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Downstream effects of the pandemic? Spatiotemporal trends of quaternary ammonium compounds in suspended particulate matter of German rivers

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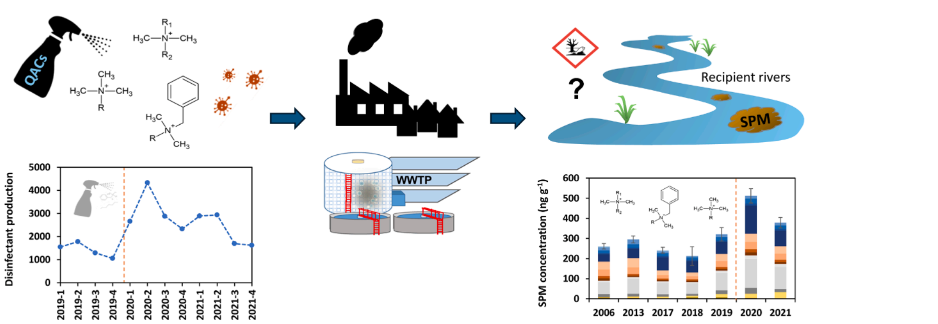
HIGHLIGHTS

- QACs were ubiquitously detected in suspended particulate matter.
- During the pandemic concentrations increased in two of three investigated rivers.
- QAC concentrations correlated with pandemic markers and mineral content.
- Spatial and monthly variation exceeded long-term concentration changes.
- Long-chained QACs reach ecotoxicological effect thresholds for sediment.

GRAPHICAL ABSTRACT



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ABSTRACT

During the SARS-CoV-2 pandemic, the preventive use of antimicrobials such as quaternary ammonium compounds (QACs) surged worldwide. As cationic and surface-active biocides, QACs are only partly removed during wastewater treatment and may cause adverse ecological effects in the downstream environment. To understand the environmental consequences of increased disinfectant use during the pandemic, we investigated spatiotemporal QAC concentration trends in the suspended particulate matter (SPM) of three diverse German rivers. Covering pooled annual SPM samples from 2006–2021 and monthly samples from 2018–2021 collected by the German Environmental Specimen Bank, 31 QACs were quantified by high performance liquid

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chromatography–mass spectrometry. ∑QAC concentrations in annual samples differed by more than tenfold between rivers in the order Saar (average 6.7 μg/g) > Rhine (0.9 μg/g) > Mulde (0.3 μg/g). The strongest potential pandemic imprint was however observed in the Mulde (+67 %) and Rhine (+22 %). Besides pandemic dynamics, also seasonal variation and mineral content of SPM tentatively affected QAC concentrations. Exceedance of predicted no-effect concentrations for sediment suggest ecotoxicological risks for long-chained QACs already before the pandemic. Our study thus highlights the importance of monitoring the environmental effects of antimicrobial use during pandemics and calls for an urgent minimization of non-essential applications.

1. Introduction

During the SARS-CoV-2 pandemic, the use of disinfectants such as quaternary ammonium compounds (QACs) increased worldwide to minimize transmission of the coronavirus [1–3]. About half of the products on the US EPA's List N of Disinfectants for Coronavirus or those approved for medical use by the German Association of Applied Hygiene contained QACs, in particular the compounds ATMAC-C16, BAC-C12 to -C18, and DADMAC-C10 (Fig. 1) [4,5]. However, excessive use of antimicrobials such as QACs could exacerbate pre-existing (eco-)toxicological risks, including the spread of antimicrobial drug-resistance (AMR) which is sometimes projected to become another, yet 'silent', pandemic of the 21st century [2,3,6]. The SARS-CoV-2 pandemic can thus be regarded as an involuntary global 'field trial' of the environmental impact of increased disinfectant use.

QACs comprise a diverse class of emerging cationic and biocidal surface water contaminants, some of which are high production volume chemicals [1,7–11]. They are characterized by an amphiphilic head-tail structure with a permanently positively charged quaternary nitrogen attached to at least one hydrophobic substituent, often an alkyl- or aryl-group (Fig. 1). This confers QACs favorable surface-active, antistatic and antimicrobial properties warranting their large-scale use not just in disinfectants and sanitizers but also cosmetics, washing and cleaning products, pesticide formulations and processing aids [10,11]. Alkyltrimethyl-, benzylalkyl- and dialkyldimethyl-ammonium compounds (ATMAC, BACs and DADMACs; Fig. 1) are among the most common alkyl-substituted QACs used across healthcare, households, agriculture and industry [1,9–11].

Despite their numerous beneficial applications, QACs increasingly raise concerns about their aquatic toxicity [9–12] and human health risks [11,13–15] as they exert non-specific biocidal activity via disruption of cell membrane integrity [16,17]. Daphnids, algae and other invertebrates are among the most sensitive aquatic organisms with- lowest observed observed adverse effect/ no observed observed adverse effect concentrations (LOECs/NOECs) reported as low as as low as 1 μg/L for DADMACs in algae and 0.06 μg/L for BACs in invertebrates [12]. Human toxicity was initially assessed to be limited to local irritation, inflammation and asthma [13,18]. However, recent mouse and in vitro studies also observed developmental, reproductive, immuno- and neurotoxic effects [11,13,17]. Since QACs can enter the human blood stream [13,19], breast milk [20], and cross the blood-brain barrier in rodents [14], cellular and potentially systemic effects in humans cannot be excluded [11,13].

Moreover, QACs in the environment may also indirectly impair ecosystem services and human health due to their broad-spectrum antimicrobial activity on viruses, fungi, Gram-positive and Gram-negative bacteria [10,16,21]. Inhibitory effects on microorganisms can lower key microbial functions [9,10]] such as methanogenesis [22,23], denitrification [22,24,25], hydrogenase activity [24], respiration [26] or mineralization [27,28]. In addition, QACs are known to induce microbial adaptations including enhanced biofilm formation, expression of efflux pumps or membrane lipid alterations [10,29,30]. This could contribute to the propagation of AMR via co-selection for antibiotic resistance genes (e.g. by horizontal gene transfer) or expression of multi-drug efflux pumps [10,29,30]. AMR is projected to become one of the leading causes of death by 2050 [6], and may also be promoted in surface waters that harbor a diverse microbiome potentially exposed to wide range of anthropogenic antimicrobial chemicals [31,32].

QACs typically enter the aquatic environment via 'down-the-drain' disposal into wastewater treatment plants (WWTPs), direct industrial discharge, livestock farm effluent [33], runoff from wood and roofs treated with preservatives [34], or from agricultural soils after pesticide or biofertilizer applications [1,9–11,35–37]. In WWTPs, QACs are partially removed by transfer to sewage sludge [36–38] and

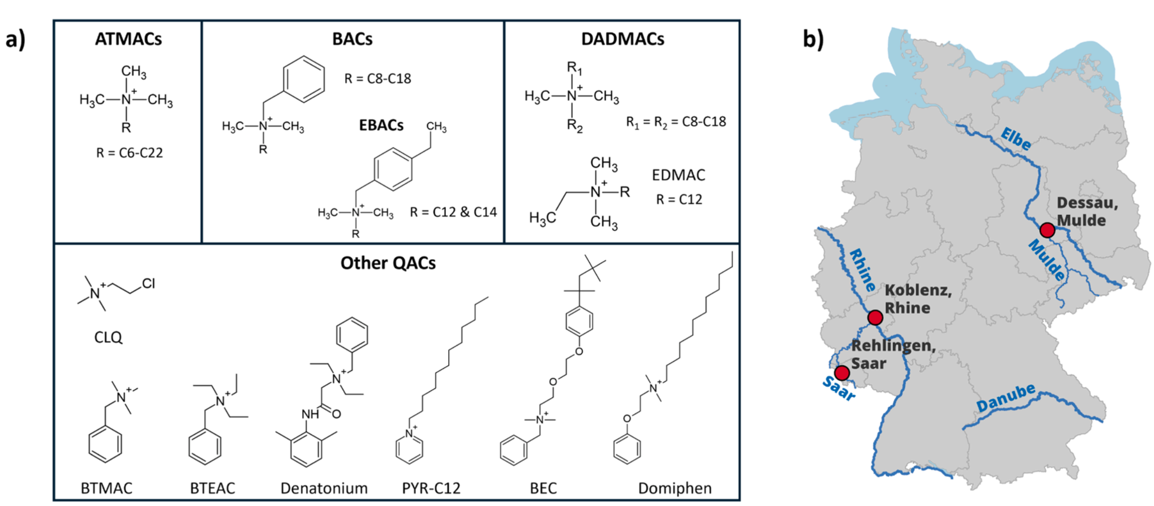


Fig. 1. Overview of QACs (a) and SPM sampling sites (b) investigated in this study. Counter anions (Cl, Br or benzoate) are omitted from the chemical structures. ATMACs: alkyl-trimethylammonium compounds, (E)BACS: (Ethyl-)benzyl-alkylammonium compounds, DADMACs: dialkyl-dimethylammonium compounds, EDMAC: ethyl-dimethylammonium, CLQ: chlormequat, BTMAC: benzyl-trimethylammonium, BTEAC: benzyl-triethylammonium, PYR-C12: dodecylpyridinium, BEC: benzethonium chloride. The map of German rivers was created in QGIS v.3.36.2. Source layers © GeoBasis-DE / BKG (2024) reused according to dl-de/by-2-0. Map lines delineate study areas and do not necessarily depict accepted national boundaries.

biodegradation by tolerant microorganisms [9,26,28,39–41]. However, because of their excessive incoming concentrations, antimicrobial activity and strong particle affinity, QACs may still be continuously emitted in low amounts with WWTP effluent, especially in association with wastewater particles [28,42].

In rivers, QACs either occur dissolved or partitioned to sediment and suspended particulate matter (SPM) due to their amphiphilic properties. While QACs are in principle water-soluble owing to their positive charge, solubility decreases markedly for alkyl chains larger than C14 [43]. Adsorption to soil or sludge, on the other hand, was favored for longer-chained homologues [24,44] since QACs show a strong affinity for both mineral and organic sorbates, driven by ionic and hydrophobic interactions [10,45,46]. QAC concentrations in solid matrices interfacing anthropogenically impacted surface waters were therefore generally high, especially for long-chained BACs and DADMACs. Total QAC levels ranged up to 84 μg/g in urban stormwater particulates from 2010–2011 [47], 114 μg/g in urban estuarine sediments collected in 1998 in the USA [48], 6.8 μg/g in Austrian river sediment from 2004 [49] and 5.5 μg/g in German floodplain soils collected between 2016–2020 [50]. Consequently, a high affinity of QACs to SPM can also be expected. SPM of wastewater-impacted rivers may thus be an ideal matrix for monitoring QAC concentration trends and the environmental imprint of altered emissions during the SARS-CoV-2 pandemic. SPM also functions as a transport medium, food source for aquatic organisms and habitat for microbes, underlining its ecotoxicological relevance. Moreover, SPM favors biofilm formation and co-localization of microorganisms with adsorbed antimicrobials, making it a potential hotspot for the development and spread of AMR in the aquatic environment [51].

Recent non-target and target screening studies already detected QACs in SPM of German rivers with reported concentrations of BAC-12, BAC-C14, DADMAC-C8 and DADMAC-C10 in the range of 18 ng/g to 5.5 μg/g between 2013–2019 [8,52]. However, quantification of a comprehensive set of different QAC homologues required to estimate risks to aquatic ecology and human health is still lacking. Moreover, the effect of altered disinfection and consumption patterns during the SARS-CoV-2 pandemic warrants further investigation. During the first lockdown in Germany, national disinfectant and QAC sales surged by up to 80 % compared to 2019 [53], with so far unknown consequences for the aquatic environment.

Retrospective studies from across the world have reported a nearly twofold increase in mean QAC concentrations in household dust [54] and human blood [19] after the onset of the pandemic, a 331 % increase in QAC loads in influent wastewater from Athens between 2019 and 2020 [55], as well as tentative increases in QAC concentrations in primary sludge from Connecticut, USA [56]. Mahony et al. furthermore observed significant positive correlations between QAC sales in 2020 and concentrations in both raw and treated wastewater in the USA [57]. In addition, Mohapatra et al. found associations between SARS-CoV-2 viral loads and concentrations of disinfectants as well as BAC-C12 in university campus wastewater in Singapore [58]. While the abovementioned studies have focused on residential exposure, to our knowledge the effect of the pandemic on QAC concentrations in wastewater-impacted downstream environments and the ecotoxicological implications thereof have not yet been investigated. This information is crucial to assess whether current regulations on the permitted use of QACs and existing emission control technologies (e.g. sewage treatment) are sufficiently protective against adverse environmental effects even under high-use scenarios.

This study therefore analyzed spatiotemporal QAC concentration trends in SPM of the three German rivers Saar, Rhine and Mulde (**see** Fig. 1) between 2006–2021. We hypothesized that QAC concentration levels and trends in SPM are influenced by 1) the molecular structure of QACs, 2) QAC emissions (market volumes and altered use during the pandemic), and 3) environmental factors such as SPM composition, weather conditions, and wastewater share of the rivers. Specifically, we postulated the following:

1) More hydrophobic (i.e. long- or double-chained, benzyl-substituted) QACs are found at higher concentrations due to stronger adsorption to SPM particles (∑DADMACs>∑BACs>∑ATMACs).
2) Higher QAC concentrations (and a stronger effect of the pandemic) are observed at SPM sampling sites with a higher wastewater share (Saar > Rhine > Mulde) as previously observed for pharmaceuticals [59], plasticizers [60] as well as per- and polyfluoroalkyl substances (PFAS) [61].
3) After the onset of the pandemic, concentrations of QACs commonly used as disinfectants increase, especially ATMAC-C16, BAC-C12 to -C18 and DADMAC10. With a short temporal delay (e.g. due to residence time in WWTPs), monthly QAC concentrations follow the trends in national disinfectant sales and pandemic markers (COVID-19 incidence and mortalities).
4) Due to their cationic nature, QAC concentrations correlate positively with TOC and clay mineral content of SPM.

To test these hypotheses, concentrations of 31 QACs were quantified retrospectively in archived SPM samples from the German Environmental Specimen Bank by high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS) after ultrasonic extraction. Pre-pandemic QAC concentrations in SPM were compared to levels in 2020 and 2021 in both monthly SPM samples from 2018–2021 as well as pooled annual samples from 2006–2021. In addition, relationships between QAC concentrations, national QAC production volumes, COVID-19 incidence, COVID-19 mortalities, and physicochemical SPM characteristics were investigated to identify chemical properties, anthropogenic and natural influences driving QAC concentrations. Potential eco-toxicological implications were assessed by comparing the range of quantified QAC concentrations to predicted no-effect concentrations (PNECs) for sediment-dwelling organisms assuming concentration-addition for mixture effects. This study thereby helps to understand the downstream effects of increased disinfectant use during the SARS-CoV-2 pandemic and the potential ecotoxicological implications of QAC contamination in the aquatic environment.

2. Materials and methods

2.1. Sampling sites

Three German rivers, the Saar, Rhine and Mulde, were selected to study spatiotemporal trends in QAC concentrations between 2006 and 2021. They differ in length, geographical location, catchment characteristics, potential QAC emission sources and effluent load, and were thus considered to be a good representation of German rivers. The Rhine is a major European transboundary river that rises in Switzerland and forms part of the borders of Switzerland, Liechtenstein, Austria, France and Germany before flowing through the Netherlands into the North Sea. With a length of 865 km in Germany, it is the largest German river and the most important north-south waterway, draining large areas of Southern and Western Germany. Although the Rhine supplies drinking water to around 20 million people, it is still affected by chemical emissions from agriculture, shipping, industrial and municipal wastewater [62]. The sampling site in the city of Koblenz is thus representative of large rivers with medium levels of pollution from a variety of sources.

Both Saar and Mulde are comparatively small rivers. With a total length of 246 km, the Saar flows in roughly equal parts through France and Germany. Its German catchment area, the Saarland conurbation, has a long history of industrial activities and was a hot spot for SARS-CoV-2 infections in Germany during the early phase of the pandemic. At the SPM sampling site Rehlingen, located downstream of the industrial town of Dillingen, the Saar carries a high proportion of wastewater (about 15 %) [59,63]. It is thus representative of rivers with high QAC emissions and legacy pollution. In contrast, the Mulde is a largely rural river located in the federal states of Saxony and Saxony-Anhalt. It rises in the

Ore Mountains near the Czech-German border and passes the largest contiguous forest area in central Germany, the Düben Heath ('Dübener Heide'), before emptying into the Elbe after 124 kilometers. At the sampling site Dessau, just upstream of the Elbe, the Mulde has a low estimated wastewater share of just about 3 %. The only large industrial sites in the region are in Bitterfeld-Wolfen 37 km upstream of the town of Dessau [61]. The Mulde thus represents rivers with a largely rural catchment and relatively low QAC emissions. Further details on the sampling sites are provided in Table 1.

2.2. SPM sampling and storage

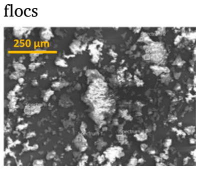
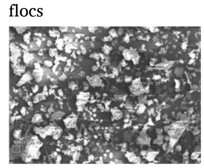
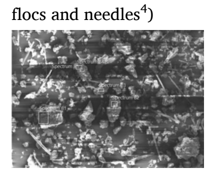
SPM sample collection, processing and basic characterization was conducted by the German Environmental Specimen Bank (umweltprobenbank.de) following standardized operating procedures [64,65]. The German Environmental Specimen Bank is a systematic long-term program sampling, analyzing and archiving a fixed set of environmental matrices from coastal, freshwater and terrestrial ecosystems in support of environmental and chemical management [110].

In brief, SPM samples were collected at monthly intervals with sedimentation boxes that are either free-hanging in the river at roughly 1 m depth (location Rehlingen/Saar) or installed within measuring stations of the German Federal Institute of Hydrology (Koblenz/Rhine) or the State Agency for Flood Protection and Water Management of Saxony-Anhalt (Dessau/Mulde). The samples were sieved to a particle size < 2 mm, pre-homogenized and frozen on site with liquid nitrogen prior to transportation to the archive, where they were lyophilized, ground and homogenized before storage over liquid nitrogen at below –130 °C. Without leaving the cooling chain, monthly samples were stored both individually and pooled into one annual composite sample. The composite samples were stored at below –130 °C in an inert atmosphere. Samples for QAC analyses were shipped on dry ice to the University Gießen and stored at –20 °C until analysis.

2.3. Physicochemical characterization of SPM

During each sampling, the total SPM yield per sampling interval, water temperature, SPM texture, color, odor as well as H_2S and CO_3^{2-} content were recorded. The SPM content was determined from fresh river water by gravimetric vacuum filtration according to DIN 38409–2 [66]. For free-hanging sedimentation boxes, 2–3 water samples were collected from different water depths [65]. For the annual composite SPM samples, the pH in 0.01 M $CaCl_2$, total carbon (TC), inorganic carbon (TIC) and organic carbon (TOC) contents and particle size fractions (sand, silt and clay) were measured according to DIN EN 15933, DIN EN 15936 and DIN ISO 11277 [67–69]. Metal and some element contents were obtained from the public data portal of the German Environmental Specimen Bank. Further elemental SPM composition was

Table 1
River, sampling site and physicochemical SPM characteristics. The pH, TC, TOC, TIC as well as P, Fe and Hg content and SPM texture were analyzed by the German ESB. Further elements were determined semi-quantitatively by EDX analysis for selected SPM particles as described in Materials and Methods Section 2.3. Sample characteristics are given as median with the range in bracket.

River	Saar	Rhine	Mulde
Length (total km)	246	1320	147
Sampling site	**Rehlingen**	**Koblenz**	**Dessau**
Area	Saarland conurbation	Urban, Rhein-Main area	Largely rural in nature park *Düben Heath*
Industry	Historical: steel, coal and iron, current: metal, electronics, automotive	diverse	Paper and chemical industry at Bitterfeld-Wolfen (37 km upstream)
Wastewater share	~15 %[1], 6 km distance to nearest WWTP	~5 %[2]	~3 %
Physicochemical sample characteristics			
pH[1]	6.8 (6.7 –7.2)	7.1 (6.9 –7.2)	6.9 (6.5 –7.3)
TC (%)[1]	6.2 (5.5 –8.4)	6.1 (5.5 –7.3)	5.4 (2.8 –8.1)
TOC (%)[1]	6 (5.2 –8.2)	3.9 (2.9 –5.3)	5 (2.8 –7.9)
TIC (%)[1]	0.3 (0.1 –0.3)	2.2 (1.9 –2.6)	0.3 (0.2 –0.5)
Sand (%)[1]	2.3 (1.9 –13.5)	6.4 (3.9 –7.5)	11 (6.7 –21.9)
Silt (%)[1]	59.8 (59.1 –62)	73.2 (61.8 –75.1)	69.6 (60.1 –78.8)
Clay (%)[1]	36.1 (27 –38.6)	21.2 (18 –32.2)	16.4 (12.5 –22.1)
P (mg/g dw)[2]	2.7 (2.1 –3.3)	1.1 (0.8 –1.1)	1.7 (1.4 –2.2)
Fe (mg/g dw)[2]	37.4 (28.8 –43.2)	20 (14.6 –20.6)	26.4 (19.9 –31.3)
Hg (µg/g dw)[2]	0.2 (0.1 –0.3)	0.2 (0.2 –0.2)	1.4 (1 –1.6)
C[3]	26.5 (13.3 –61.4)	23.1 (14 –73.4)	24.9 (7.8 –92.5)
N[3]	0.3 (0 –10.1)	0 (0 –2.3)	0 (0 –5.2)
O[3]	49.2 (26 –59.2)	54.3 (21.6 –60.6)	51.8 (6.7 –63.6)
Al[3]	5.4 (0.5 –8.7)	3.8 (0.8 –6.3)	2.1 (0.1 –10.8)
Si[3]	10.7 (0.9 –19.1)	10.7 (2.3 –31.1)	18.2 (0.3 –28.2)[4])
Si:Al	2.0	2.8	8.9
Na[3]	0.1 (0 –0.3)	0.1 (0 –1.7)	0.1 (0 –0.7)
K[3]	0.9 (0.1 –3.6)	0.6 (0.1 –1.3)	0.3 (0 –2.7)
Mg[3]	0.7 (0.1 –7.4)	0.9 (0.1 –6.3)	0.2 (0 –2.1)
Ca[3]	0.4 (0 –1.1)	1.9 (0.4 –13.2)	0.2 (0 –0.6)
Morphology	flocs	flocs	flocs and needles[4])
EDX images			

1. Analyzed for annual samples 2006-2020
2. Analyzed for annual samples 2006-2019

3. Relative abundance (at%) with respect to all elements analyzed by EDX (C, N, O, Na, Mg, Al, Si, P, S, Cl, K, Ca, Fe, Ti)
4. Needles containing higher amounts of Si (average 24 at% vs. 17 at% in flocs)

determined semi-quantitatively by energy dispersive X-ray (EDX) analysis (Ultim Max 170, Oxford Instruments, Abingdon, United Kingdom) on a high-resolution scanning electron microscope (GeminiSEM 560, Carl Zeiss Microscopy GmbH, Jena, Germany). For further details please see Text S1, Fig. S1 and Tables S1-S3.

2.4. QAC analysis

2.4.1. Extraction

The QAC extraction procedure was a slight modification of the protocol developed by Heyde et al. for soil [70]. In brief, a 0.5 g (dry weight) SPM sample was extracted by three cycles of 10 min shaking with acidified acetonitrile (0.1 % HCl) followed by 10 min ultrasonication and 10 min centrifugation at 2000 rpm (Rotanta R360, Hettich, Germany). The pooled supernatants were concentrated to 1 mL of extract using a Syncore Polyvap evaporator (BÜCHI Labortechnik GmbH, Essen, Germany), diluted 1:1 with acetonitrile and fortified with 20 μg/L of deuterated internal standards (ISTDs; D_9-ATMAC-C10; D_7-BACs: C8, C12, C14, C18; D_6-DADMACs: C8, C10, C12, C14) before analysis by HPLC-ESI-MS/MS. Further details on the utilized chemicals, reagents, internal standards and method performance are given in Text S2, Table S4 and Supplementary file 2.

2.4.2. Identification and quantification by HPLC-ESI-MS/MS

A set of 31 QACs was investigated by targeted multi-residue analysis (Table S5), including ATMACs of chain length C6-C22, BACs of length C8-C18, ethylbenzylalkyl (dimethyl)ammonium compounds (EBACs; mixture of C12-C14), DADMACs C8-C18, dodecylethyldimethyl ammonium chloride (EDMAC-C12), benzethonium chloride (BEC), chlormequat chloride (CQC), dodecylpyridinium chloride (PYR-C12), denatonium benzoate, domiphen bromide, benzyltriethyl ammonium chloride (BTEAC) and benzyltrimethyl ammonium chloride (BTMAC). Further information on the preparation of a QAC multi-standard, supplier, purity and CAS-numbers of individual analytes is provided in Text S3 and Table S5. For QAC analysis, 20 μL of the sample extract was injected into an Exion LC system coupled to a QTRAP 4500 MS/MS (Sciex, Darmstadt, Germany). Analyte separation was achieved on a CSH phenyl-hexyl-column (130 Å, 150 mm length, 2.1 mm ID, 3.5 μm particle size, Waters Chromatography Ireland Limited, Wexford, Ireland) equipped with the corresponding guard column by employing a two-step gradient from 0–100 % acetonitrile according to Heyde et al. [70]. Ultrapure water with 50 mM ammonium formate and 10 mM formic acid served as aqueous mobile phase. The MS was operated in positive electrospray ionization mode and QACs were measured by scheduled multiple reactions monitoring (sMRM) with 2–3 transitions per compound. For correct identification, fragment ions were obtained by direct injection of standard solutions into the MS. While some mass transitions were adopted from Heyde et al. [70], MS parameters of all compounds were optimized again on the instrument used for this study.

Notably, EBACs-C_n and BACs-C_{n+2} share the same precursor mass, and both showed the benzyl radical at $m/z = 91.1$ as most abundant ion fragment. Although previously published methods have used the benzyl radical as a quantifier ion [70,71], this was not suitable for the current study since EBACs-C_n and BACs-C_{n+2} eluted as partially overlapping peaks (for XICs see Fig. S2). Instead, the dimethyl-n-alkyl ammonium cation ($m/z = 212.3$ for BAC-C12 and $m/z = 240.3$ for BAC-C14) was used as a selective quantifier for BACs and the ethylbenzyl radical at $m/z = 119.1$ for EBACs. By preparing separate standards for BACs and EBACs, we could attribute the latest eluting benzyl radical peak to the longer-chained BACs and the earlier two ones to the ortho- and para-isomers of EBACs (Fig. S2). Thus, the benzyl radical could still be used as a qualifying fragment for BACs. Further details on the LC gradient profile, MS settings and other measured transitions are provided in Table S6 and Supplementary file 2, respectively.

QACs were quantified by external ten-point calibration between 0.1 μg/L to 200 μg/L in acetonitrile using the area/ISTD area ratio. If linearity was not given over the entire range, calibration curves were split to include at least five levels at the lower and upper end, resulting in a very good linear fit (R^2 0.98–0.999 depending on the analyte). For positive identification, the ion ratio tolerance was set to a maximum of 30 % deviation from the calibrants. The instrumental limits of detection (IDL) and quantification (IQL) were calculated from solvent blanks across all measurements (Fig. S3) as:

$$\mathrm{IDL} = \mathrm{mean}_{\text{solvent blank}} + 3 * \text{standard deviation}_{\text{solvent blank}} \tag{1}$$

$$\mathrm{IQL} = \mathrm{mean}_{\text{solvent blank}} + 5 * \text{standard deviation}_{\text{solvent blank}} \tag{2}$$

IDLs ranged from 0.10 μg/L for EBAC-C12 to 6.9 μg/L for BAC-C12 (median 0.13 μg/L) and are given in Supplementary file 2. Peak review, quality assurance and calculation of QAC concentrations were performed in Sciex OS v.2.0.0.

2.4.3. Quality assurance

2.4.3.1. Background contamination. To avoid laboratory cross-contamination, all reusable glassware in contact with SPM samples or QAC extracts was rinsed with acidified acetonitrile prior to use. Reusable analytical syringes were thoroughly cleaned with solvents of different polarity before and after sample processing. QAC background levels were monitored in each extraction batch by processing two method blanks and one solvent blank (acetonitrile) alongside the samples. The method detection and quantification limits (MDL, MQL) were calculated analogous to the IDL and IQL considering the method blanks from all extraction batches (see Fig. S4) and can be found in Supplementary file 2. The MDLs ranged from 0.02 ng/g for EBAC-C14 to 7.2 ng/g for DADMAC-C10.

2.4.3.2. Reproducibility. Method reproducibility across different extraction days was monitored using an SPM sample from the river Elbe as reference material (Supplementary file 2). This sample had also been archived by the German Environmental Specimen Bank and contained 18 QACs above MDL, ranging in concentration from 1 to 301 ng/g. Interday variation for these homologues (excluding long-chained DADMACs) ranged from 10 % to 34 %, showing good reproducibility of the extraction method. In addition, all annual SPM samples were extracted in triplicate and on one day per river to increase confidence in temporal trend measurements. As less material had been archived for the monthly samples, only some timepoints could be extracted in triplicate or duplicate (see Supplementary file 2).

2.4.3.3. Recovery. Method recovery was assessed for SPM from each of the three investigated river to detect potential matrix-dependent differences in recovery. To this end, the annual 2017 sample of each river was spiked with 200 ng/g of QAC standards. To ensure a homogenous QAC distribution, the samples were first covered with pentane, then spiked with the QAC standard solution in acetonitrile, and finally mixed for 24 h on a horizontal shaker. The extraction was performed one week after solvent evaporation to mimic the conditions of untreated SPM samples more closely. For each spiked sample and corresponding unspiked native sample, triplicates sets were extracted. Recovery was then calculated after background-subtraction as:

$$\mathrm{Recovery}(\%) = 100 * \frac{\text{concentration}_{\text{spiked sample}} - \text{concentration}_{\text{sample background}}}{\text{concentration}_{\text{nominal}}} \tag{3}$$

The spiking level was selected to fall within the range of measured QAC background concentrations across all rivers to represent recovery at environmentally relevant conditions. The recovery rates obtained may therefore be less accurate for highly abundant QACs such as ATMAC-22, BAC-C12 and DADMAC-10 in the Saar. Recovery of

DADMAC-C18 could not be reliably assessed in any river and DADMAC-C18 was therefore only considered semi-quantified.

Recovery rates of all other QACs (excluding DADMAC-C18) ranged from 26 % to 67 % (median 48 %) for the Mulde, 29 % to 93 % (median 60 %) for the Rhine, and 25 % to 148 % (median 80 %) for the Saar (Fig. S5). Differences in recoveries between the rivers may be due to a different composition of the SPM matrix, as also evidenced by different degrees of matrix effects between the rivers (Fig. S6), and different analyte background concentrations, which were notably higher for the Saar. Average recoveries exceeding 100 % were likely due to the uncertainty of the estimates for QACs with high background levels. Overall lowest recoveries were found for the most hydrophilic QACs (e.g. ATMAC-C6, BTEAC and CLQ). Despite some variations, recovery rates for ATMACs, BACs and DADMACs $\geq$ C8 showed no obvious dependency on the alkyl chain length. Thus, although recoveries differed slightly between rivers and QAC homologues, differences in absolute measured concentrations were much greater and therefore unlikely to be introduced by the extraction method used.

2.4.3.4. Matrix effect. To account for matrix-induced alterations of the analyte signal and instrumental drift, all samples were fortified with 20 µg/L of the nine deuterated ISTDs before measurement. Matrix effects were quantified for both the ISTDs and analytes relative to the calibrants in acetonitrile as:

$$\text{Signal alteration}(\%) = 100 * \frac{\text{area}_{\text{spiked sample}} - \text{area}_{\text{sample background}}}{\text{area}_{\text{spiked blank}}} - 100 \tag{4}$$

For all rivers, moderate to strong ionization suppression (−19 to -59 %) was observed (Fig. S6). Matrix effects were overall strongest in the Saar.

2.5. Simplified ecotoxicological risk assessment

A simplified risk assessment was conducted by calculating the risk quotient (RQ) of measured QAC concentrations in SPM (MEC) to PNECs for freshwater sediment organisms published in ECHA registration dossiers [39,40,72] (MEC/PNECs). RQs > 1 indicate a potential risk for aquatic organisms and risk quotients < 1 no risk. For simplicity, the measured SPM concentrations were assumed to be identical to sediment concentrations, and both MECs and PNECs were considered representative of 100 % bioavailability. Experimental PNECs for sediment dwellers were however only publicly available for very few QACs. For most registered homologues or mixtures, PNECs were estimated based on a read-across approach from other registered QACs or from toxicity to aquatic organisms by applying the equilibrium partitioning method [73]. In the latter case, the distribution coefficient of QACs between water and sediment were often not experimentally determined but only estimated either from structural analogues (i.e. read-across), K_{oc}, or in a few cases modelled by regression based on the K_{ow}. Besides single-compound risk quotients, also different mixtures of ATMACs, BACs and DADMACs were considered. Due to similar acute and chronic toxicity thresholds, the shared mechanism of action [11], and often identical established toxicity thresholds for homologues or mixtures of different alkyl chain lengths, additive mixture toxicity was assumed.

Although there is currently no European regulatory guideline for integrating selection of AMR into the chemical risk assessment, the AMR Industry Alliances of the pharmaceutical manufacturing industry recently suggested an approach based on minimal inhibitory concentrations (MICs) for antibiotics [74]. This approach to calculate a PNEC-MIC was adapted here to estimate the risks of QACs in SPM to contribute to the selection of AMR. As the MIC is the lowest concentration at which a 100 % reduction in microbial growth is observed, an assessment factor of 10 was applied to extrapolate to a PNEC corresponding to the minimal *selective* concentration at which resistant, mutant strains with a growth advantage are selected [74,75]. MICs for BAC-C12 and DADMAC-C10 in aqueous solution were taken from Heyde et al. [76]. For a protective assessment, the lowest MICs obtained among four tested bacterial strains (i.e. *Escherichia coli* ESBL37B15_13_1E, *Escherichia coli* ConF4, *Pseudomonas fluorescens* DSM 50090 T, and *Enterococcus faecalis* DSM 20478 T) were used for further calculations. MICs determined in aqueous solution were converted to theoretical concentrations per g of solid by applying the equilibrium partitioning method [77] using averaged soil sorption coefficients from regulatory assessment for BAC-C12 [78] and DADMAC-C10 [79]. The calculated PNEC-MICs and corresponding risk quotients therefore only represent a rough estimate of the potential QAC concentrations at which selection for resistant mutations could occur.

2.6. Statistical analysis

Differences in QAC concentrations between rivers were assessed by one-way ANOVA with Welch's correction for heteroscedasticity, and in case of non-normality by Kruskal-Wallis tests (Table S7). Normality was assessed from quantile-quantile plots of groupwise normalized z-residuals (Fig. S7). For post hoc comparison, Games-Howell or nonparametric Conover-Iman tests were conducted (Table S8). Differences in QAC concentrations between pre-and post-pandemic were assessed by independent samples one-sided Welch test (Table S9). P-values obtained by Welch-tests, ANOVA and post hoc comparisons were separately corrected for multiple testing by applying the Holm-Bonferroni method.

To identify which anthropogenic and natural factors potentially affect QAC dynamics, Spearman correlations were calculated between QAC concentrations, pandemic markers (COVID-19 incidence [80] and mortality [81] at national and federal state level), market trends (national production, import and export of QAC salts and ammonium-based disinfectants [82]), as well as physicochemical SPM characteristics. The monthly COVID-19 incidence and mortality was calculated based on the daily new cases reported by the Robert-Koch-Institute between the start of case reporting in January 2020 and December 2021. The exact period of SPM sampling was used as the reference time frame for each month and in line with the approach by the Robert-Koch-Institute, a constant population was assumed. QAC market data were only available from the German Federal Statistical Office at annual resolution for the years 2013–2021 and at quarterly resolution for the years 2019–2021. Monthly data points in 2018 and annual data from 2006 therefore had to be omitted from the correlation analysis. Quarterly QAC production data were matched to the corresponding months of SPM sampling and all available QAC production data were used without further censoring. Physicochemical SPM characteristics including particle size, pH, TOC, TIC and total C were only determined for the period 2006–2020 and contents of other elements for the period 2006–2019. All missing time points were omitted from the correlation analysis. More detailed information on the data source, unit, available time frame, and reason for inclusion of each tested parameter are given in Supplementary file 2 and Table S10.

For all statistical analyses, (technical) replicates were averaged into one mean value and treated as a single observation. Samples collected at different time points were assumed to be independent since there was no direct physical link between them. The significance level was set to $\alpha = 0.05$. All statistical tests were conducted using R v.4.3.3.

3. Results and discussion

3.1. QAC homologue patterns

The rivers Saar, Rhine and Mulde showed a very similar distribution of QAC homologues (Fig. 2) despite large differences in absolute concentration levels (ranging from 212 ng/g to 8.1 µg/g across all rivers and sampling years; see Fig. 3). Alkyl-substituted QACs accounted for over 90 % of the $\sum$QAC in all three rivers. They were dominated by ATMAC-C18 and ATMAC-C22 (together comprising 13–41 % of

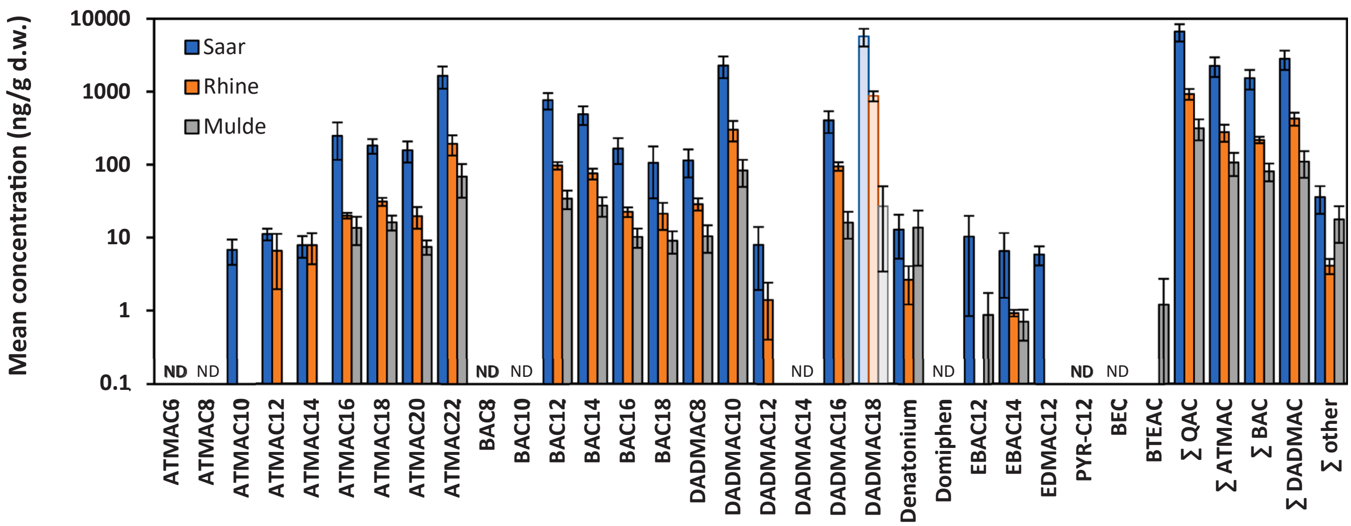


Fig. 2. Mean concentrations of QAC homologues detected in pooled annual SPM samples of the rivers Saar, Rhine and Mulde over the period 2006–2021. DADMAC-C18 is shown in hollow bars as it was only measured semi-quantitatively and is not included in ∑QAC and ∑DADMAC concentrations. ND indicates QACs measured <LOD in all rivers. The graph is truncated at the lowest LOD (0.1 ng/g) and missing bars for individual rivers indicate mean concentrations <LOD. If for one river more than 50 % of the concentrations in annual samples were <LOD, also mean concentrations were set to <LOD. Otherwise, single concentrations <LOD were treated as zero.

∑QACs), BAC-C12 and BAC-C14 (10–31 % of ∑QACs), as well as DADMAC-C10 and DADMAC-C16 (23–60 % of ∑QACs) (Fig. S8). Tentatively highest concentrations of 0.1–8.2 µg/g were measured for DADMAC-C18, nearly reaching the same concentrations in the Saar and Rhine as all other QACs combined (Fig. 2, Supplementary file 2). However, due to poor chromatographic peak shapes and reproducibility, DADMAC-C18 was only considered semi-quantified and excluded from the ∑QAC concentrations. Among the 'other' QACs analyzed in this study, denatonium, EBAC-C12 and EBAC-C14 were detected most frequently with concentrations between 0.4–32 ng/g. EDMAC, BTMAC, BTEAC and PYR-C12 were only rarely present in the low ng/g range while CLQ and domiphen were not detected at all (Fig. 2). Literature data for these homologues are scarce; however, denatonium has already been recognized as an emerging contaminant in German rivers [8,83]. Moreover, while a direct comparison of QAC homologue patterns between studies focusing on different sets of analytes is not possible, ATMAC-C22 [84], BAC-C12 and -C14 [49] as well as long-chained DADMACs [48,49,85,86] were also identified as the most abundant QACs in prior sediment screenings.

The observed QAC homologue distribution can be explained by a combination of market volumes and homologue-specific adsorption affinities. ATMAC-C16, ATMAC-C20 +C22 and two mixtures containing mostly BAC-C12 +C14 or DADMAC-C16 +C18 are the alkyl-substituted QACs registered in the EU with the highest annual tonnages (1000 – 10,000 t/a), while among the 'other' QACs, denatonium has the highest market volume (100 – 1000 t/a) [72]. This seems directly reflected in measured SPM concentrations. In addition, for ATMACs the alkyl chain length and thus hydrophobicity showed a significant positive correlation with measured concentrations in the Saar (spearman's $\rho = 0.82$, $p = 0.03$) and Rhine ($\rho = 0.90$, $p = 0.01$) (Table S11). Longer alkyl chains are known to increase the adsorption of ATMACs and BACs to organic and mineral sorbents [24,44,46,87,88], both of which are important structural components of SPM (see Table 1). Sorption affinity affects not only the water-SPM partitioning, but surface binding or sequestration into (clay) mineral interlayers could also limit the availability of QACs to microbial [10,89,90] and photolytic degradation. Therefore, both stronger particle-association and enhanced persistency may contribute to the high abundance of long-chained QACs in SPM. Double-chained DADMACs are comparatively hydrophobic irrespective of chain length, and thus differences in market volumes may be the main reason behind their homologue distribution.

3.2. Spatial differences

The Saar, Rhine and Mulde showed striking discrepancies in absolute concentration levels (Fig. 2), reaching statistical significance for ∑QACs and nearly all individual homologues (one-way Welch ANOVA: $p < 0.05$, for further details see Tables S7 and S8). Clearly highest ∑QAC concentrations of 4.4–8.1 µg/g were measured in the Saar, followed by the Rhine with concentrations about one order of magnitude lower between 0.6–1.1 µg/g. Lowest overall concentrations were measured in the Mulde, ranging from 210 to 510 ng/g. The same pattern was observed for most individual QACs; only denatonium, EBAC-C12, EDMAC, BTEAC and BTMAC were more abundant in the Mulde than Rhine (Supplementary file 2). While data on QAC contamination in SPM are scarce, these findings are in good agreement with concentrations estimates by Köppe et al. for the years 2006–2018 [8] and previously quantified ∑QAC concentrations up to 8.7 µg/g in SPM of the Saar [52]. Moreover, they fall in the same range as concentrations in river sediments from Austria (~0.1–10 µg/g ∑QACs) [49], Northern Europe (~0.02–25 µg/g ∑QACs) [85], Belgium (~10–70 µg/g of DADMACs C14-C18) [91] and the USA (~20–200 ng/g BAC-C12 and -C14) [92]. The strong spatial variation in this study may result from a combination of SPM properties in each river, predominant land use in the catchment areas, and varying wastewater share at the sampling sites (Table 1).

3.2.1. Potential influence of SPM composition on QAC concentrations

Among the three investigated rivers, SPM of the Saar was characterized by the largest clay fraction and highest contents of TOC, Fe and Al. In contrast, SPM of the least polluted river Mulde showed the highest sand and Si contents (Table 1). QAC cations are well known for their strong affinity to negatively charged clay minerals [10,24]. In comparison, uncharged or low-charged sand and silt particles with a lower specific surface area provide fewer binding opportunities. Thus, the observed differences in QAC concentrations between the rivers may partly be explained by varying contents of clay (Saar > Rhine > Mulde; see Table 1), sand (Mulde > Rhine > Saar) and silt (Rhine ~ Mulde > Saar) in SPM. Spearman correlations indeed revealed statistically significant relationships between SPM particle size and QAC concentrations across all annual SPM samples from 2006–2020 (for 2021 no

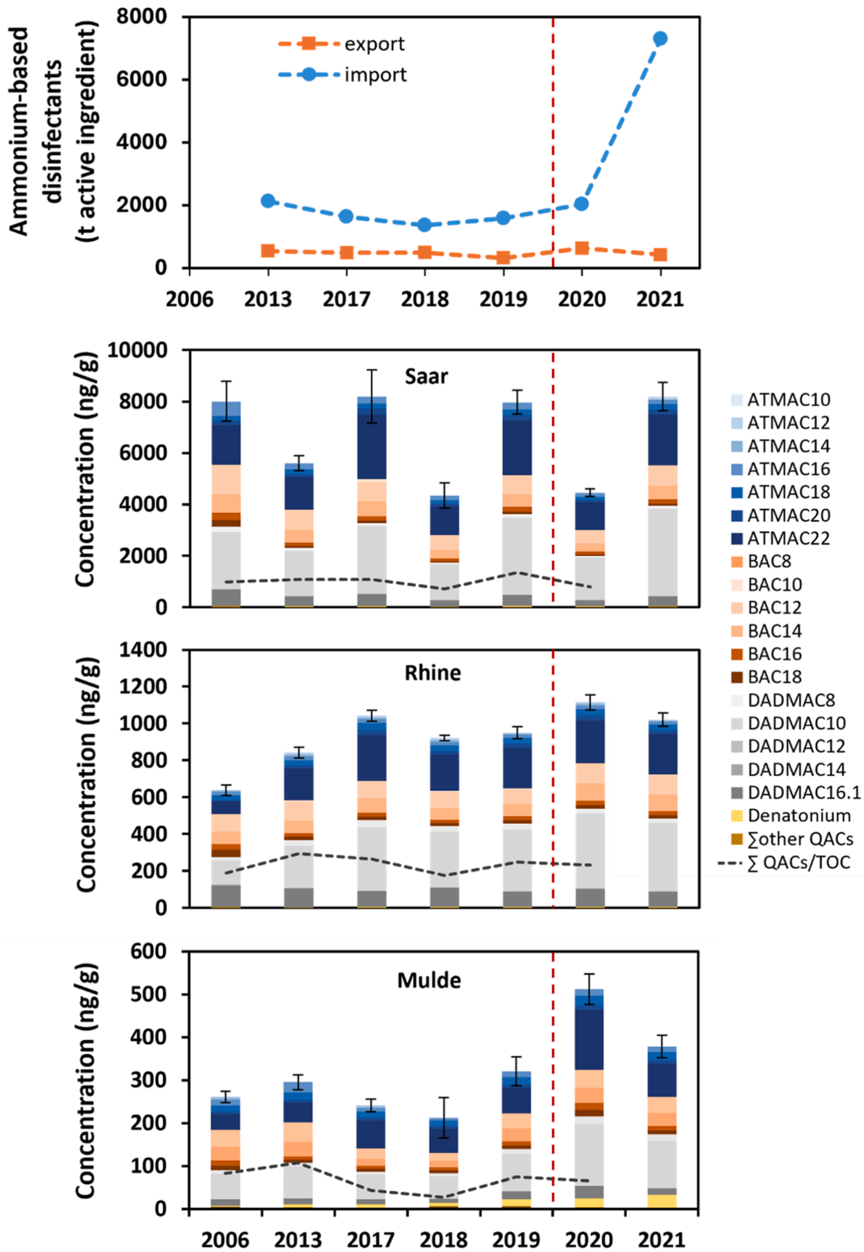


Fig. 3. QAC market trends in Germany and QAC concentrations in annual SPM samples from 2006–2021. Market data were obtained from the Federal Statistical Office of Germany (DESTATIS, 2024) and only publicly available from 2013. Vertical dashed lines indicate the begin of the SARS-CoV-2 pandemic in Germany in March 2020. Gray dashed lines show ∑QAC concentrations normalized for the varying TOC content of SPM (normalized to 1 % TOC). No TOC data were available for 2021. Error bars indicate the standard deviations of ∑QACs measured in three separately processed subsamples.

texture data were available; Table 2). Most QACs showed significant positive correlations with the clay fraction of SPM ($\rho = 0.593 - 0.855$, $p < 0.05$). In contrast, sand content, and for some homologues also silt content, were negatively correlated with QAC concentrations (Table 2). Within the same river, however, no correlations between QAC concentrations and other SPM components were found (Supplementary file 2), possibly due to the comparatively small samples size.

Previous studies have also reported hydrophobic interactions between the alkyl chain of QACs and organic matter in soil and sediment: For example, Sarkar et al. observed lower desorption in TOC-rich soil [24] while Li & Brownawell measured highest QAC concentrations in TOC-rich sediments [48]. Also in this study, peak QAC concentrations coincided with highest TOC contents in SPM of the Saar. However, only the concentrations of four QAC homologues, namely ATMAC-C20, DADMAC-C12, EBAC-C12 and denatonium, showed significant positive correlations with the TOC content across all rivers (Table 2). This may be because ionic rather than hydrophobic interactions could dominate the binding of QACs to SPM. Positive correlations were instead found between Fe content and the concentrations of ATMACs, short-chained DADMACs and denatonium (Supplementary file 2), suggesting that iron (hydr)oxides/minerals may act as sorbents for QACs in SPM. Iron can, however, also occur bound to phyllosilicates of SPM [51], and thus, the apparent correlations between Fe content and QAC concentrations may be mediated by sorption to clay minerals.

Concentrations of a few QACs, including ATMAC-C12, DADMAC-C12, EBAC-C12, denatonium and EDMAC-C12, were also positively associated with the P content of SPM (Supplementary file 2). Positive correlations between QAC and P contents have also been reported for German lake sediments and may be indicative of wastewater discharge or agricultural runoff [93].

Table 2
Spearman correlation coefficients (ρ) between QAC concentrations in riverine SPM across all rivers and SPM composition. Only significant associations ($p < 0.05$) are shown, and significance is indicated as * ($p < 0.05$), * * ($p < 0.01$), * ** ($p < 0.001$) and * ** * ($p < 0.0001$). Pairwise complete observations were used and all QAC measurements <LOD omitted. Numbers of pairs: 11–18. Results including exact p-values for all comparisons are provided in **Supplementary file 2**.

QAC	Clay	Sand	Silt	TOC	TIC	TC
	ρ (p-value)					
∑ ATMAC	0.787***	−0.775***				
∑ BAC	0.8****	−0.798****	−0.498*			
∑ DADMAC	0.75***	−0.763***				
∑ other QACs				0.721***	−0.782***	
∑ QAC	0.752***	−0.754***				
ATMAC12	0.748**	−0.741**				
ATMAC16	0.779***	−0.61**	−0.692**			
ATMAC18	0.765***	−0.769***				
ATMAC20	0.8****	−0.779***		0.498*		0.478*
ATMAC22	0.798****	−0.783***				
BAC12	0.829****	−0.775***	−0.579*			
BAC14	0.812****	−0.789***	−0.515*			
BAC16	0.759***	−0.787***				
BAC18	0.742***	−0.763***				
DADMAC8	0.783***	−0.725***	−0.505*			
DADMAC10	0.736***	−0.756***				
DADMAC12	0.855***		−0.682*	0.773**	−0.755**	
DADMAC16	0.783***	−0.777 ***	−0.47*			
DADMAC18	0.889****	−0.746***	−0.622**			
EBAC12	0.873***	−0.818**		0.655*	−0.714*	0.655*
EBAC14	0.593**	−0.662**				
Denatonium				0.603*	−0.6*	
EDMAC12	0.762*	−0.762*				

Overall, correlation results suggest that differences in SPM composition contribute to the spatial variation between rivers. In particular, the mineral components of SPM appear to play an important role in modulating the binding affinity, and thus fate and transport of QACs under environmental conditions.

3.2.2. *Potential influence of catchment characteristics*

As hypothesized, maximum QAC concentrations were measured at the site Rehlingen/Saar with the highest wastewater proportion of 15 % [59,63] (Table 1). Furthermore, site Rehlingen is situated within the Saarland conurbation (Fig. 1), an area strongly characterized by industrial activity. High QAC concentrations in SPM of the Saar may therefore originate from both municipal and industrial wastewater or resuspension of historically polluted sediment. Compared to other sampling sites of the German Environmental Specimen Bank, elevated levels of other anthropogenic pollutants such as pharmaceuticals [59], plasticizers [60] and PFAS [61] have also been reported for SPM from the Saar. In contrast, the Rhine serves as a drinking water source for about 20 million people [62] and has experienced a reduction in chemical pollution from municipal and industrial wastewater. However, due to its large and diverse catchment area, QACs can still be emitted from settlements, industry and agriculture. Livestock farm effluent in particular has recently been identified as major source of DADMAC-C10 in a Japanese watershed, exceeding the contribution from WWTPs [33]. Moreover, downstream enrichment of pollutants has previously been observed in SPM of the Rhine, e.g. for pharmaceuticals [59]. Therefore, at the sampling site in the city of Koblenz at river km 590, both upstream and direct urban emissions may contribute to QAC contamination. Of all the investigated rivers, the Mulde has the most rural catchment located largely within the nature park Düben Heath. For the sampling site Dessau, only a few potential QAC emission sources were identified including a municipal WWTP, upstream chemical and paper industry [61], or roof or façade runoff after biocidal treatment with e.g. BACs [34]. The comparatively low anthropogenic impact and wastewater share can therefore explain why QAC concentrations were more than one order of magnitude lower in the Mulde compared to the Saar.

3.3. *Temporal changes and potential effect of the SARS-CoV-2 pandemic*

3.3.1. *Annual variation of QAC concentrations*

Not just the absolute concentrations but also annual patterns in ∑QAC concentrations shown in Fig. 3 differed between rivers. Concentrations in the Saar fluctuated strongly year-to-year with no apparent long-term change. In contrast, the Rhine and Mulde showed a gradual increase in ∑QAC concentrations over time, tentatively both before and during the pandemic. Within the same river, most QAC homologues showed similar time profiles and strong positive correlations with each other (**Supplementary file 2**). This suggest that common emission sources and fate processes determined the QAC concentrations in SPM. Interestingly, TOC-normalized QAC concentrations (gray dashed lines in Fig. 3) showed a common trend between all rivers with a dip in 2018 followed by an increase in 2019. Fluctuations in absolute concentrations in the Saar could thus potentially be explained by varying organic matter contents.

To depict the potential imprint of the pandemic on the river-specific annual dynamics of each QAC more clearly, percentual changes in average QAC concentrations between pre- and post-pandemic SPM samples are summarized in Fig. 4. Due to the small sample set with only two independent post-pandemic samples per river, however, statistical testing hardly revealed any significant differences (Table S9).

The Saar exhibited strong year-to-year variation in QACs concentrations (Fig. 3). Peak ∑QAC concentrations around 8–8.1 µg/g were measured in 2006, 2017, 2019 and 2021 while in the other years concentrations only reached 4.4–5.6 µg/g. Maximum absolute concentrations of ∑QACs, ∑DADMACs and DADMAC-C10 were measured in 2021, but considering the strong interannual variation, this may not necessarily be linked to the SARS-CoV-2 pandemic. Surprisingly, averaged over the first two pandemic years 2020 and 2021, the concentrations of most QACs even decreased slightly by −1 to −48 % compared to average pre-pandemic levels (Fig. 4). This was also observed for the key disinfectant ingredients ATMAC-C16 and BACs C12-C18. A potential explanation could be lowered industrial QAC usage in the Saarland conurbation during the pandemic, for example in restaurants, canteens and recreational facilities. Only DADMAC-C10 showed a slight increase of + 15 %, possibly due to its very popular use in commercially available

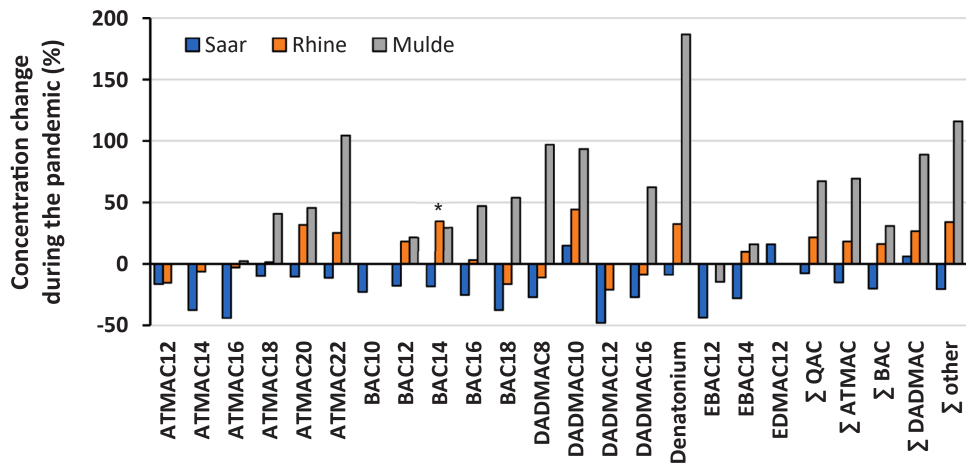


Fig. 4. Percentage change in QAC concentration in SPM between pre-pandemic (mean of years 2006–2019) and post-pandemic begin (mean of 2020 and 2021). The asterisk (*) indicates statistically significant differences for $p < 0.05$ (one-sided t-test with Welch correction). Please note that due to the small sample size statistical significance was not reached in most cases.

antimicrobial products against SARS-CoV-19 [4,5].

In the Rhine, ∑QAC concentrations rose by 60 % over the entire sampling period from 2006–2021. (Fig. 3). The most pronounced increases were observed for ATMAC-C20 (+199 %), ATMAC-C22 (+216 %), DADMAC-C10 (+185 %) and especially denatonium (+ >700 %). Increases in ∑QAC concentrations occurred mainly between 2006–2017 while in 2018 and 2019, most QACs showed a small dip in concentrations. ∑QACs as well as ∑BACs and ∑DADMACs reached peak levels in 2020, leading to an overall 25 % increase in ∑QACs in 2020/2021 compared to average pre-pandemic levels (Fig. 4). This may result from increased disinfectant use during the initial phase of the pandemic since higher post-pandemic concentrations were most notable for the disinfectant ingredients BAC-C12 (+18 %), BAC-C14 (+35 %, $p = 0.013$) and DADMAC-C10 (+44 %). Moreover, they prevailed through both 2020 and 2021. After pandemic begin, maximum concentrations were also measured for the bitter substance denatonium as well as ATMAC-C20 and ATMAC-C22 frequently used in cosmetics. Since concentrations of these QACs and DADMAC-C10 already started to increase in 2006, peak ∑QAC concentrations in 2020 may not be attributable to the SARS-CoV-2 pandemic alone.

In the small river Mulde, the strongest potential pandemic imprint was observed (Fig. 3). Compared to average pre-pandemic levels, ∑QACs (+67 %) as well as ∑ATMACs, ∑BACs, ∑DADMACs and ∑other QACs showed elevated concentrations in 2020/2021. The most affected homologues included long-chained ATMACs C18-C22 (+41 % to +105 %), BACs C12-C18 (+23 % to +54 %), DADMACs C8-C10 (+93 to +97 %), DADMAC-C16 (+63 %) and denatonium (+187 %; see Fig. 4). Like for the Rhine, however, concentrations already gradually rose between 2006–2019 (+23 % for ∑QACs), especially due to ATMAC-C22 (+63 %), DADMAC-C8 (+62 %), DADMAC-C10 (+45 %) and denatonium (+621 %). Therefore, increasing concentrations in 2020/21 cannot be unambiguously linked to pandemic dynamics but may also be affected by other anthropogenic or natural factors. For example, a similar increasing trend was observed in the sediments of a small lake in the USA and attributed to a gradually increasing wastewater share [86]. For the Rhine and Mulde, no time-resolved data on wastewater share are available. However, even continuous low-level QAC discharge could increasingly accumulate in sediment over time - and from there partition to SPM.

3.3.2. *Intra-annual variation*

Besides annual variation, the Saar, Rhine and Mulde also showed distinct fluctuations in monthly QAC concentrations between 06/2018 and 12/2021. Within the same river, up to 6-fold differences in ∑QAC concentrations were observed (Fig. 5). Similar variations in ∑QAC concentrations spanning roughly one order of magnitude were also measured for WWTP influent and effluent sampled monthly during the pandemic in the USA [57] and for DADMAC-C10 measured quarterly in a Japanese watershed in 2023 [33]. In this study, the maximum ∑QAC concentrations in monthly SPM samples were about twice as high as in the pooled annual samples. For some homologues, the differences even ranged up to fourfold. These findings highlight the importance of monitoring intermittent contaminant levels at high temporal resolution.

Among all rivers, monthly ∑QAC concentrations fluctuated the most by up to 6-fold (142 to 801 ng/g) in SPM from the Mulde. Even stronger differences up to 11-fold were observed for ∑BACs, ranging from 19 to 217 ng/g. In contrast, in the Saar, ∑QAC concentrations varied relatively little up to 3-fold (4.7–14 μg/g). In both rivers, the minimum and maximum QAC concentrations were measured in different months each year. Interpretation of these temporal patterns is complicated by the varying availability of monthly samples each year. However, although no regular temporal pattern was discernible, the concentrations were generally consistent with annual samples.

Contrary to the Mulde and Saar, monthly SPM samples from the Rhine were analyzed at regular quarterly intervals (Fig. 5). QAC concentrations typically peaked in December around 1.1–1.8 μg/g ∑QACs and were lowest around 0.5–0.8 μg/g ∑QACs in June or September. The exception was 2021, when concentrations almost plateaued from December 2020 to June 2021. This may be due to the occurrence of the third 'wave' of the pandemic in early 2021. The apparent seasonal pattern in the Rhine may result from lower temperatures and solar irradiance in winter, which affect chemical and biological fate processes [94]. This is supported by significant negative associations between mean water temperatures during SPM sampling and measured concentrations of ATMAC-C18, DADMAC-C10, DADMAC-C16, denatonium, BEC, as well as ∑QACs, ∑BACs and ∑ other QACs in the Rhine (see Table S12). Colder temperatures result in lower biological activity [51], which could reduce the biodegradation of QACs and decrease biomass production and thus total SPM yield, leading to higher contaminant concentrations. Some QACs indeed showed negative associations with SPM yield per sampling period (normalized to grams per day) in the Rhine and Saar but not Mulde (Table S12). The same was previously also observed for concentrations of hydrophobic organic contaminants in suspended sediments [95]. In addition, adsorption of QACs was shown to proceed exothermically, so that lower temperatures could favor the partitioning of dissolved QACs from river water to SPM [96]. Other potential factors could be increased runoff and river flow velocities in winter, which promote sediment transport and resuspension, or higher

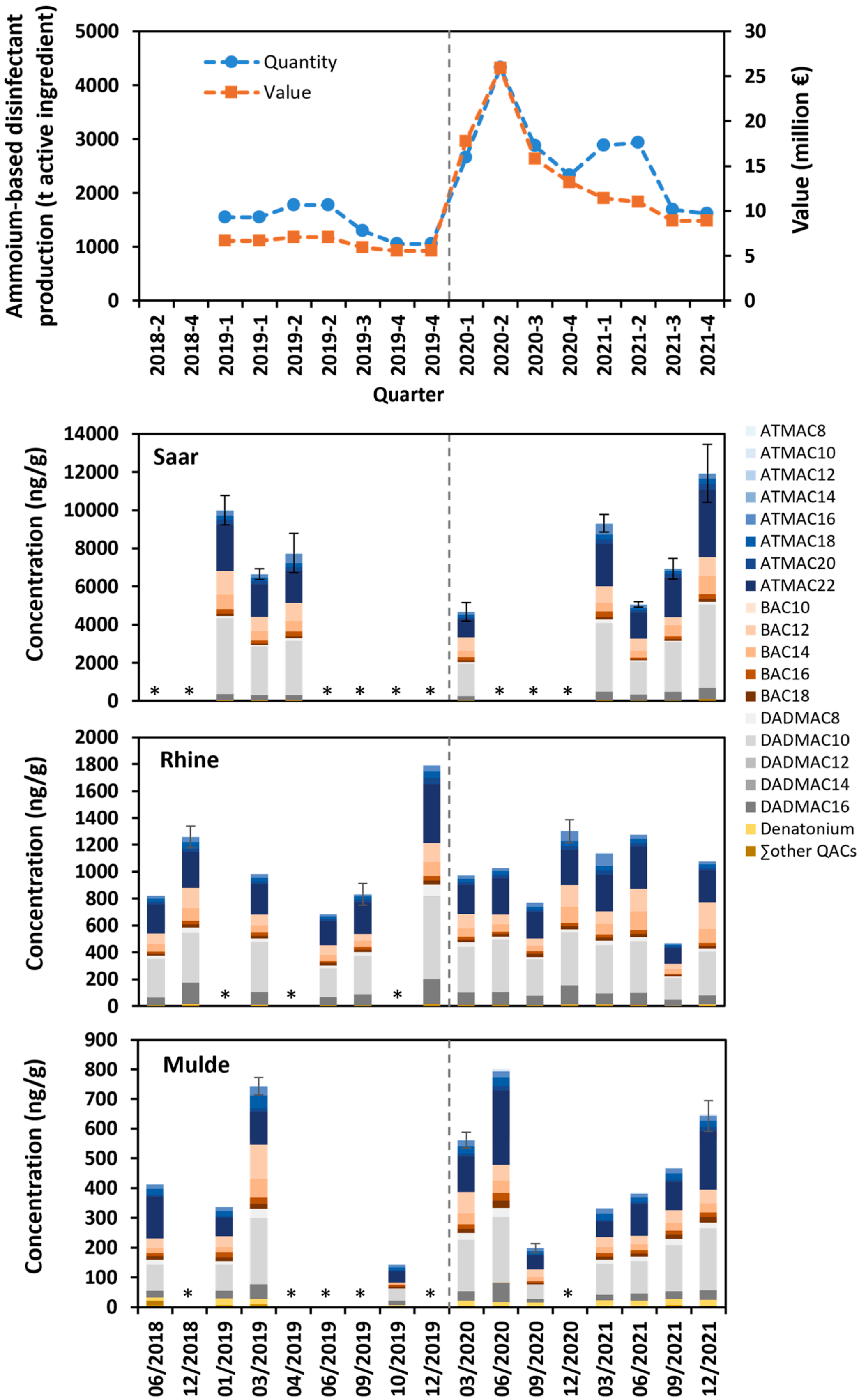


Fig. 5. Production of ammonium-based disinfectants in Germany (top) and measured QAC concentrations in monthly SPM samples. Market data were obtained from the Federal Statistical Office of Germany [82]. Vertical dashed lines indicate the begin of the SARS-CoV-2 pandemic in Germany in March 2020. For time points marked by asterisks (*) no samples were available for analysis. Error bars indicate the standard deviations of ∑QACs measured in separately processed subsamples.

infection rates which increase the use of antimicrobials.

3.3.3. Link between QAC concentrations and pandemic markers

Combined analysis of all rivers revealed positive associations of COVID-19 incidence and COVID-19-attributable mortalities with the SPM concentrations of some QACs, including ATMAC-C22, BAC-C12-C14, DADMAC-C14, ∑QACs, ∑ATMACs and ∑BACs (Table 3a). More and stronger associations were found between QAC concentrations across all rivers and pandemic markers of the previous month. This could be explained by delayed consumer responses to pandemic dynamics (e.g. in hospitals and clinics) or a time lag between QAC use and release into the aquatic environment due to e.g. hydraulic retention in WWTPs. However, the associations varied between rivers (Table 3b), possibly because of differences in sample availability or the influence of environmental fate processes.

In the Saar, only a few QACs, including ATMAC-C18, BAC-C18, DADMACs C12-C14 and EBACs C12-C14, showed strong positive Spearman correlations with COVID-19 incidence in the Saarland or at national level in the same month (Table 3b). No associations were found with pandemic markers of the previous month. In the Rhine, the opposite was observed: Only ATMAC-C18, ∑ATMACs and ∑QACs showed significant positive associations with COVID-19 mortalities in the month of SPM sampling. In comparison, a larger number of QACs correlated positively with COVID-19 mortalities in the previous month, including ATMACs C18-C22, BAC-C16, DADMAC-C8 and BEC as well as ∑QACs, ∑ATMACs and ∑other QACs (Table 3b). In the Mulde, peak ∑QAC concentrations in 2020 and 2021 coincided with the first and fourth wave of the pandemic in Germany and may be partially driven by increased disinfectant use. However, this pattern was only observed for individual DADMACs, long-chained BACs (C16-C18) and very long-chained ATMACs (C20–22), but not for other key antimicrobials such as ATMACs C16-C18 or BACs C12-C14. Moreover, surprisingly, significant positive Spearman correlations with pandemic markers were only observed for the bitter agent denatonium and ∑other QACs (Table 3b). Consequently, the rising QAC concentrations in the 2020 and 2021 annual samples cannot be clearly linked to increased disinfectant use alone. In all rivers, other changes in consumer behavior during the pandemic or longer-term market dynamics may have played a role as well.

Overall, while market data and pandemic markers correlated with concentrations of some QACs, there was no consistent pattern across all rivers, QAC homologues, or even disinfectant ingredients. Similar mixed results have been reported in previous studies. Mahony et al. had tentatively identified QAC sales but not COVID-19 incidence or viral load as a predictor of WWTP loadings in the USA [57]. Moreover, Mohapatra et al. found that concentrations of BAC-C12 but not DADMAC-C10 or BEC correlated with SARS-CoV-2 viral loads in university campus wastewater in Singapore [58]. In all studies, a combination of factors, especially other uses of QACs than in disinfectants, may have partly masked the direct link between QAC concentrations and SARS-CoV-2 pandemic markers. For example, discharge from animal husbandry, in particular swine farming, was recently identified as a key source of DADMAC-C10 [33] and was likely unaffected by the SARS-CoV-2 pandemic. Detailed source identification was however beyond the scope of this study and should be addressed in future research.

Table 3a
Spearman correlations between QAC concentrations in riverine SPM across all rivers and German QAC production volumes or pandemic markers in the same and the previous month/quarter. Only significant associations ($p < 0.05$) are shown, and significance is indicated as * ($p < 0.05$) and * * ($p < 0.01$). Pairwise complete observations were used and all QAC measurements <LOD omitted. Numbers of pairs: 20–27. ρ: Spearman correlation coefficient.

River	Variable 1	Variable 2	ρ (significance)	
			same month	1-month shift
Combined	QAC-salt production	BTEAC	−0.701*	
		DADMAC14		−0.847*
	Incidence (national)	DADMAC14	1*	
	Mortailities (federal state)	ATMAC16		0.728**
		ATMAC18		0.669**
		ATMAC20		0.613*
		ATMAC22	0.45*	0.654**
		BAC12	0.447*	0.672**
		BAC14	0.488*	0.615*
		BAC16		0.625**
		DADMAC8		0.544*
		DADMAC10		0.637**
		DADMAC16		0.578*
		EBAC12		0.613*
		BEC		0.699*
		∑ QAC	0.517*	0.674**
		∑ ATMAC	0.462*	0.672**
		∑ BAC	0.464*	0.647**
		∑ DADMAC		0.637**

Table 3b
Spearman correlations between QAC concentrations in riverine SPM of individual rivers and German QAC production volumes or pandemic markers in the same ad the previous month/quarter. Only significant associations ($p < 0.05$) are shown, and significance is indicated as * ($p < 0.05$) and * * ($p < 0.01$). Pairwise complete observations were used and all QAC measurements <LOD omitted. Numbers of pairs: 5–7 (Saar), 8–11 (Rhine), 7–9 (Mulde). ρ: Spearman correlation coefficient.

River	Variable 1	Variable 2	ρ (significance)	
			same month	1-month shift
Saar	QAC-salt production	BAC18		−0.757*
		DADMAC12		−0.775*
		DADMAC14		−0.847*
	QAC-disinfectant production	BAC14	−0.778*	
	Incidence (federal state)	DADMAC12	1*	
	Incidence (national)	ATMAC18	1*	
		BAC18	1*	
		DADMAC14	1*	
		EBAC12	1*	
		EBAC14	1*	
		∑other QACs	1*	
Rhine	QAC-salt production	BTEAC	−0.943*	
	QAC-disinfectant production	DADMAC8		−0.627*
	Mortailities (federal state)	ATMAC18	0.762*	0.893*
		BEC		0.821*
		∑ QAC		0.786*
		∑ ATMAC		0.786*
		∑other QACs		0.821*
	Motalities (national)	ATMAC18	0.786*	
		ATMAC20		0.821*
		ATMAC22		0.893*
		BAC16		0.821*
		DADMAC8		0.964**
		∑ QAC	0.786*	
		∑ ATMAC	0.786*	0.857*
Mulde	QAC-disinfectant production	BAC12		−0.881**
		EBAC12		−0.881**
	Incidence (federal state)	Denatonium	0.786*	
		∑other QACs	0.821*	
	Incidence (national)	Denatonium	0.786*	
		∑other QACs	0.821*	
	Mortailities (federal state)	Denatonium	0.793*	
		∑other QACs	0.829*	

3.4. Risk assessment

For many alkyl-substituted QACs, sediment PNECs in the ECHA dossiers were not directly based on experimental data but derived by applying the equilibrium partitioning method, often combined with a read-across approach from other registered substances. Moreover, water-SPM, water-sediment or water-soil partitioning coefficients essential for estimating bioavailability and correct application of the equilibrium partitioning method [97] were experimentally determined only for the mixture of ATMACsC20-C22 [98], BACs C12-C16 [99] and DADMAC-C10 [79]. Hence, there is considerable uncertainty in the obtained ecotoxicological effect values of individual alkyl-substituted QACs as well as their mixtures for sediment organisms. In some cases, no PNECs were provided with the justification that no exposure of sediment was expected (DADMAC-C8 [100], mixture of DADMACs C8-C10 [101]). Screening studies from across the world have however demonstrated the opposite and reported accumulation of these compounds in sediments from China, Japan, the USA, Austria, Northern Europe and Greenland [48,49,85,102]. Information on sediment toxicity or homologue-specific partitioning behavior is also rare in the scientific literature [9,11,12], highlighting a crucial gap in the ecotoxicological risk assessment of QACs in solid compartments.

For most QACs and QAC combinations, calculated RQs were 1–3 orders of magnitude below effect levels (Table 4), both before and after the onset of the SARS-CoV-2 pandemic (Supplementary file 2). However, concentrations of long-chained ATMACs and DADMACs exceeded established effect thresholds. Notably, for the ATMAC mixture of C18-C22 much lower PNECs were established than for the mixtures of C20-C22 or C12-C18. This is because for the C18-C22 mixture further data from more toxic structural analogues, namely unsaturated ATMACs and ATMAC-methyl sulfates, were considered in the overall assessment although these are not registered components of the mixture. The stricter PNEC values suggest exceedance of safe levels of long-chained ATMACs by up to 2.5-fold to 42-fold in all the three German rivers. Risk quotients were hardly affected by the SARS-CoV-2 pandemic in the Saar (pre-pandemic: 39, post-pandemic: 31) and Rhine (pre-pandemic: 3.9, post-pandemic: 3.7) but increased by twofold in the Mulde (pre-pandemic: 1.03, post-pandemic: 2.5; Supplementary file 2). Based on the assessment for ATMACs C20-C22, however, only in the Saar ecotoxicological effect levels were reached. Ambiguities such as these complicate the risk assessment for QAC mixtures and highlight the need for further empirical hazard data. For BACs and 'other' QACs, no risks were identified (RQs <0.5 both pre- and post-pandemic). However, the estimated PNEC values and sorption coefficients applied in the equilibrium partitioning method may not correctly reflect partitioning and bioavailability of all chain lengths, and this should be accounted for in the future. Concentrations of DADMAC-C16 nearly reach effect levels in the Saar, while combined with estimated concentrations of the semi-quantified DADMAC-C18, PNECs are exceeded by up to twofold in the pre-pandemic period, and by up to 1.6-fold post-pandemic begin (Supplementary file 2). Long-chained, highly sorptive ATMACs and DADMACs were thus present in SPM at concentrations exceeding currently established effect thresholds for the EU. Further refinement of sediment toxicity data is however urgently needed for a more reliable risk assessment. While estimated risks were similar between pre- and post-pandemic for the Saar and Rhine, they increased by up to twofold in the Mulde after pandemic begin.

Comparison of theoretical PNEC-MICs to SPM concentrations of BAC-C12 and DADMAC-C10 did not show any evident risks for selection of AMR (Table 4), neither pre- nor post-pandemic begin (Supplementary

Table 4
Calculated risk quotients (RQ) for exposure of freshwater sediment or SPM to different QACs and QAC mixtures. Predicted no-effect concentrations (PNEC) for reference substances were obtained from the European Chemicals Agency and compared to maximum measured QAC concentrations in annual SPM samples (MEC_{max}). For CLQ, BEC, domiphen no PNECs were available. Only for some QAC mixtures regulatory assessments were carried out PNECs for microbial selection of resistant mutations (PNEC-MIC) were calculated as described in Section 2.5. See footnotes for further information. For mixtures highlighted in bold, RQs exceed 1 indicating potential risks for sediment organisms.

QACs and mixtures	Reference substance ECHA assessment	**PNEC** (µg/g)	$MEC_{max,\ SPM}$ (µg/g)			**$RQ_{sediment}$**		
			Saar	**Rhine**	**Mulde**	**Saar**	**Rhine**	**Mulde**
∑ATMAC (C10-C18)[1]	QACs, C12-C18 (even numbered) alkyltrimethyl chloride	68	0.762	0.079	0.041	0.011	0.0012	0.0006
∑ATMAC (C20-C22)	**QACs, C20 −22-alkyltrimethyl, chloride**	**1.25**	**2.74**	**0.274**	**0.150**	**2.2**	**0.22**	**0.12**
∑ATMACs (C18-C22)	**QACs, C18 −22 (even numbered) alkyltrimethyl, chlorides**	**0.07**	**2.95**	**0.313**	**0.174**	**42**	**4.5**	**2.5**
BAC-C12	Benzododecinium chloride	6.8	1.13	0.110	0.046	0.17	0.02	0.01
BAC-C14	Miristalkonium chloride	5.61	0.73	0.093	0.036	0.13	0.02	0.01
∑BAC (C12-C14[2]	Benzyl-C12 −14-alkyldimethyl-ammonium chlorides	68	1.86	0.203	0.079	0.027	0.003	0.0012
BAC-C18	Benzyldimethyl(octadecyl)ammonium chloride	0.64	0.26	0.04	0.01	0.41	0.064	0.023
∑BAC (C16-C18[2]	QACs, benzyl-C16-C18 (even numbered)-alkyldimethyl, chlorides	68	0.548	0.07	0.031	0.0081	0.001	0.0005
∑BAC (C8-C18)[3]	QACs, benzyl C12-C16 (even numbered)-alkyldimethyl chlorides	68	2.41	0.247	0.108	0.035	0.0053	0.0032
∑EBAC (C12-C14)	QACs, C12 −14-alkyl[(ethylphenyl)methyl] dimethyl, chlorides	6.81	0.049	0.004	0.003	0.0072	0.0006	0.0004
∑DADMAC (C8-C10)	QACs, di-C8 −10-alkyldimethyl, chlorides; DDAC	n.d./62[4]	3.51	0.43	0.16	0.06	0.01	0.003
∑DADMAC (C16-C18)	QACs, di-C16 −18-alkyldimethyl, chlorides	55	8.82	1.26	0.07	0.16	0.02	0.001
∑DADMAC (C8-C16)	QACs, di-C12 −18-alkyldimethyl, chlorides	5.5	3.91	0.53	0.19	**0.71**	0.10	0.03
∑DADMAC (C8-C18)	**QACs, di-C12 −18-alkyldimethyl, chlorides**	**5.5**	**11.2**	**1.42**	**0.199**	**2.0**	**0.26**	**0.04**
Denatonium	Denatonium benzoate	25	0.028	0.004	0.031	0.001	0.0002	0.0012
BTMAC	Benzyltrimethylammonium chloride	1.12	<LOD	<LOD	0.004	na	na	0.004
BTEAC	Benzyltriethylammonium chloride	0.118	<LOD	0.0007	0.001	na	0.006	0.0112
EDMAC-C12	C12 −14 (even-numbered)-alkylethyldimethyl, ethyl sulphates	9.27	<LOD	<LOD	<LOD	na	na	na
PYR-C12	1-dodecylpyridinium chloride	0.362	<LOD	<LOD	<LOD	na	na	na
QACs	**Reference**	**PNEC-MIC (µg/g)**	**$MEC_{max,\ SPM}$ (µg/g)**			**RQ_{SPM}**		
			Saar	**Rhine**	**Mulde**	**Saar**	**Rhine**	**Mulde**
BAC-C12	[76]	8519[5]	1.13	0.11	0.05	0.00013	0.00001	0.00001
DADMAC-C10	[76]	438[6]	3.40	0.41	0.14	0.0078	0.0009	0.0003

1. ATMACs C10-C18: PNECs partly based on read-across approach from BACs
2. PNECs based on assessment for C12-C16 mixture, identical values for mixtures of BACs C12-C14 and BACs C16-C18 mixture
3. Same PNEC as for C12-C16 mixture assumed
4. "no exposure of sediment expected" according to ECHA for DADMAC-C8 (CAS 5538-94-3) and the mixture of DADMACs C8-C10 (CAS 68424-95-3), hence values for DADMAC-C10 adopte
5. Based on MIC for most sensitive tested bacteria: *Escherichia coli* (Gram-negative)
6. Based on MIC for most sensitive tested bacteria: *Escherichia coli* (Gram-negative) and *Enterococcus faecalis* (Gram-positive)

file 2). However, it should be noted that PNEC-MICs for SPM were only estimated based on soil sorption coefficients, which do not fully describe the dynamic equilibrium of QAC partitioning between water and SPM. Moreover, it remains unclear to what extent QACs sorbed to different SPM components are bioavailable to microorganisms. Notably, Heyde et al. observed that sorption to smectite clay minerals buffered the acute antimicrobial activity of QACs, resulting in elevated nominal MIC values, while the same was not observed for kaolinite [76]. Furthermore, MICs of the four tested bacterial strains may not be representative of the entire pathogenic microbiome in surface waters, nor was a distinction made between Gram-positive and typically more tolerant Gram-negative bacteria. The calculated PNEC-MICs therefore only represent a rough estimate of the potential QAC concentrations at which selection for resistant mutations could occur, and the applied approach requires further improvement before it can provide a valid, generic threshold for AMR selection.

3.5. Recommendations for a safer use of QACs

While disinfectant application can be a crucial public health measure for infectious disease control, excessive and uncontrolled use may have dire consequences for human and environmental health. Therefore, rising trends of QAC concentrations in SPM are concerning and call for a thorough re-evaluation of the broad use of antimicrobials across an ever-increasing number of applications. The current study demonstrated that on top of the general upward trend, not just residential [19,54] but also environmental exposure to QACs increased during the SARS-CoV-2 pandemic. To prevent further environmental harm, we suggest the following: Firstly, QACs should be applied according to the 'essential use approach', i.e. only when essential for maintaining public safety or societal functioning, and when no alternatives exist [103,104]. This permits the targeted and controlled application of QACs as disinfectants in healthcare while in residential settings, less hazardous disinfectants such as ethanol or normal hand soaps and surface cleaners may often suffice [11]. QACs used as antistatics or surfactants in consumer products and industry could also be replaced by safer alternatives such as the more biodegradable esterquats [105,106]. In addition, recommendations for preventive measures during pandemics should be evidence-based and regularly updated according to known transmission pathways. SARS-CoV-2, for example, was found to spread primarily via droplet infection and not surfaces. Hence, massive surface disinfection in public areas may have had little added benefit compared to other preventative measures such as frequent aeration, maintaining general hygiene and social distancing, but drastically increased human and environmental exposure to QACs [107]. Clear communication of the potential risks of excessive antimicrobial use can guide authorities and the public to make more informed choices about top-down and individual-level preventive measures. Finally, when resorting to disinfectant use, measures should be taken to minimize the risk of spreading AMR. Application according to the label recommendations (dose, frequency and target use), avoiding repeated treatments with the same substances, and maintaining good aeration are key steps to foster a diverse microbiome and prevent the formation of environmental niches for tolerant pathogens [108].

4. Conclusion

This study confirmed QACs as a major group of contaminants in SPM in German rivers. As hypothesized, concentrations were affected by the chemical structure of QACs, emission dynamics (market volumes and pandemic markers) and environmental factors (temperature and SPM yield, texture, and composition), but the contribution of each differed between sampling sites. SPM of all three investigated rivers Saar, Rhine and Mulde showed a strikingly similar distribution of the 31 analyzed QAC homologues, which was dominated by long-chained and thus more hydrophobic ATMACs (C18-C22) and DADMACs (C16-C18), as well as two key disinfectant ingredients (BAC-C12 and DADMAC-C10). Despite this common pattern, the ∑QAC concentrations differed by up to two orders of magnitude between the rivers, following the hypothesized pattern Saar > Rhine > Mulde. This high spatial variability is likely linked to the different wastewater share at the sampling sites, population density and predominant (historical) land use in the catchment areas, as well as the clay fraction and possibly TOC content and composition of mineral phases of SPM. Identifying catchment-specific emission sources was however not the main aim of this study and should be investigated in future studies using broader sampling strategies with a higher spatial resolution.

Surprisingly, the tentative imprint of the SARS-CoV-2 pandemic on QAC concentrations was mostly visible in the small river Mulde and not just limited to key disinfectant ingredients. Besides DADMACs-C8 and -C10, strongest increases in concentrations were observed for the cosmetic ingredients ATMACs C20 and -C22 as well as the bitter agent denatonium, which already showed a rising trend in the Mulde before the pandemic. Moreover, the potential effect of the pandemic was far exceeded by spatial and intra-annual variation in QAC concentrations. Opposite to the trend in absolute concentrations, the magnitude of concentration changes during the pandemic and of monthly variation followed the order Mulde > Rhine > Saar. This suggests that less polluted rivers may be more susceptible to intermittent pollution releases and highlights the need for spatiotemporally resolved monitoring to capture peak contaminant concentrations.

Based on a simplified risk assessment, in the Saar long-chained ATMACs and DADMACs reach ecotoxicological effect thresholds for sediment set by the ECHA both before and after the begin of the SARS-CoV-2 pandemic. This suggest that current regulation may not be sufficiently protective and further steps should be taken to minimize QAC emissions, in particular by limiting non-essential applications. Important directions of future research include source identification, development of enhanced removal technologies and synthesis of 'greener', less toxic and more biodegradable alternatives. Moreover, the current risk assessment should be improved by closing significant knowledge gaps regarding experimentally derived partitioning behavior, bioavailability and toxicity of many different QAC homologues as well as their mixtures and degradation products in surface water-sediment or water-SPM systems. Furthermore, indirect effects on microorganisms, including (functional) microbial diversity and the potential contribution of QACs to the spread of AMR, are currently not addressed in regulatory safety assessment but warrant urgent attention. This is particularly relevant for surface water SPM, which forms an important habitat for microorganisms where tolerant pathogens could acquire drug-resistance and then enter drinking water resources, putting also human health at risk.

The current findings confirm particle-association of QACs to SPM as vector for their environmental fate by facilitating transport from emission sources, distribution within surface waters, and potential entry into floodplain soils after SPM deposition. Although to a lesser extent than previously reported in residential environments or wastewater, the SARS-CoV-2 pandemic also appeared to have increased the QAC concentrations in SPM of two of the investigated rivers, showing that its chemical imprint likely reached downstream environments. This demonstrates the need for a holistic consideration of the ecological side-effects of chemical-based public health interventions, especially for biocidal compounds such as QACs that could contribute to the global threats of biodiversity decline and an impeding AMR crisis. While trends were assessed retrospectively in this study, future broad-scale increases in chemical use, e.g. due to pandemics, should be accompanied by close temporal monitoring for early detection and mitigation of potential side-effects in the environment.

CRediT authorship contribution statement

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used DeepL in order to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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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Supplementary materials

Supplementary File 1.
Supplementary file 2.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.136237.

Data Availability

Data will be made available on request.

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