 presented a similar tendency to THM4 with significantly higher levels in chlorinated tertiary treatments than the other technological processes (Fig. 2). Remarkably, the formation of HAA9 in chlorine/UV exhibited a high variation, with values between 34.6 and 2583.9 μg L^{-1} for quartiles 1 and 3 (Q1 and Q3, respectively), in the boxplot of the treated effluents. Fig. 2 illustrates the significant differences in the content of HAA9 between chlorine and PAA or UV, while no differences were observed in the combined chlorine/UV treatment. Punctually, effluents treated with PAA and UV also showed high HAA9 values in two of the samplings (99.1 and 66.8 μg L^{-1}, respectively) as it happened with THMs.

When the chlorate residues in the effluent samples were examined, it was observed that the levels were relatively high in the single (chlorine) or combined treatments (chlorine/UV) (media levels of 3.9 and 5.8 mg L^{-1} and outliers of 258.9 and 283.9 mg L^{-1}, respectively) (Fig. 3). As expected, in the non-chlorinated treatments, the chlorate residues were significantly lower ($p < 0.001$) with the minimum levels in PAA alone or combined with UV. Chlorate levels were also measured in the tertiary effluent of the electroactive biofilter (WWTP-M) as the inactivation protocol was applied before the analysis. As expected, the chlorate level was very low and significantly different from the chlorinated treatment (Fig. 3).

3.4. Improvements in the WWTPs through the appropriate disinfection technologies to reduce DBPs

DBPs were evaluated twice a month in four WWTPs, and once a month in eleven WWTPs and the DBPs fluctuated considerably along the seven months of sampling as a function mainly of the disinfection treatment applied. Results on the content of each DBP group analyzed in each WWTP over the sampling period are shown in Supplementary information: THM4 in Fig. S2, HAA9 in Fig. S3 and chlorate in Fig. S4. Results revealed that WWTPs reduced the generation of DBPs by changing the disinfection treatment. Two WWTPs (B and E) are presented in more detail in Figs. 4–6. When UV or PAA alone or combined replaced chlorine or chlorine/UV, THM levels decreased to extremely low levels, even lower than the MRL permitted for drinking water (Fig. 4, Fig. S2). TCM variation in chlorinated tertiary effluents showed the same pattern as total THM4, with higher concentrations correlated with the chlorine dosages and residuals (Fig. 4). Similar behavior was observed for BDCM and CDBM as when chlorine was added, their concentrations increased, although to a lesser extent. The significance of this trend was enhanced by the consistency of this observation in all the WWTPs treated with chlorine or chlorine/UV (Fig. S2). When the WWTPs were compared, the highest accumulation of THM4 varied considerably among those WWTPs using chlorine or chlorine/UV as tertiary treatment, with maximum levels as low as 120 μg L^{-1} in WWTP-K or as high as 4530 μg L^{-1} in WWTP-H (Fig. S2).

When the WWTPs were examined regarding the formation of HAAs, a similar trend to that of THM4 was observed. For HAA9 formed, the effluents of WWTPs showed that HAA concentrations increased when chlorine was added either alone or combined with UV (Fig. 5, Fig. S3). HAA levels increased severely in chlorine-treated effluents, reaching concentrations up to 2600 μg L^{-1} in the WWTPs- C, G, H and J (Fig. S3). The high accumulation of HAA9 in the WWTP-E at a sampling time is also remarkable, showing a maximum level up to 6300 μg L^{-1}, which largely exceeded the MRL established by USEPA and EU for drinking water (60 μg L^{-1}). DCAA and TCAA were detected in all chlorine or chlorine/UV treated samples, showing WWTP-B, C and E the treatment plants with the highest levels. MCAA and DBAA levels followed the same trend as HAA9 as they were the second group that most contributed to HAA9. In contrast, MBAA, BCAA, BDCAA CDBAA and TBAA were, except in some samplings, the HAAs detected in the smallest proportion. It is remarkably mentioning that when the raw wastewater from the same WWTPs was treated with either PAA or UV alone or in combination (PAA/UV), HAAs levels decreased extraordinary to values mostly lower than the MRL for drinking water.

Regarding chlorate levels, the lowest concentrations detected for chlorate were when non-chlorinated treatments were used as tertiary treatments, contrary to what happened with chlorine and chlorine/UV independently of the sampling time (Fig. 6, Fig. S4). WWTP-B, E and G were identified as the ones with the highest levels of chlorate (>250 mg L^{-1}), exceeding deeply MRL allowed by the EU for drinking water, due to the treated effluent samples with chlorine alone or combined with UV. As expected, except in the first sampling, chlorate levels were not detected in the tertiary effluent of WWTP-M as the only WWTP that did

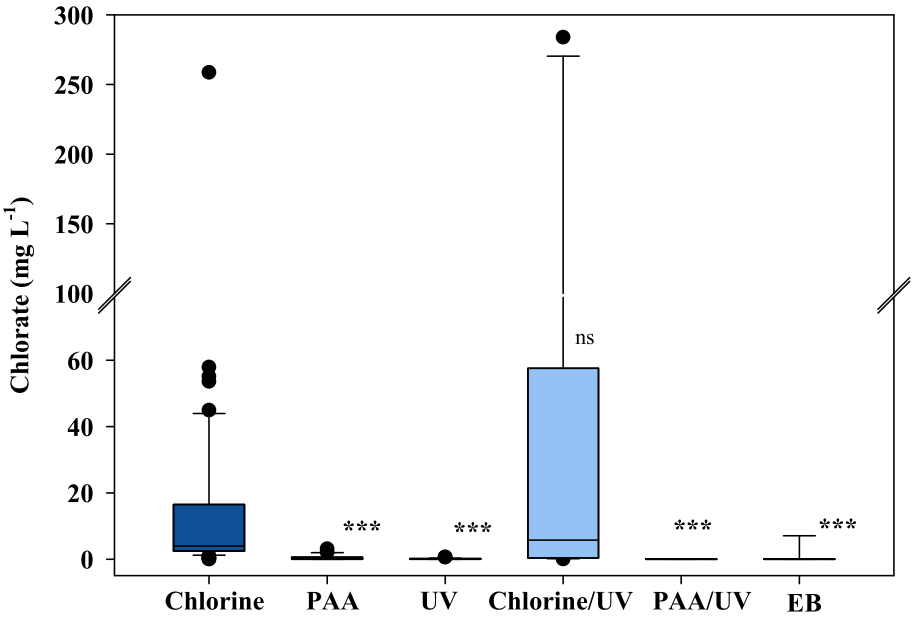

Fig. 3. Changes in chlorate levels in the effluent samples of WWTPs grouped by disinfection technologies. The horizontal line in the box represents the median value and the low and upper sides show percentiles 25 and 75, respectively. The whiskers from the box show percentiles 10 and 90 calculated according to Cleveland method. Symbols (●) represent outliers. The stars near symbols indicate significant differences at $P < 0.05$ when comparing chlorine with PAA, UV, chlorine/UV and PAA/UV, according to Mann-Withney *U* test: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), ns: not significant.

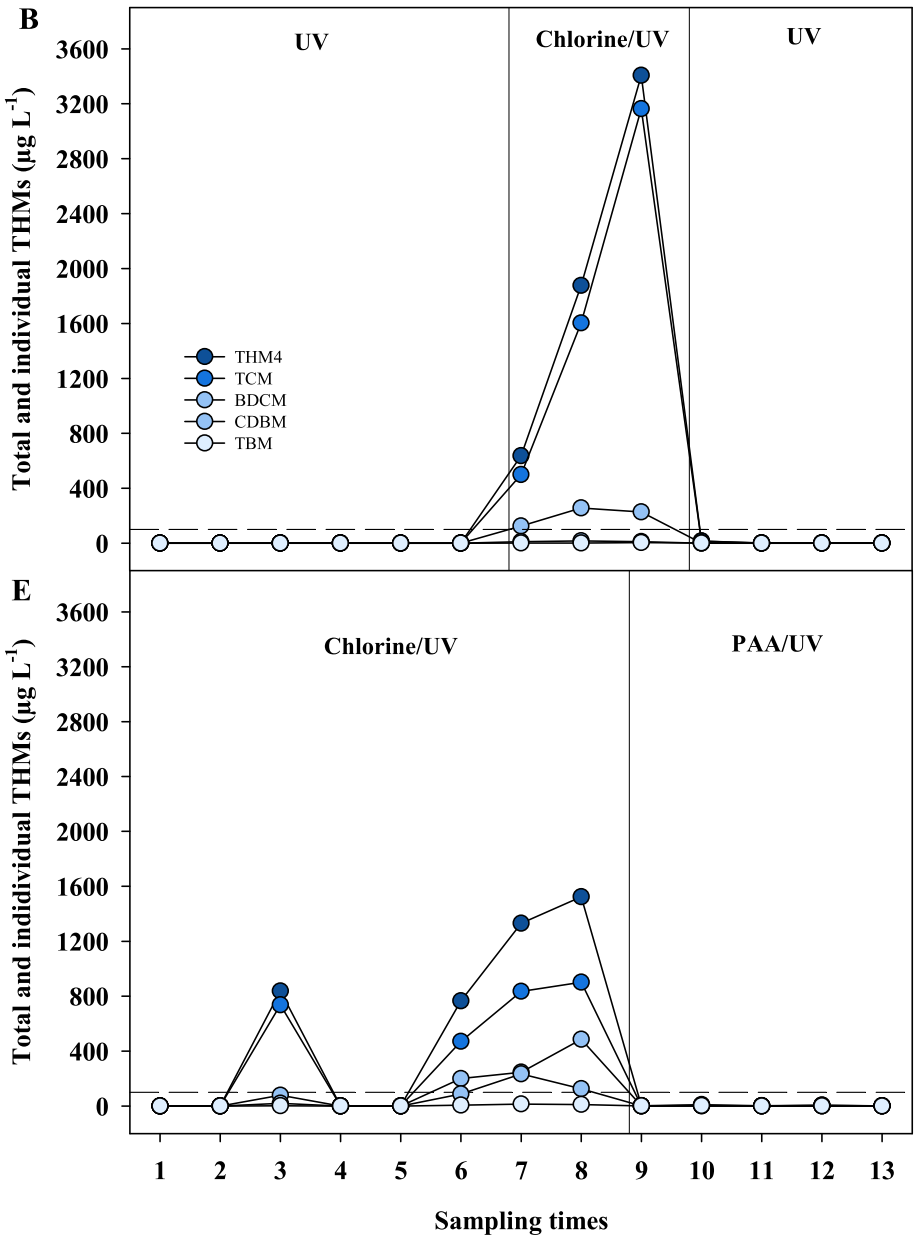

Fig. 4. Total and individual THMs over 13 samplings in WWTP-B and WWTP-E in which vertical lines separate the disinfection technologies. Dashed line shows the maximum residue level (MRL) of 100 µg L^{-1}, established by the EU for drinking water.

not use any disinfection system. Remarkably, at the end of the study, the tertiary treatment of all the WWTPs changed to PAA or UV or their combination (PAA/UV), achieving a significant reduction of DBPs.

4. Discussion

The selection of wastewater disinfection technologies is extremely important in reducing microbiological hazards without generating chemical risks due to the presence of DBPs. Chlorine disinfection has been widely employed in municipal wastewater plants worldwide (Li et al., 2017a, 2017b, 2017b). As wastewater effluents are much more complex matrices than drinking water, DBPs in chlorinated wastewater effluents are usually present in high amounts (Du et al., 2017). Effluent organic matter (EfOM) has more hydrophilic compounds than natural organic matter (NOM), which favors the incorporation of halogen groups and the DBP formation (Coble, 1996; Filloux et al., 2012; Leenheer et al., 2000; Vakondios et al., 2014; Zhong et al., 2019). Bulman and Remucal (2020) reported for chlorine-treated drinking water averages of 12.5 µg L^{-1} and 12.6 µg L^{-1} for total THMs and HAAs, respectively, while in chlorinated wastewater effluents, we observed an average of 329 µg L^{-1} and 418 µg L^{-1} for total THMs and HAAs, respectively. Li et al. (2019) showed lower values for THMs (28 µg L^{-1}) and HAAs (47 µg L^{-1}) in chlorinated WWTP effluents. When we compared chlorine/UV treatment with chlorine, we observed that the formation of THMs and HAAs was higher. However, as the ranges widely varied between samples, no significant differences were observed between both treatments. These results agree with those reported by Hua et al. (2021), who observed a significant increase of 11% in the formation of both THMs and HAAs in chlorine/UV to that obtained by chlorination. On the contrary, Bulman and Remucal (2020) observed that THM formation decreased during chlorine photolysis compared to dark chlorination. Our results evidenced that when considering the application of chlorine/UV for wastewater treatment, the increase in the formation of THMs and HAAs was a major concern. Combination of chlorine/UV has a synergetic effect of oxidizing organic contaminants as it generates reactive oxidants such as chlorine radical ($Cl^{\bullet}$), dichloride

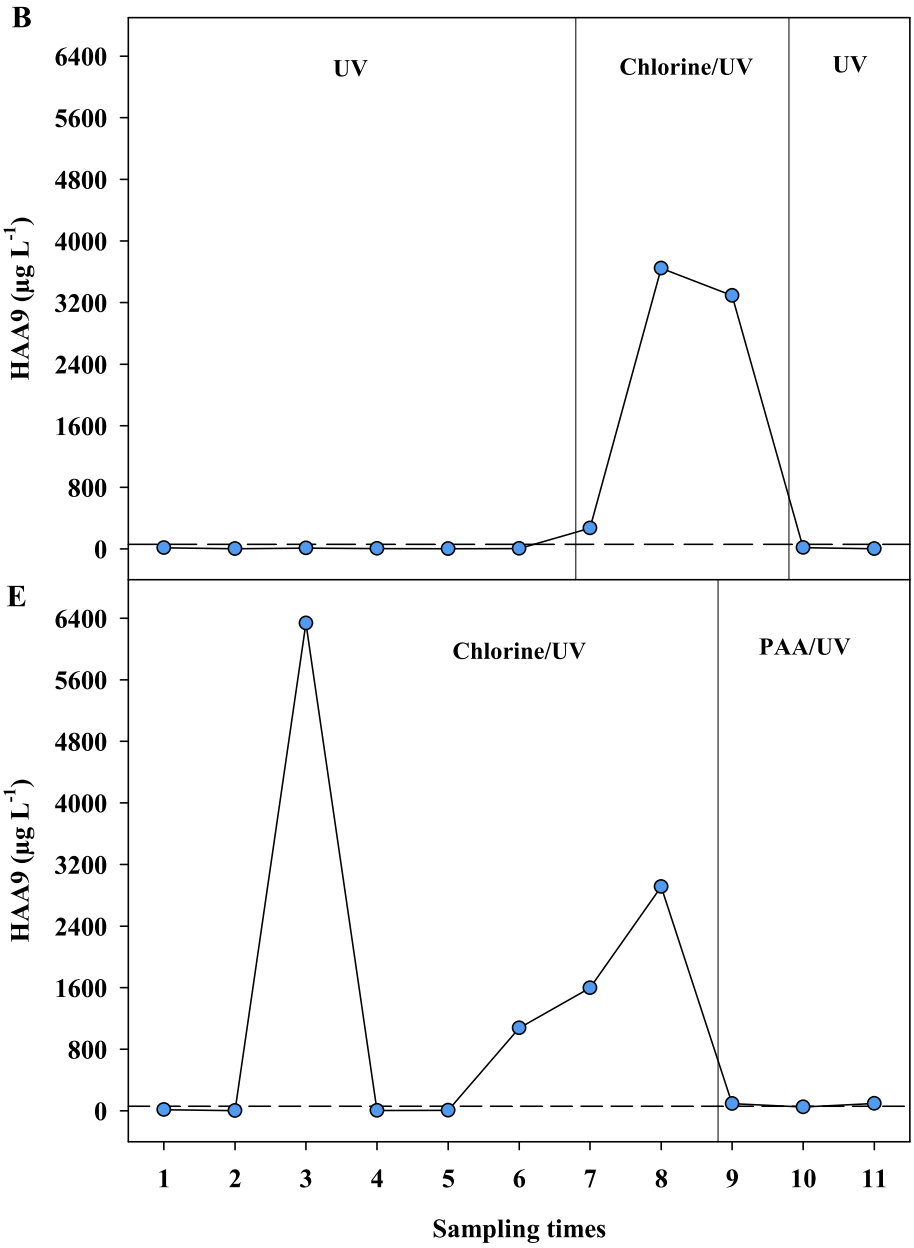

Fig. 5. Content of HAA9 over 11 samplings in WWTP-B and WWTP-E in which vertical lines separate the disinfection technologies. Dashed line shows the Maximum Residue Level (MRL) of 60 μg L^{-1}, established by the United States Environmental Protection Agency (USEPA) and the EU for drinking water.

radical anion ($Cl_2^{\bullet-}$), and hypochlorous acid as well as hypochlorite ion plus a high proportion of hydroxyl radicals ($^{\bullet}HO$) (Fang et al., 2014; Bulman and Remucal, 2020). Several studies have reported the significant role of these reactive chlorine species and the greatest activity of $^{\bullet}OH$ enhancing the degradation of micropollutants but increasing the formation of chlorinated DBPs (Wang et al., 2016; Guo et al., 2017; Hua et al., 2019, 2021).

Several studies have described that THMs could serve as a surrogate for overall DBP exposure (Furst et al., 2019). In the present study, THMs tracked well with HAAs and chlorate concentrations in most sampling points. For HAAs, we quantified the formation of HAA9 in the tertiary effluents treated with chlorine and chlorine/UV even though regulations for drinking water only consider HAA5. HAA9 were generated under all treatment conditions, including those carried out by the direct photolysis with or without PAA but only punctually probably due to the elevated use of domestic chlorinated disinfectants as the most dominant DBP group (Li et al., 2021). Domínguez-Henao et al. (2018) also reported that with high concentrations of PAA, halide ions can be oxidized to hypohalous acids and they can react with organic matter forming halogenated DBPs, particularly brominated DBPs in solutions enriched with bromide (Booth and Lester, 1995; Dell'Erba et al., 2007; Luukkonen and Pehkonen, 2017; Shah et al., 2015). On the other hand, the reactive chlorine species can be partially responsible for the increase in the formation of HAAs among other species (Bulman and Remucal, 2020). Our results showed that the combination of chlorine and UV light promoted the formation of HAAs in a quantity that was always higher than that of THMs. It has been reported that THMs increased when pH increased while HAA was favored under acidic conditions (pH < 6) (Hung et al., 2017).

Few studies have examined the formation of chlorate as a byproduct in chlorinated treated wastewater. Most of these studies deal with chlorine dioxide/UV and lower organic chlorinated DBPs in wastewater and drinking water disinfection (Zhong et al., 2019; Wang et al., 2021; Zhao et al., 2021). These studies revealed that chlorate was formed via

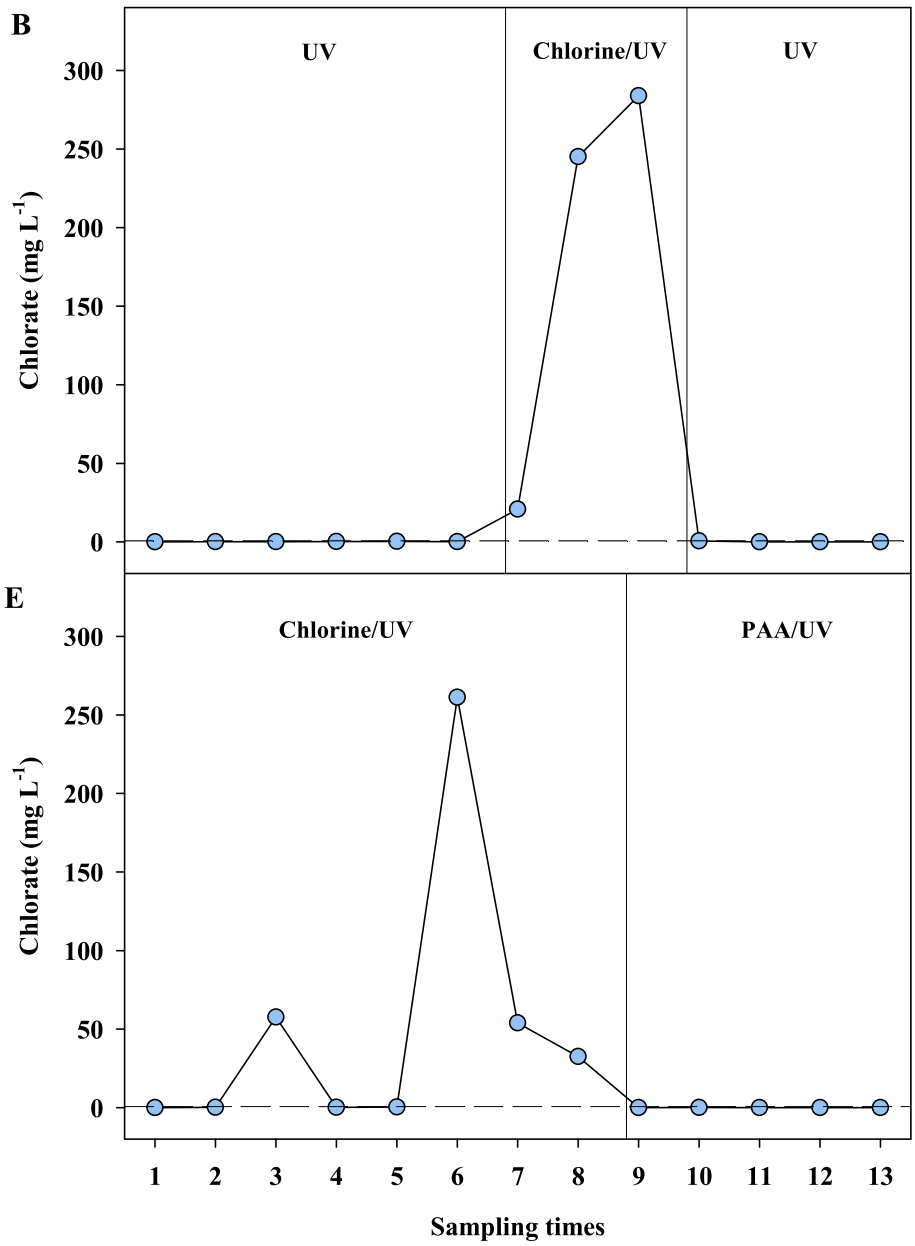

Fig. 6. Content of chlorate over 13 samplings in WWTP-B and WWTP-E in which vertical lines separate the disinfection technologies. Dashed line shows the Maximum Residue Level (MRL) of 0.7 mg L^{-1} established by the EU for drinking water.

the photolysis of ClO_2 and that the radical $^{\bullet}HO$ contributed to its formation (Wang et al., 2021). Our results agree with those reported that the UV photolysis of chlorine increased chlorate formation. However, contrary to our observations, in hospital wastewater, Luo et al. (2020) detected that Cl_2/UV disinfection process could effectively reduce the dosage of chlorine and decreased DBPs. The extent to which chlorine disinfection results in the formation of DBPs mainly depends on the content and type of organic matter present, the quantity of chlorine used, pH, temperature, and reaction time (Chowdhury et al., 2009). To reduce chlorate formation, chlorine concentration can be reduced from 10 mg L^{-1} to 5 mg L^{-1} as long as the microbial safety is assured (Wang et al., 2019).

Other alternative disinfectants such as individual or combined PAA and UV radiation are increasing in use. Still, much less is known if they can lead or not to the formation of DBPs or distinctly different byproducts, as a consequence of the different reactive intermediates formed (Varanasi et al., 2018; Alexandrou et al., 2018; Manoli et al., 2019). Domínguez-Henao et al. (2018) concluded that most DBPs formed in with PAA are carboxylic acids with limited or non-existent formation of halogenated compounds, aldehydes, epoxides and N-nitrosamines. The results obtained in our study show that PAA can be considered an excellent alternative as it allows fulfilling the absence of potentially harmful DBPs. Even though PAA is the more consistent alternative to chlorine, future research should deepen on different relevant aspects for PAA use, such as toxicity tests. Several studies indicate that PAA produces almost no toxic DBPs or mutagenic products derived from the reaction with organic matter present in wastewater (Sánchez et al., 2020). Our results showed that the combined treatment chlorine/UV significantly promoted the formation of DBPs and the same WWTPs with PAA alone or combined with UV reduced DBPs by 95–100%.

Darnell et al. (2004) examined several inactivation methodologies for SARS-CoV, including treatments based on UV light, gamma irradiation, heat application, formaldehyde and glutaraldehyde treatment, plus

pH adjustment and a lysis step through a phenol and guanidine isothiocyanate. Among all, two methods were selected and tested as the ones with the most negligible interferences in the analysis of DBPs, considering the chemical properties of the compounds analyzed (THMs, HAAs and chlorate). However, both inactivation methodologies based on a lysis step and PFA addition degraded THM4 and HAA9. Results for THMs were as expected due to the volatile characteristics of these DBPs, which showed a labile nature during centrifugation and filtration processes, being the loss of these compounds higher when the microbial inactivation agents were added. In the case of HAA9, the addition of lysis buffer and the PFA along with the hydrophilic and strong acidic character could have influenced slight losses during the inactivation process or the manipulation of the samples during centrifugation and filtration at low HAA concentrations. The only DBP analyzed in the raw wastewater samples was chlorate, as samples were inactivated before the DBP analysis without significant losses (USEPA, 2015).

Due probably to the excessive use of chlorinated derivatives by the population during the sampling period of the COVID-19 pandemic, chlorate levels were extremely high with considerable variability in some of the samples (WWTP-I). Chlorate formation remains a concern if treated wastewater is used for agricultural irrigation. The excessive use of chlorinated disinfectants in pandemic times to reduce the contamination rate must be considered because of the considerable risk of increasing with a chlorinated disinfection technology.

Regulation of DBPs has focused on drinking water, with defined guidelines placed on the most commonly occurring compounds (four THMs, five HAAs, chlorite, and bromite) (WHO, 2017). Currently, there are no regulatory limits worldwide for monitoring DBPs in reclaimed water. Disinfection byproducts are considered as part of the additional requirements that could be identified as potential hazards after a risk assessment (EC, 2020a). Some DBPs can be of concern if the treated wastewater is used for agricultural irrigation (Alcalde-Sanz and Gawlik, 2017). The irrigation with reclaimed chlorinated water can accumulate chlorate in fresh lettuce (Garrido et al., 2020). The knowledge gap about the presence and concentrations of byproducts in wastewater is a concern. The impact associated with chlorine and chlorine/UV and the potential formation of DBPs have been clearly shown in the present study. Alternative technologies for the same WWTPs are described for the first time with limited formation for DBPs to levels even lower than those regulated for drinking water. While the wastewater used in the present study had an inherently high DBP formation potential due to the high organic matter content, the results indicated that the regulated DBPs could be diminished when using the individual application of PAA or UV or their combination.

5. Conclusions

Most of the previous work on disinfection technologies for wastewater has been performed at a small scale. In our study, full-scale operations in 15 WWTPs with individual or simultaneous application of chlorine, PAA and UV were examined. When each WWTP changed from chlorinated disinfection treatments to PAA or UV alone or combined, the presence of DBPs in the effluents was reduced to negligible values. Our results indicate the risk posed by chlorine and chlorine/UV as tertiary treatments of WWTPs, increasing the presence of organic regulated DBPs (THMs and HAAs) and chlorate as an inorganic byproduct in the effluents. When evaluating the individual treatments for each DBP, levels fluctuated as a function of the raw wastewater quality but confirmed the strong impact when chlorine was used. High levels of DBPs, including THMs, HAAs and chlorate, were formed with chlorine and even higher when combined with UV. This study provides data on the benefits of PAA as an alternative to chlorine for its implementation in WWTPs to meet future regulatory compliance requirements. Of particular importance from a regulatory viewpoint is that PAA and UV alone or combined could be the best option to provide minimum values for the DBPs evaluated, as long it ensures the required microbiological quality. The analysis of THMs and HAAs cannot be performed in the raw wastewater due to safety concerns as the inactivation method of phenol and guanidine isothiocyanate described for SARS-CoV-2 reduced THMs and HAAs, unlike chlorate that was able to be quantified in the raw wastewater and effluent samples. The high levels of chlorate detected in the raw wastewater emphasize even more the relevance of selecting the disinfection technology base on reducing as much as possible DBPs formation.

Credit author statement

Sofía Albolafio: Methodology, Formal analysis, Writing-Original draft Alicia Marín: Methodology, Formal analysis Ana Allende: Project administration, Conceptualization Francisca García: Resources Pedro J. Simón-Andreu: Resources Manuel Abellán Soler: Resources María I. Gil: Supervision, Writing- Reviewing and Editing.

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.132583.

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