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Ecotoxicity and rapid degradation of quaternary ammonium compounds (QACs) subjected to combined vacuum UV and UV-C treatment

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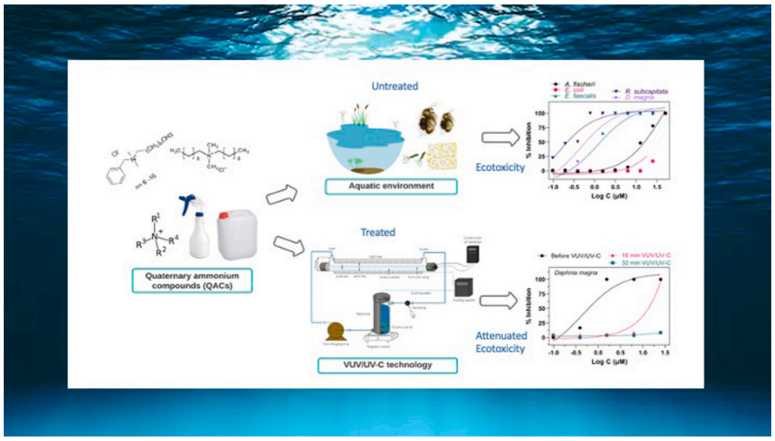
HIGHLIGHTS

- QACs exhibit high toxicity to aquatic organisms and a potential for trophic transfer.
- VUV/UVC irradiation rapidly degrades QACs within minutes.
- VUV/UVC irradiation can mitigate the ecotoxicity of QACs.
- VUV/UVC oxidation is an effective technology without requiring catalysts.

GRAPHICAL ABSTRACT



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ABSTRACT

Quaternary ammonium compounds (QACs) are active ingredients in a palette of commercially available disinfectants, sanitizers, and biocides. QACs are widely used because of their broad-spectrum antimicrobial properties but the ubiquitous uses have resulted in frequent detection in aquatic and terrestrial matrices including domestic wastewater, surface waters, urban soils and sediments. An increased domestic QACs consumption has increased the environmental occurrence, and investigation of mitigation methods and effects on non-target organisms are in demand. In this study, we examined the potential ecotoxicity of six QACs and investigated the effect of combined vacuum UV (185 nm) and UV-C (254 nm) irradiation (VUV/UVC) on degradation and mitigation of ecotoxicity of QACs. The study showed that combined VUV/UVC irradiation facilitated rapid degradation of benzalkonium chloride, benzethonium chloride, didecyldimethylammonium chloride, dodecyltrimethylammonium chloride, and hexadecyltrimethylammonium chloride. The estimated half-lives varied between 2 and 7 min, and degradation was affected by the initial QAC concentrations, the UV fluence, and the water matrix. The potential ecotoxicity of QACs and VUV/UVC treated QACs was examined using a battery of

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test organisms that included the luminescent bacterium *Aliivibrio fischeri*, the gram-negative and gram-positive bacteria *Escherichia coli* and *Enterococcus faecalis*, the freshwater microalga *Raphidocelis subcapitata*, and the crustacean *Daphia magna.* The potential for trophic transfer of QACs was investigated in a simplified aquatic food web. Test organisms from different trophic levels were included to assess adverse effects of bioactive compounds in VUV/UVC treated samples including transformation products. The study showed that several QACs were highly toxic to aquatic test organisms with EC50 and/or EC20 values < 1 μM. VUV/UVC treatment of QACs resulted in substantial photolysis of the parent compounds and comprehensive mitigation of the ecotoxicity potential. VUV/UVC represent an attractive oxidation technology for abatement QACs in contaminated water because the process does not require addition of catalysts or precursors.

1. Introduction

Quaternary ammonium compounds (QACs) are man-made cationic chemicals with antimicrobial properties. QACs are the foundation of many commercially available biocidal formulations, and they can be found in a wide variety of products such as personal hygiene and cosmetic products, ophthalmic solutions and similar medical-use formulations, fabric softeners, wood preservatives, and a range of general cleaning and disinfection products (Arnold et al., 2023; Gerba, 2015; Juergensen et al., 2000). QACs contain at least one hydrophobic hydrocarbon chain linked to a positively charged nitrogen atom, and other alkyl groups which are mostly short-chain substituents such as methyl or benzyl groups and as such are strong cationic surfactants. QACs can be clustered into 4 main groups: the group 1 alkyl or hydroxyalkyl (straight chain) substituted QACs; the group 2 non-halogenated benzyl substituted QACs; the group 3di- and tri-chlorobenzyl substituted QACs, and the group 4 QACs with unusual substituents (Kaj et al., 2014; Zhang et al., 2015).

QACs have been demonstrated to be efficient in inactivation of numerous pathogens including protozoa, fungi, bacteria and vira such as the SARS-CoV-2 virus (Hora et al., 2020). As a result, the use of QACs has increased concurrently with the emergence of the COVID-19 pandemic (Arnold et al., 2023; Hora et al., 2020). However, the availability of QACs on the global market had already increased prior to the COVID-19 pandemic due to the ban in many countries of triclosan use in over-the-counter antiseptic products (McNamara and Levy, 2016). Furthermore, QACs are used in many biocide products for outdoor use including formulations for cleaning and disinfection of facades, tiles, patios, greenhouses, and roof material. The antimicrobial properties of QACs towards fungi has also resulted in use in wood preservatives (Tatarazako et al., 2002; Xiao and Kreber, 1999). The mode of action of QACs in most microorganisms starts with the adsorption to the negatively charged surfaces of membrane lipid bilayers of microbial cells which impairs membrane properties and function eventually resulting in the exosmosis of cellular contents and cell death (Wessels and Ingmer, 2013).

As a result of their ubiquitous societal use, QACs are frequently detected in aquatic and terrestrial matrices such as wastewater, surface waters and sediments, and urban soils. Because of the chemical structure, QACs easily adsorb to negatively charged particles e.g., sludge, soil, and sediments (van Wijk et al., 2009). Although substantial amounts (>90%) are often successfully removed during conventional wastewater treatment due to biodegradation and sorption, significant concentrations are often detected in the treated effluents and aquatic environment (Hora et al., 2020; Mohapatra et al., 2023; Tezel 2009). Benzalkonium chloride (BAC) is arguably the most common QAC, and its concentrations in wastewater of hospitals and laundries in some European countries can reach the mg/L levels (Martínez-Carballo et al., 2007). In addition, QACs such as BAC and didecyldimethylammounium chloride (DDAC) are approved in some countries for use in cleaning and biocide products for out-door use and may therefore enter aquatic and terrestrial ecosystems directly due to leaching and wash-off. Even though QACs are believed to be less environmentally harmful than their alternatives, their increasing load into the environment is concerning due to their relative persistence and toxicity towards non-target organisms. QACs are potentially toxic to many environmental bacteria (e.g., gram-positive bacteria), fungi, protozoans, algae, small invertebrates such as daphnids, and fish (Zhang et al., 2015). In addition, there is a growing concern that QACs can lead to development of antimicrobial resistance (Hora et al., 2020; Mohapatra et al., 2023). Hence, there is an increasing interest in methods that can be used for mitigation of QACs in waste streams to attenuate environmental impacts on non-target organisms.

UV-based advanced oxidation processes (AOPs) are becoming increasingly popular in water treatment due to the efficient degradation of many pollutants. The underlying principle of most UV-based AOPs is the in-situ generation of highly reactive species such as hydroxyl radicals (•OH), that react with and oxidize organic or inorganic target pollutants. Hydroxyl radicals are among the strongest known oxidizing agents known in chemistry and Vacuum UV (VUV) irradiation of aqueous solutions will generate different radicals including •OH without the need for additions of catalysts or radical precursors (Zoschke et al., 2014; Zhang et al., 2022). The VUV process leading to •OH includes several key steps including activation of H_2O to generate primary •OH via homolysis or photochemical ionization, and a secondary process that involve activation of H_2O_2 formed by recombination of primary •OH to produce secondary •OH via photolysis of H_2O_2 (Zhang et al., 2022). Many commercial VUV low-pressure mercury lamps emit radiation at both 185 nm (VUV) and at 254 nm (UVC). A combined VUV-UVC treatment has the potential for both direct and indirect photolysis as the UVC light penetrates further into aqueous solutions than VUV who is often limited to <10 mm closest to the UV lamp (Zhang et al., 2022). Hence, UVC could contribute with direct photolysis of pollutants in combined VUV-UVC systems if the target molecule can absorb UV light that promotes excitation and electron transfer whereas VUV will contribute with direct photolysis and indirect photolysis caried out by photochemically produced reactive intermediates. However, the reaction kinetics and degradation efficiency will depend on different factors such as contaminant concentration and chemical structure, water matrix, and the intensity of the UV exposure (Duca et al., 2017; Zoschke et al., 2014; Zhang et al., 2022). In addition, very few studies have included toxicological analyses of UV treated aqueous QACs. This is relevant because degradation of the parent QAC molecules does not necessarily imply total mineralization of the parent compound and complete attention of toxicity.

In the present study, we examined the potential ecotoxicity of 6 QACs belonging to the alkyl or hydroxyalkyl (straight chain) substituted QACs and non-halogenated benzyl substituted QACs. The degradation kinetics of aqueous QACs were examined during combined VUV/UVC irradiation with different UV doses and in different water types. Finally, we assessed the potential ecotoxicity of QACs after VUV/UVC irradiation, and test organisms from different trophic levels were included to better assess possible effects of bioactive transformation products remaining in the samples after the VUV/UVC treatment. We are not aware of comparable studies that addresses the potential of VUV/UVC treatment and degradation of QACs coupled with a multi-species toxicity assessment. Such studies are relevant because the mere removal of parent QAC molecules does not necessarily imply complete attenuation

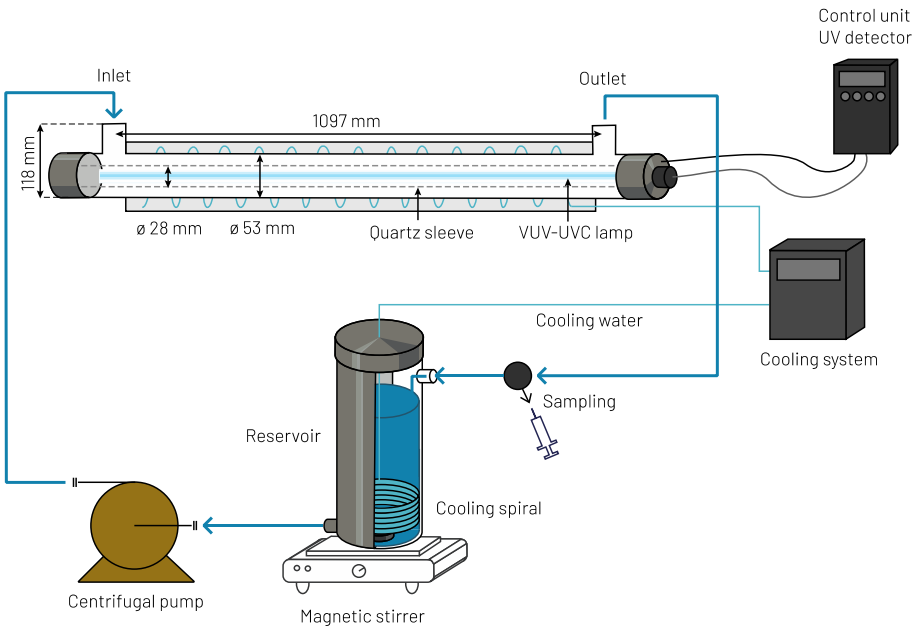

Fig. 1. VUV/UVC setup used for treatment of water with different QACs.

of toxicity.

2. Materials and methods

2.1. Chemicals

Alkyldimethylbenzylammonium chloride (Benzalkonium chloride, BAC) (CAS 63449-41-2, purity >99%) was obtained from Sigma-Aldrich (Denmark). Didecyldimethylammounium chloride (DDAC) (CAS 7173-51-5, purity ≥95.0%) was obtained from Glentham Life Sciences Ltd (UK). Benzethonium chloride (BZC) (CAS 121-54-0, purity >97.0%), Dodecyltrimethylammonium chloride (DTAC) (CAS 112-00-5, purity >98.0%), Hexadecyltrimethylammonium chloride (HTAC) (CAS 112-02-7, purity >95.0%) and Diallyldimethylammonium chloride (DADMAC) (CAS 7398-69-8, 60% in water) were purchased from TCI Europe (Belgium).

2.1. VUV/UVC irradiation of QACs in water

The effect of vacuum UV irradiation on QACs in different water matrices, with emphasis on drinking water, was investigated in a continuous-flow VUV photoreactor (ULTRAAQUA A/S, Aalborg, Denmark). The VUV photoreactor consisted of a tubular stainless-steel reactor with an inner diameter of 53 mm, a length of 1270 mm and a reactor volume of 1.7 L (Fig. 1). The photoreactor was connected to a 2.3 L stainless steel reservoir and a diaphragm pump operated at 2 L/min with recirculation (Siebec, pompe M7). The reservoir was equipped with a magnetic stir bar to facilitate mixing, and a stainless-steel cooling spiral operated at 10 °C to prevent heating. The UV photoreactor was equipped with a low-pressure high output amalgam VUV Hg lamp with a 1050 mm length and a 19 mm diameter (UltraTherm 200 W LPHO TOC UV, Ultraaqua A/S, Denmark). The UV lamp simultaneously emitted VUV (185 nm) and UVC (254 nm) at a radiation flux of 14W and 56W, respectively (1:4 ratio). The VUV/UVC lamp was located inside a high purity 28 mm diameter synthetic quartz tube transparent to both UV wavelengths. The theoretical thickness of the water film around the quartz sheath was 12.5 mm. The reactor was equipped with an UVC sensor and the maximum irradiance in drinking water was 340 W/m^2 for UVC corresponding to about 85 W/m^2 of VUV.

VUV irradiation experiments were conducted by loading the photoreactor and reservoir with a total of 4 L of QAC solution prepared in drinking water with different nominal concentrations (10 μM, 100 μM, and 1000 μM). The water was recirculated for 32 min and 50 mL samples were collected at the outlet of the photoreactor before turning on the VUV lamp (0 min) and at 1, 2, 4, 8, 16, and 32 min after turning on the VUV lamp corresponding to combined VUV/UVC doses (fluence) of 0, 0.25, 0.75, 2, 4, 8, and 16 J/cm^2.

2.3. Degradation of QACs

A fast photometric method was used for routine quantification of quaternary ammonium cations in degradation experiments and measurements were subsequently confirmed by HPLC with UV detection (Summit - Dionex Corporation). The HPLC was equipped with a Luna 5μ C18 100 Å column (250 × 4.60 mm) and acetonitrile/water (50:50 v:v) was used as mobile phase. The photometric method was based on formation of mixed dye-surfactant aggregates between quaternary ammonium cations and 2″,4″,5″,7″- tetrabromofluorescein (Eosin Y) in acetate buffer at pH 4.0 (ion-pair formation). The Eosin-QAC product was stable at pH 4.0 for >120 min. The procedure is a modified version of methods described by Kovács-Hadady and Fábián (1998) and Ma et al. (2014). Quaternary ammonium cations were quantified as changes in absorbance at 556 nm (A_{556}). Absorbance was measured in an Epoch Microplate Reader (BioTek) using 96-well clear Nunclon microplates (Thermo Scientific). The results from.

2.4. Toxicity test with the luminescent bacterium Aliivibrio fischeri

Toxicity screening of QAC samples was examined in a standard inhibition test with the luminescent bacterium *Aliivibrio fischeri* (ISO 11348-1, 2007). *A. fischeri* DSM 7151 was incubated in white 96-well plates (CulturPlate, PerkinElmer) together with serial 2-fold dilutions of QAC resulting in 10 different nominal concentrations and 8 replicates. Changes in bioluminescence were quantified after 30 min using a Victor X2 Multilabel Plate Reader (PerkinElmer).

2.5. Toxicity test with the gram-positive bacterium Enterococcus faecalis

The toxicity of QAC samples to gram-positive enterococci was examined in a newly developed inhibition test with *Enterococcus faecalis*

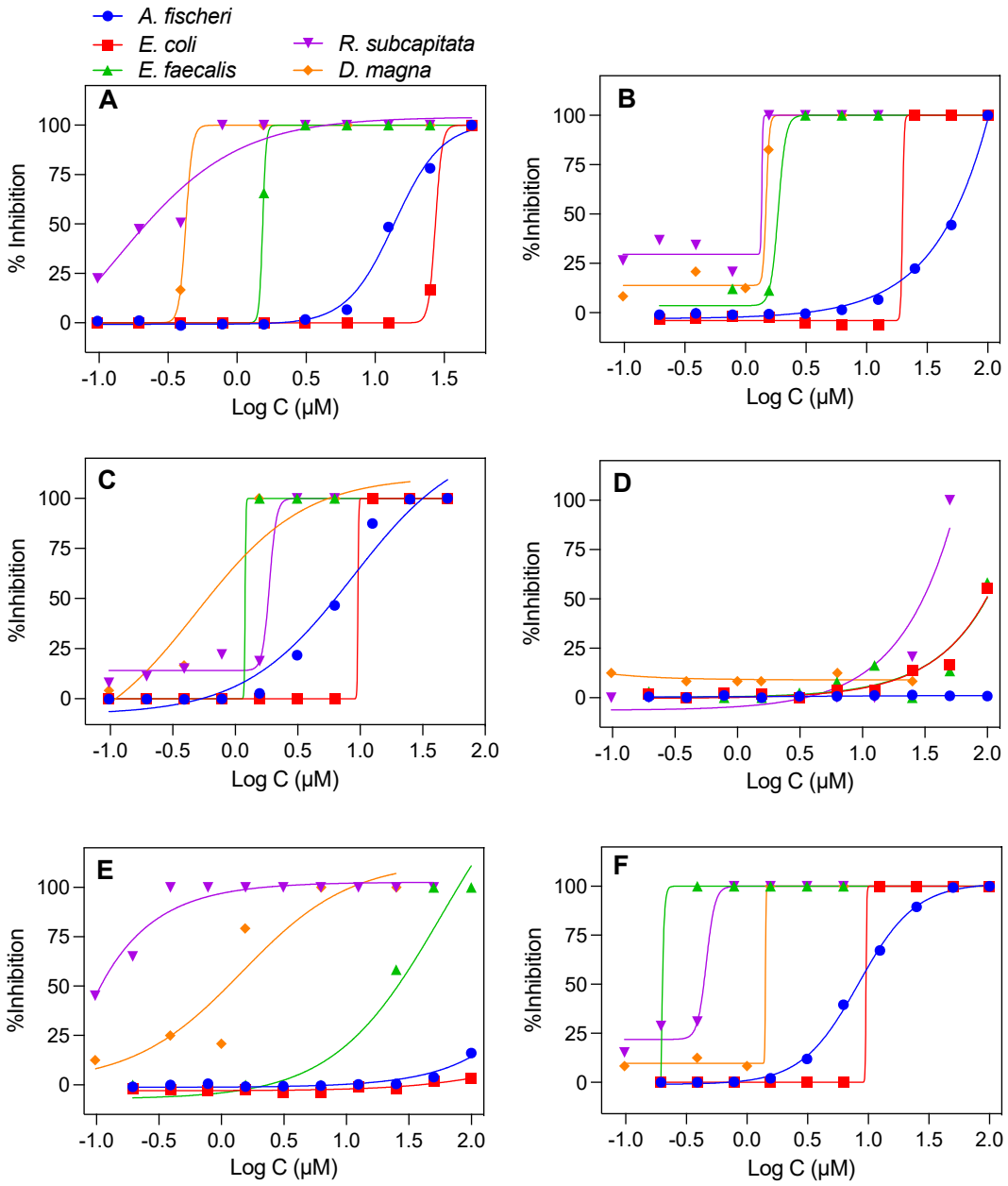

Fig. 2. Toxicity of 6 different QACs to a battery of test organisms that included *A. fischeri*, *E. coli*, *E. faecalis*, *R. subcapitata* and *D. magna*. *A: BAC; B: BZC; C: DDAC; D: DADMAC; E: DTAC; F: HTAC.*

401 (EF-TOX). The endpoint was inhibition of bacterial growth after 22 h of exposure to the test substance at 36 °C. Prior to the experiment, *E. faecalis* was cultivated at 36 °C in intestinal enterococci test medium (IE medium) with the following composition: Peptone 5 g/L; Tryptose 5 g/L, NaCl 5 g/L; K_2HPO_4 3.4 g/L; KH_2PO_4 1.6 g/L; Sodium azide 0.2 g/L; 4-methylumbelliferyl-β-D-glucoside (MUF). MUF is a fluorescent substrate analogue that is hydrolyzed by the enzyme β-D-glucosidase in *E. faecalis*. Serial two-fold dilutions of QACs were made in 96-well clear Nunclon microplates (Thermo Scientific) using 100 µL QAC solution serially diluted in 100 µL IE medium. The serial dilutions of QACs were followed by addition of 100 µL of diluted *E. faecalis* culture in IE medium (1:100000 dilution). The final liquid volume was 200 µL in each well and 10 different concentrations of QACs were analyzed simultaneously. Four replicates were included for blanks (medium only), controls (no QACs), and each QAC concentration. The EF-TOX microplates were incubated for 22 h ± 1 °C at 36 °C on a shaker at 180 rpm and then the absorbance at 620 nm was measured for each well using the Epoch Microplate Spectrophotometer (BioTek). Changes in fluorescence for each well were quantified using a Victor X2 Multilabel Plate Reader (PerkinElmer). The toxicity of QACs to *E. faecalis* was examined before and after exposure of aqueous QACs solutions to VUV/UVC irradiation.

2.6. Toxicity test with the gram-negative bacterium Escherichia coli

The toxicity of QAC samples to gram-negative Enterobacteriaceae was examined in a toxicity test with *Escherichia coli* ATCC 25922 (EC-TOX). The endpoint was inhibition of bacterial growth after 18 h of exposure to the test substance at 36 °C. Prior to the experiment, *E. coli* was cultivated at 36 °C in *Escherichia coli* test medium (EC medium) with the following composition: Tryptose 5 g/L, NaCl 5 g/L; Sorbitol 1.0 g/L; K_2HPO_4 2.7 g/L; KH_2PO_4 2.0 g/L; 4-methylumbelliferyl-ß-D-glucuronide (MUG). MUG is a fluorescent substrate analogue that is hydrolyzed by the enzyme β-D-glucuronidase in *E. coli*. Serial two-fold dilutions of QACs were made in 96-well clear Nunclon microplates (Thermo

Scientific) using 100 μL QAC solution serially diluted in 100 μL EC medium. The serial dilutions of QACs were followed by addition of 100 μL of diluted *E. coli* culture in EC medium (1:1,000,000). The final liquid volume was 200 μL in each well and 10 different concentrations of QACs was analyzed simultaneously. Four replicates were included for blanks (medium only), controls (no QACs), and each QAC concentration. The EC-TOX microplates were incubated for 18 h ± 1 °C at 36 °C on a shaker at 180 rpm and then the absorbance at 620 nm was measured for each well using the Epoch Microplate Spectrophotometer (BioTek). Changes in fluorescence for each well were quantified using a Victor X2 Multi-label Plate Reader (PerkinElmer). The toxicity of QACs to *E. coli* was examined before and after exposure of aqueous QACs solutions to VUV/UVC irradiation.

2.7. Toxicity test with the green microalga Raphidocelis subcapitata

The toxicity of QACs to phytoplankton was examined in inhibition tests with the freshwater unicellular green microalgae *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*). The endpoint was inhibition of growth measured after 72 h of incubation according to ISO 8692 (2012). *R. subcapitata* (Micro-BioTests Inc.) was cultivated in algal test medium at 22 ± 2 °C and continuous illumination at 6500 lux (ISO 8692, 2012). Two-fold dilutions of QACs were prepared in 96-well clear Nunclon microplates (Thermo Scientific) by serially diluting 150 μL of the chemical in 150 μL algal test medium. Serial dilutions of QACs were followed by addition of 150 μL diluted *R. subcapitata* culture (1:50) resulting in a final liquid volume of 300 μL in each well. Plates were incubated for 72 h at 22 ± 2 °C on a shaker at 60 rpm with continuous illumination (6500 lux). Growth was measured after 0, 24 h, 48 h and 72 h as absorbance at 450 nm using an Epoch Microplate Spectrophotometer (BioTek). The bioassay with *R. subcapitata* included eight replicates of blanks (medium only), controls (no test chemical), and 10 concentrations of QACs. The toxicity of QACs to *R. subcapitata* was examined before and after exposure of aqueous QACs solutions to VUV/UVC irradiation.

2.8. Toxicity test with the crustacean Daphnia magna

The toxicity of QACs to zooplankton was examined in inhibition tests with the crustacean *D. magna* (ISO 6341, 2012). The toxicological endpoint was inhibition of mobility determined by visual inspection of the animals (ISO 6341, 2012). *D. magna* STRAUS was cultivated from a laboratory clone originating from pure culture ephippia. The bioassay was conducted using 24-well clear microplates where each well contained one animal and 2 mL of diluted contaminant (1 mL of ISO freshwater medium: 1 mL of contaminant solution). The mobility of each animal was determined after 24 h and 48 h (ISO 6341, 2012). The toxicity of QACs to *D. magna* was examined before and after exposure of aqueous QACs solutions to VUV/UVC irradiation.

2.9. Data analysis and statistics

The toxic response measured for all endpoints were expressed as inhibition (I) relative to control samples: $I = 1 - (R_i/R_c)$, where R_i and R_c are responses measured for inhibited and control samples, respectively. Control samples also included water samples with UV exposure but without QACs to assess any toxicity associated with active species generated during the UV irradiation.

Concentration-response curves were fitted to a log-logistic model using iterative non-linear regression:

$$Response = A1 + \frac{A2-A1}{1 + 10^{(Log\ EC50-C)p}} \quad [1]$$

where A1 is the bottom asymptote, A2 is the top asymptote, C is the toxicant concentration (mg/L), EC50 is the median effective concentration (mg/L), and p is a model parameter representing the slope of the curve. Iterative non-linear regressions and calculation of 95% confidence limits for EC50 values were performed using Prism 8.0.1 (Graphpad Software) and Origin 2019b (9.65, OriginLab Software).

Table 1
Median effective concentration (EC50) and EC20 for test organisms exposed to different QACs. "-" indicates that it was not possible to establish an EC value.

QAC	Organism	EC20 (μM)	EC50 (μM)
BAC	*A. fischeri*	7.96	13.76
	E. coli	13.19	27.33
	E. faecalis	0.12	1.53
	R. subcapitata	0.42	0.46
	D. magna	0.32	0.43
BZC	*A. fischeri*	19.06	30.40
	E. coli	1.25	19.79
	E. faecalis	1.69	1.87
	R. subcapitata	1.22	1.37
	D. magna	0.47	1.49
DDAC	*A. fischeri*	3.40	6.34
	E. coli	0.84	9.60
	E. faecalis	0.51	1.20
	R. subcapitata	1.21	1.90
	D. magna	0.38	0.43
DADMAC	*A. fischeri*	–	–
	E. coli	50.31	54.50
	E. faecalis	50.31	54.50
	R. subcapitata	24.96	26.90
	D. magna	–	–
HTAC	*A. fischeri*	7.80	8.30
	E. coli	9.79	9.60
	E. faecalis	0.14	0.19
	R. subcapitata	0.41	0.46
	D. magna	0.42	1.42
DTAC	*A. fischeri*	88.8	165.0
	E. coli	24.91	38.94
	E. faecalis	23.72	24.77
	R. subcapitata	0.19	0.20
	D. magna	0.46	1.40

3. Results and discussion

3.1. Ecotoxicity of common QACs

The *in vivo* toxicity of 6 common QACs was assessed using a battery of different test organisms that included *A. fischeri*, *E. coli*, *E. faecalis*, *R. subcapitata* and *D. magna* (Fig. 2 and Table 1). The traditional test organism *A. fischeri* was generally the least responsive to different QACs (Fig. 2 and Table 1). The test organisms that exhibited the greatest sensitivity to QACs were *E. faecalis, R. subcapitata* and *D. magna* with EC50 values ranging from 0.2 to 1.9 μM for BAC, DDAC, BZC, DTAC, and HTAC. These findings emphasize the relevance of including species of test organisms from different trophic levels when conduction ecotoxicity assessments of QACs. For example, the multi-species tests identified freshwater algae as some of the mores sensitive test organisms for different QACs. Previous studies have also indicated that algae may be particularly sensitive to QACs (Mohapatra et al., 2023; Zhang et al., 2015). The negatively charged algal cell wall appears to strongly bind QACs with a net positive charge although some variation may occur among algal species (Mohapatra et al., 2023; Zhang et al., 2015). In the present study, DADMAC was generally the least toxic QAC with EC20 and EC50 values > 10 μM and >30 μM, respectively. BAC, DTAC and HTAC were generally the most toxic QACs with EC20 and EC50 values < 0.5 μM for several test organisms (Table 1). This is partly in line with previous observations suggesting that QACs with longer carbon chains often possess a greater potential for ecotoxicity (Mohapatra et al., 2023).

The potential for trophic transfer of BAC was investigated in a simplified aquatic food web consisting of *Daphnia magna* incubated in mesocosms with potential food items such as the microalga *R. subcapitata* or the bacterium *E. coli*. The microorganisms were pre-

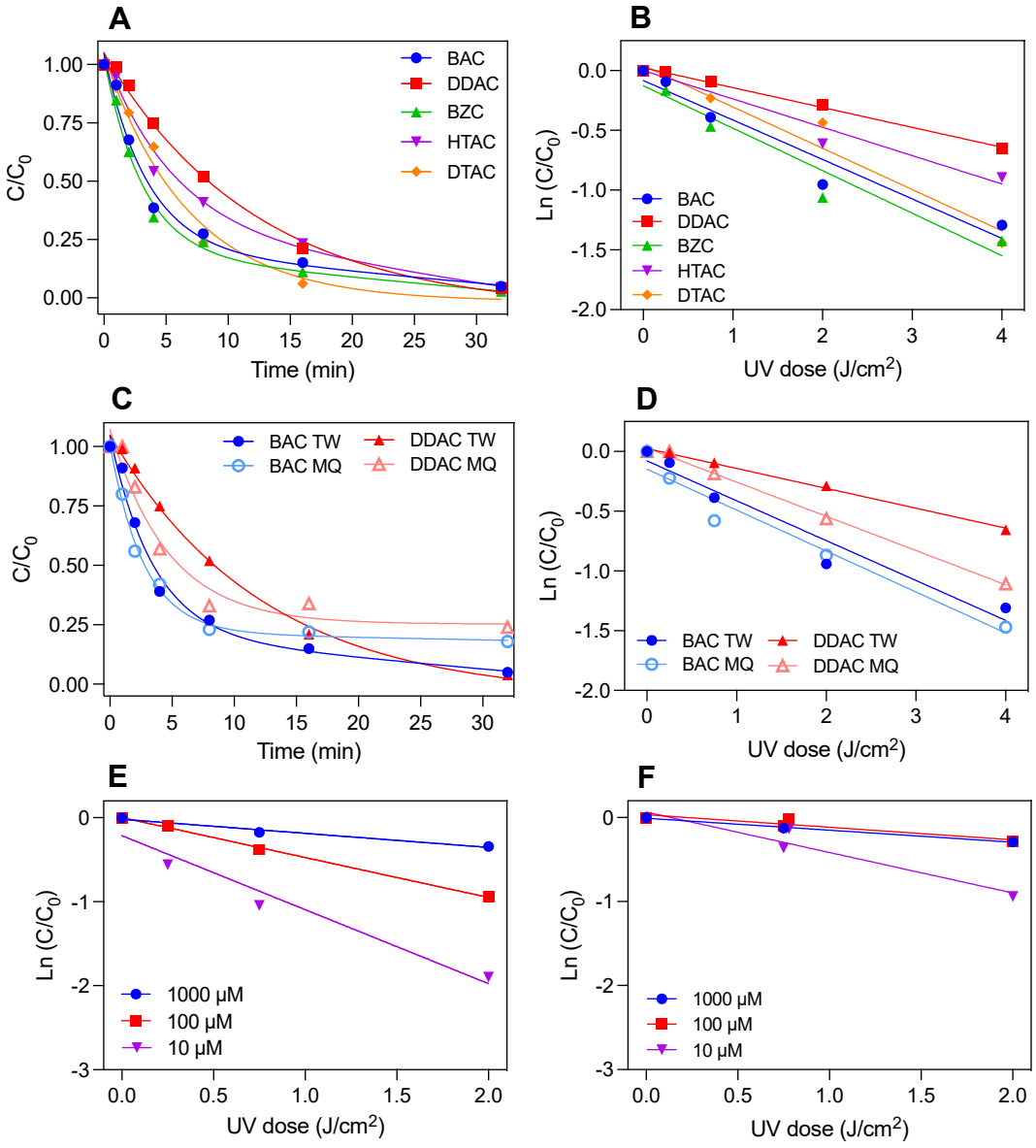

Fig. 3. VUV/UVC mediated degradation of QACs. Degradation of 100 µM solutions of BAC, DDAC, BZC, DTAC and HTAC in tap water (A) and initial pseudo first order degradation (B). Comparison of BAC and DDAC degradation (100 µm) in tap water (W) and in Milli-Q water (mQ) (C and D). Effect of the initial QAC concentration on VUV/UVC mediated degradation of BAC in tap water (E) and DDAC in tap water (F).

exposed for 24 h to different BAC concentrations (0–25 µM) and washed twice in artificial freshwater (ISO 6341, 2012) before being fed to *D. magna*. The results indicated that detectable concentrations of BAC were transferred from live and dead microorganisms to *D. magna* and that the viability of *D. magna* was affected significantly after 24 h for BAC levels <0.5 µM compared to controls without BAC (Kruskal-Wallis, $p < 0.05$).

Environmental concentrations of QACs have been reported at levels between 1 and 60 µg/L in surface waters and treated wastewater, and up to the mg/L level in non-treated wastewater and hospital wastewater (Arnold et al., 2023; Hora et al., 2020; Zhang et al., 2015). For example, up to 170 µg/L of BAC and DDMAC has been reported for municipal wastewater (Zhang et al., 2015), and up to >200 µg/L DDAC and >2000 µg/L of BAC in hospital wastewater (Kreuzinger et al., 2007). These µg/L to mg/L values for environmental QACs are clearly in the range of the reported EC20 values for acute toxicity observed in the current study (Table 1). *R. subcapitata* and *D. magna* were the organisms most sensitive to BAC and DDAC, and the EC20 values for these organisms corresponded to QAC concentrations of 90–119 µg/L and 138–438 µg/L, respectively. Furthermore, even lower effect concentrations for non-target organisms have been reported in some studies with EC values < 20 µg/L (Arnold et al., 2023). In addition, QAC concentrations in surface water sediments have been reported at levels >50 mg/kg and concentrations in municipal biosolids can reach levels >500 mg/kg (Hora et al., 2020). Hence, it is very likely that environmental QACs have adverse effects on non-target organisms in some ecosystems (e.g., in urban environments). Such adverse effects may be potentiated by synergistic interactions at low concentrations with other environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs), anionic surfactants and heavy metals (Mohapatra et al., 2023; Zhang et al., 2015).

3.2. Degradation of QACs by VUV/UVC irradiation

The potential for degradation of selected QACs in water by combined VUV/UVC irradiation was examined for different compounds, water

Table 2
Pseudo first order reaction rate coefficients ($k = \text{min}^{-1}$) for VUV/UVC mediated degradation of BAC and DDAC at different initial concentrations and water matrices. "-": not determined.

Initial QAC Concentration	Pseudo First Order Reaction Rate Coefficients (min^{-1})		
	MILLI-Q water	Tap water	1:1 mixture of Milli-Q and Tap water
10 μM BAC	1.10	0.46	–
100 μM BAC	0.29	0.25	0.25
1000 μM BAC	0.06	0.09	–
10 μM DDAC	1.24	0.22	–
100 μM DDAC	0.15	0.08	0.10
1000 μM DDAC	0.10	0.07	–

types and initial QAC concentrations (Fig. 3). BAC, BZC, DDAC, DTAC and HTAC in water were degraded to <50% within 10 min at an initial starting concentration of 100 μM (Fig. 3A and B). After 32 min of VUV/UVC treatment, the achieved degradation efficiency was relatively similar for BAC (95.0%), BZC (97.2%), DDAC (96.0%), DTAC (97.4%) and HTAC (95.5%) (Fig. 3A). However, the initial degradation kinetics varied for the different QACs (Fig. 3B). The initial degradation followed pseudo first order kinetics and the initial first order rate coefficient for BAC and BZC were relatively similar at 0.25 min^{-1} and 0.27 min^{-1} corresponding to initial half-lives (T50) of 2.8 min and 2.7 min, respectively. Degradation of DDAC, DTAC and HTAC were slightly slower with initial first order rate coefficient of 0.10 min^{-1}, 0.0.11 min^{-1} and 0.16 min^{-1} corresponding to T50 values of 4.3–6.9 min. This difference may be partly explained by the different structure of the 2 groups of QACs where DDAC, DTAC and HTAC belong to the groups 1 alkyl or hydroxyl (straight chain) substituted QACs whereas the BAC and BZC contains elements from the group 2 non-halogenated benzyl substituted QACs (Kaj et al., 2014; Zhang et al., 2015). This observation is supported by a study by Lee et al. (2020) where UV irradiation of aqueous QACs (0.96 mW/cm^2) resulted in <5% degradation of DTAC in 10 min whereas dodecyl di-methyl benzyl ammonium chloride (DDBAC) was degraded by approximately 60% after 10 min treatment.

VUV/UVC degradation of QACs in the current study proceeded without addition of catalysts or radical precursors. Interestingly, the reaction velocities and degradation efficiencies were in the same range as UV AOP processes were QACs are degraded after addition of precursors (oxidants) such as persulfate (UV/PS), persulfate and copper (UV/PS/Cu) and chlorine (UV/Cl) (Huang et al., 2017; Lee et al., 2019, 2020; Xiao et al., 2022). In the present study, the efficient degradation of aqueous QACs by VUV/UVC was likely due to a combination of direct photolysis of QACs via absorption of UV light, and indirect photolysis caried out by photochemically produced radicals such as •OH (Hora and Arnold, 2020; Zhang et al., 2022).

DDAC and BAC were selected for further degradation experiments. We focused on BAC and DDAC because they represent the 2 main groups of QACS (Group 1 and 2) and because they are the most commonly used QACs in the EU and approved for indoor and outdoor uses by the European Environment Agency (EEA). BAC and DDAC are registered as biocides for e.g., disinfection, human and veterinary hygiene, preservation for liquid systems and preservation of construction materials by the European Chemical Agency (ECHA).

The VUV-UVC mediated degradation of BAC and DDAC was different in tap water compared to Milli-Q water (Fig. 3C and D). The initial first order rate coefficient was slightly greater in Milli-Q water compared to tap water for 100 μM BAC and 100 μM DDAC (Table 2). Furthermore, the initial first order rate coefficient was 2-fold and 5-fold greater in Milli-Q water compared to tap water for 10 μM BAC and 10 μM DDAC, respectively (Table 2). The faster initial degradation in the ultrapure Milli-Q water at low QAC concentrations was likely due to the presence of natural quenchers in the tap water. The municipal tap water consisted of hard groundwater (12°dH) abstracted from chalk aquifers, and no water treatment or disinfection was employed. The tap water therefore contained natural levels of potential quenchers such as non-volatile organic carbon (0.99 mg/L NVOC), bicarbonate (264 mg/L HCO_3^-) and nitrate (1.3 mg/L NO_3^-). Such organic and inorganic water constituents are known to scavenge hydroxyl radicals and can therefore affect the efficiency of VUV mediated degradation of micropollutants (Duca et al., 2017; Zhang et al., 2022).

The VUV/UVC mediated degradation of BAC and DDAC in water was affected by the initial QAC concentration (Fig. 3E and F). The pseudo first order rate coefficient (k) was higher for initial QAC concentrations of 10 μM compared to 100 μM and 1000 μM regardless of water type (Table 2). In Milli-Q water and tap water with a starting concentration of 10 μM BAC, the initial first order rate coefficients corresponded to half-lives of only 0.30 min 1.5 min, respectively. The rate coefficient in Milli-Q water was 4 times and 18 times higher at an initial BAC concentration of 10 μM compared to 100 μM and 1000 μM (Table 2). Similarly, the rate coefficient was 8 and 12 times faster at an initial DDAC concentration of 10 μM compared to 100 μM and 1000 μM (Table 2). In tap water, the effect of the initial QAC concentration on degradation kinetics was less pronounced and less relative attenuation was observed at the highest QAC concentration (Table 2). However, it should be noted that although the pseudo first order rate coefficient (k) was higher at lower initial QAC concentrations, more BAC and DDAC was actually removed at 100 μM and 1000 μM because the initial substrate concentrations were 10–100 times greater (k·C). Overall, our findings suggest that combined VUV/UVC irradiation can degrade common QACs, and that the photolysis is more efficient at lower initial QAC concentrations indicated by a higher rate coefficient, but more compound is removed at higher concentrations because of a higher molar conversion per time. These findings support previous observation that removal of organic contaminants by combined VUV/UVC treatment is concentration dependent and that the process is more efficient at lower concentrations (Gonçalves et al., 2021; Del Puerto et al., 2022).

3.3. Mitigation of QAC ecotoxicity by VUV/UVC irradiation

The ability of VUV/UVC irradiation to mitigate the toxicity of BAC and DDAC was assessed in assays with *A. fischeri, E. coli, E. faecalis, R. subcapitata, and D. magna*. Aqueous BAC and DDAC were exposed to combined VUV/UVC irradiation in a continuous flow through reactor (Fig. 1), and changes in apparent ecotoxicity were determined before and after VUV/UVC in samples collected after 0 min, 8 min and 32 min corresponding to a combined VUV/UVC fluence of 0 J/cm^2, 4 J/cm^2 and 16 J/cm^2, respectively. Although tap water quenched some of the reaction between radicals and QACs (Fig. 3), this water type was selected for toxicity studies because it was likely more representative of natural water types than Milli-Q water.

VUV/UVC treatment of QACs in tap water decreased the toxicity of BAC and DDAC to the gram-negative bacteria *A. fischeri* and *E. coli,* and the gram-positive bacterium *E. faecalis* (Fig. 4). The apparent decrease in toxicity increased with increasing UV exposure time and UV dose (Fig. 4). An almost complete mitigation of toxicity of 100 μM aqueous BAC to *A. fischeri* and *E. coli* was achieved within the initial 8 min of UV treatment (Fig. 4A and C). Furthermore, 32 min VUV/UVC irradiation of 100 μM BAC and DDAC resulted in complete mitigation of toxicity to *A. fischeri* and *E. coli* (Fig. 4A–D).

E. faecalis was more susceptible to BAC and DDAC than *A. fischeri* and *E. coli* and the EC50 values before VUV/UVC were only 1.5 μM and 1.2 μM, respectively (Table 1). After VUV/UVC treatment of 100 μM BAC and DDAC for 8 min, the toxicity decreased as indicated by 16-fold and 3-fold increases in apparent EC50 values, respectively. Hence, DDAC remained more toxic after 8 min of VUV/UVC treatment compared to BAC (Fig. 4C and E) which can be partly explained by the larger resilience of DDAC to initial degradation compared to BAC (Fig. 3). After 32 min VUV/UVC, the measured decreases in toxicity of BAC and DDAC to *E. faecalis* were somewhat comparable and corresponding to 17 to 22-fold lower apparent EC50 values (Fig. 4E and F). This coincided with

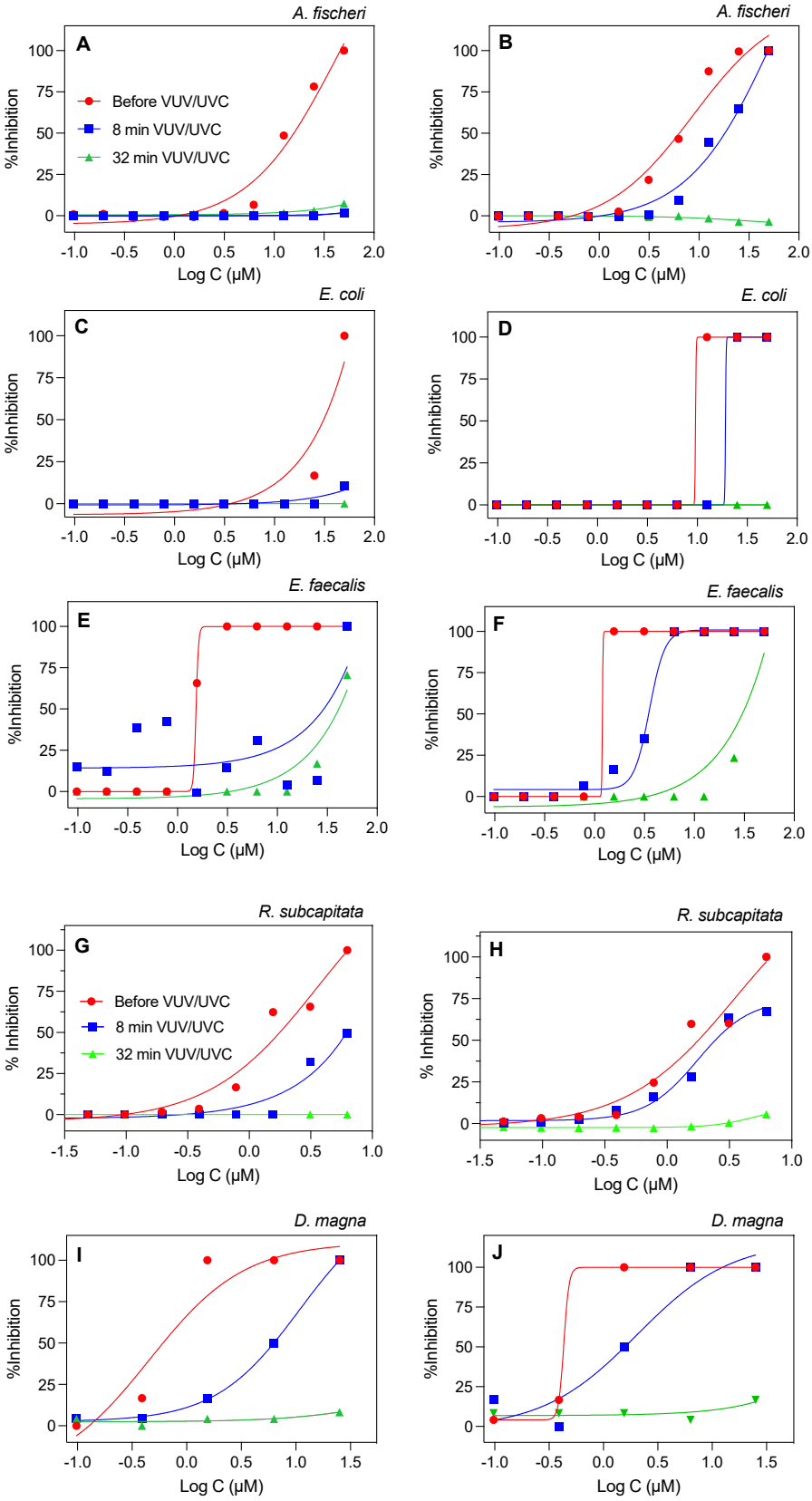

Fig. 4. Toxicity of aqueous BAC (left panels) and aqueous DDAC (right panels) before and after VUV/UVC treatment. *A. fischeri* (A and B), *E. coli* (C and D), *E. faecalis* (E and F), *R. subcapitata* (G and H) and *D. magna* (I and J).

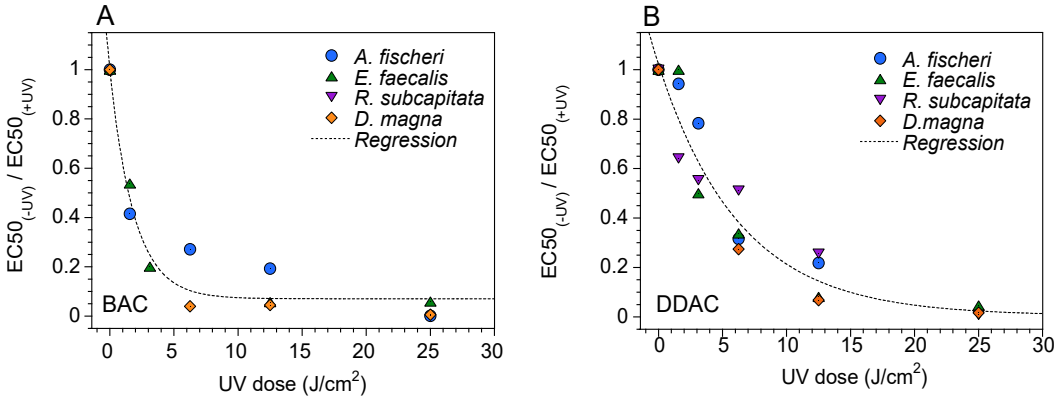

Fig. 5. Decreases in ecotoxicity of BAC (A) and DDAC (B) to different organisms after exposure of the parent compounds to VUV/UVC irradiation. Decreases in toxicity for a given UV dose are expressed as the EC50 before VUV/UVC ($EC50_{-UV}$) divided by the apparent EC50 after VUV/UVC ($EC50_{+UV}$).

a comparable degradation of >90% of both parent compounds after 32 min (Fig. 3).

VUV/UVC treatment also decreased the toxicity of BAC and DDAC to the microalga *R. subcapitata* and the crustacean *D. magna* (Fig. 4G–J). This is particularly interesting because these two test organisms were generally affected by the lowest concentrations of BAC and DDAC and therefore potentially sensitive to low remaining QAC concentrations (Table 1). Similar to the results obtained with bacteria, DDAC was more toxic after 8 min of VUV/UVC treatment compared to BAC likely due to slower initial degradation (Fig. 4H and J). However, an almost complete mitigation of toxicity of 100 μM aqueous BAC and DDAC to *R. subcapitata* and *D. magna* was achieved after 32 min VUV/UVC treatment (Fig. 4H–J).

The VUV/UVC treatment of QACs in water mitigated the apparent ecotoxicity of samples regardless of the test organism (Fig. 5). The mitigating effect was exponential and therefore most pronounced in the initial irradiation phase with UV doses up to approximately 4 J/cm^2 (Fig. 5). The mitigation effect was more pronounced for BAC compared to DDAC (Fig. 5) which likely reflect that BAC is degraded somewhat faster during VUV/UVC than DDAC (Fig. 3). These findings confirm that effect-based approaches and bioanalytical tools can complement chemical analyses by offering valuable information about the absence of potentially toxic transformation products in water samples after advanced water treatment (Escher et al., 2021; Sharma et al., 2018). Bioassays can be tailored for specific compounds and AOP processes and can therefore provide important information about whether an oxidation process resulted in potentiation or attenuation of adverse biological effects.

4. Conclusions

Several QACs examined in the current study were highly toxic to aquatic test organisms with EC50 and/or EC20 values below 1 μM. A potential for trophic transfer of BAC from microorganisms to filter feeders (e.g., daphnids) was observed. A combination of VUV and UVC irradiation of QACs in water resulted in rapid degradation of the parent compounds that followed pseudo first order kinetics with half-lives of 2–7 min. VUV-UVC mediated degradation was most likely achieved through both direct and indirect photolysis of QACs. The VUV/UVC treatment decreased the potential ecotoxicity of the QACs with decreasing toxicity with increasing UV fluence. This was attractive because removal of parent compounds does not always imply complete attenuation of toxicity. Bioassays can therefore be used an integral part of evaluating the efficacy of QAC mitigation. The efficient removal of QACs and the abatement of ecotoxicity suggested that combined VUV/UVC treatment could be a relevant technology for removal of QACs from contaminated water. VUV/UVC represents an interesting alternative to other AOP technologies because the process does not require addition of catalysts or radical precursors.

Credit authorship contribution statement

Lana Flanjak: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Visualization, Writing draft, Review & Editing. Loukia Lypirou: Methodology, Investigation. Vasilios Sakkas: Funding acquisition, Supervision, Review & Editing. Peter Roslev: Funding acquisition, Administration, Supervision, Resources, Conceptualization, Methodology, Investigation, Formal analysis, Validation, Writing draft, Review & Editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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