

<https://doi.org/10.56770/pst2026225>

OCCURRENCE OF QAC IN SOIL AND ROLE IN THE SELECTION OF ANTIBIOTIC RESISTANCE

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Submitted February 25, 2026; Revised June 18, 2026; Accepted June 22, 2026; Published July 20, 2026

ABSTRACT

The rise of multi resistant pathogenic bacteria is a pressing 21st century challenge and a significant threat to human health. To combat the proliferation of antimicrobial resistant pathogens, a more judicious use of antibiotics in medical treatments is essential. Quaternary ammonium compounds (QACs) are high production volume, surface active antimicrobial chemicals widely used in agriculture. This review comprehensively examines QAC concentrations in soils and explores how these concentrations and the fate of QACs in soils influence the proliferation of antibiotic resistance in the environment. We also discuss predicted environmental concentrations for QACs applied to integrons, clay minerals, montmorillonite, and interlayer sorption.

Keywords: QAC, Soil, Antibiotic resistance

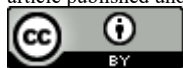
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INTRODUCTION

In agriculture, reducing the prophylactic use of antibiotics, as recommended for pig farming, can mitigate the risks of selecting and spreading multidrug resistant

pathogens. Similarly, the EU banned the use of antibiotics for growth promotion in 2006 [1-3]. As in human healthcare, the agricultural sector must prioritize improved

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hygiene to minimize antibiotic use. However, stricter hygiene standards often lead to increased disinfectant use, which in turn raises the risk of antimicrobial resistant strain development [4-8]. One of the most often used disinfectants is quaternary ammonium compounds (QACs). Significant amounts of QACs also enter agricultural fields through the application of manures, sewage sludge, or wastewater for nutrient recycling, although the environmental occurrence of QACs, including concentrations, fate, ecotoxicity, and analytical methods, has recently been reviewed with an emphasis on wastewater and aquatic environments. Therefore, by synthesizing current understanding and research needs surrounding the production, usage, detection, and environmental destiny and impacts of QACs. In particular, we concentrate on the destiny of quaternary alkylammonium compounds (QACs) and how they contribute to the emergence of antibiotic resistance in microbial communities in agricultural soils. Ultimately, we propose an agroecosystem specific research hypothesis in which the interlayer regions of expandable 2:1 clay minerals act as significant sites for QAC sequestration [9-13].

The entire class of quaternary ammonium compounds is referred to by the term QAC. Four covalently bonded organic moieties encircle the quaternary nitrogen atom in QACs, giving the nitrogen atom a persistent positive charge. They exist as salts

because a counter ion, usually chloride or bromide, balances this charge. QACs are divided into three major groups based on the ternary amine utilized during synthesis alkylation: (a) linear alkylammonium, (b) imidazole, and (c) pyridinium compounds. Each of these groups include a large number of compounds and subgroups. QACs are less basic and cannot be protonated, in contrast to primary, secondary, and tertiary amines. QACs act as water structure breakers rather than being hydrophilically hydrated, whereas primary amines can surround themselves with hydration spheres and create up to three hydrogen bonds. Examples of soil relevant QACs, often overlooked when discussing surface active and biocidal members, include diquat and paraquat. These two QACs are among the most widely used herbicides globally and have also been detected and retained in soil [14-16].

A benzyl group, two methyl groups, and one alkyl chain between C8 and C18 surround the cationic nitrogen in homologues of benzyl ammonium compounds (BACs). The primary ingredients of disinfection products, C12-C14 homologues, are linked to the highest biocidal action. When QACs reach their critical micelle concentration, they form micelles because of their amphiphilic nature. The dynamic equilibrium between surfactant molecules in micelles and those in cellular membranes is caused by this feature, which also influences the effectiveness of

numerous surfactant related applications. Eventually, this equilibrium may cause the cell membrane to dissolve, which would have biocidal effects. There are relationships between rising hydrophobicity and falling critical micelle concentration for alkyl trimethyl ammonium compounds (ATMACs) and DADMACs. However, data on critical micelle concentration are incomplete and inconsistent across authors due to varying experimental setups. While several studies have reported calculated values for the octanol–water partition coefficient for QACs, these are not valid due to the surfactant nature of this compound class [17, 18]. In conclusion, QACs are efficient surfactants and antibacterial disinfectants due to the combination of a positive charge and hydrophobic alkyl chains, as well as the resulting amphiphilic characteristics. Their chemical configurations encourage electrostatic interactions with negatively charged surfaces, such as cell walls or soil clay minerals, as well as with soil organic matter or (hydr-)oxides at higher pH levels.

APPLICATIONS OF QACS

Quaternary Ammonium Compounds (QACs) are versatile molecules found in many disinfectants and surfactants, serving a wide array of purposes. They function as softener active agents in fabric softeners and enhance the thickening, foaming, or wetting properties of surface cleaning agents, laundry detergents, and personal care products. Their

use in hair conditioning is particularly notable due to their ability to attach to the negatively charged hair cuticles [19, 20]. Beyond consumer products, QACs have significant industrial applications. They act as debonding agents in paper production, lubricants for conveyors and chains, and adhesion promoters in bitumen emulsion for road construction. In oilfield operations, QACs stabilize swelling clays, improve foaming, wetting, and suspension properties, and serve as corrosion inhibitors. The mining industry utilizes them in mineral flotation separation processes. For biostatic and biocidal applications, QACs are key ingredients in disinfectants and wood preservatives. In agriculture, they are found in pesticide formulations and are common components of disinfectants and detergents used in animal husbandry, including the dairy cattle, hog, and poultry industries. Among specific QAC types, Alkyl trimethyl ammonium chlorides (ATMACs) are primarily used in hair care products as conditioners and preservatives. Commercial products predominantly feature ATMACs with C14, C16, and C18 alkyl chain lengths. The extensive use of Dimethyl dialkyl ammonium chlorides fabric softeners raised concerns in the 1990s due to elevated concentrations and aquatic toxicity in industrialized nations. Their use in fabric softeners was drastically reduced as a result of an ecological risk assessment and a

voluntary industry phase out. Since then, they have been replaced by more biodegradable diethyl ester dimethyl ammonium chlorides (DEEMDMAC). Over 90% of industrial production is currently made up of dialkyl dimethyl ammonium [21-25]. Applications for benzalkonium chlorides (BACs) of different chain lengths are numerous and include everything from eye drops and hair washes to surface disinfectants in hospitals, food manufacturing, agriculture, and the dairy industry. In technical blends of C8–C18 homologues, BACs are commonly used. The primary ingredients of disinfection products, C12–C14 homologues, are linked to the highest biocidal action. QACs have also gained particular importance in the industrial preparation of organoclays. These modified clay minerals are used in thixotropic fluids, drilling fluids, as rheological control agents in paints, cosmetics, and refractory varnishes, and as adsorbents for organic pollutants in contaminated site remediation and protective landfill liners. Montmorillonite, a dioctahedral smectite commonly found in soil environments, is a key mineral used in organoclay preparation. Therefore, understanding the interactions between this clay mineral and QACs is crucial when assessing the environmental fate of QACs in soil [26-31].

OCCURRENCE OF QAC IN SOIL AND ENVIRONMENT

Data on the production and consumption of QACs are scarce and fragmented across numerous sources. Environmental contamination by QACs significantly surpasses that of pharmaceuticals, often by orders of magnitude. Given the diverse applications of QACs, it is unsurprising that they are detected in substantial quantities across various environmental compartments.

Soils

Studies indicate a high potential for QACs to accumulate in sediments after release into rivers or marine waters, even when initial QAC concentrations are below critical levels [32-36]. It is also likely that similar chemicals will be enriched in other ecosystems exposed to QAC sources. Significant levels of QACs may be present in agricultural soils, especially those that receive organic fertilizers or wastewater for water and nutrient recycling. However, monitoring data on background concentrations in various soils is mostly lacking, and the fate and transport of QACs in soils are still poorly studied. The use of manure, sewage sludge, and wastewater, as well as their preventative usage as biocides and adjuvants in agricultural pesticide formulations, are the main routes for QAC entry into agricultural soils. In order to improve solubility, rain fastness, and leaf penetration, QACs are frequently included as adjuvants in pesticide formulations, particularly for water insoluble chemicals.

Based on personal communications with local hog farmers and product

information on disinfectants used before restocking, the estimated concentration of DADMAC-C10 in manure was in the mg kg^{-1} range. Assuming zero biodegradation due to strong sorption and efficient sequestration, predicted QAC concentrations in agricultural soil might reach up to 3.65 mg kg^{-1} after a year of manure input. BAC homologues (carbon chain length 8–18) were identified in a recent study from Korean soils that were covered with disinfectants. The study demonstrated that BACs were retained by soils, with concentrations in the low mg kg^{-1} range found in soil samples taken six months after coverage, despite the authors' scant information regarding the types of soil or the nature of the BAC contamination. BAC-C12 and BAC-C14 were found to have the highest amounts [37, 38]. The only other available soil data point is a reported DTDMAC concentration of 15 mg kg^{-1} in soil previously amended with sewage sludge. Both studies demonstrate that soils have the capacity to store QACs over time.

Marine and Freshwater Environments

Surface water, usually from rivers, and one marine water research are examples of data for aquatic habitats. For wastewater, the mean and median cumulative QAC values are 271 and 11 lg L^{-1} , respectively, but for surface water, they are 33 and 40 lg L^{-1} . The mean and median concentrations of ATMACs in surface water (10 and 8 lg L^{-1}) are significantly higher than those found in wastewater. These figures,

however, reflect study design limitations rather than real world concentrations. Most studies aimed to validate analytical methods, with water samples typically collected from areas expected to have some degree of QAC contamination, leading to an overrepresentation of urban areas compared to pristine environments [39–41].

Wastewater systems

Industrial discharges, effluents, and influents are all considered wastewater. The cumulative values for separately determined DADMAC, ATMAC, and BAC concentrations are impacted by the occasionally arbitrary distinction between river water measured near wastewater intake and indirect industrial discharge. It is well known what happens to QACs in wastewater systems. The majority of surfactants are effectively removed by wastewater treatment facilities (WWTPs) at typical rates of 95% to 99%. However, sorption to sludge is partially responsible for this removal. With a median of 59 mg kg^{-1} and a mean of 220 mg kg^{-1} , total QAC concentrations in the eight sewage sludge investigations that are currently available approach the mg kg^{-1} limit. For DTDMAC in anaerobic sludge, the maximum reported content was 8 g kg^{-1} ; however, this concentration should be interpreted with caution. Overestimation is probably caused by the colorimetric detection process, which creates disulfine blue complexes [42–44].

Sediments

Estimating total QAC concentrations in sediments is challenging due to data being compiled from various studies using different authors, methods, and detected QAC homologues. The data reported here are not from systematic screening, and standard methods do not exist. Nevertheless, published data indicate significant accumulation of QACs in sediments of rivers and estuaries, ranging from 0.1–100 mg/Kg. DTDMAC values can exceed 25 mg/Kg. Concentrations of QACs up to 74 mg/Kg in sewage impacted estuarine sediments were found to be higher than other organic contaminants measured in the same or nearby samples. A review on 'emerging' organic contaminants in biosolids for agricultural application showed that QAC concentrations rank fourth in the upper 1g/Kg range. This is consistent with literature data and an order of magnitude larger than pharmaceutical concentrations found in biosolids.

ANALYTICAL METHODS USED FOR QAC

The chemical characteristics and related analytical challenges of quaternary ammonium compounds (QACs) make it difficult to investigate their environmental destiny and toxicity. QACs were usually measured colorimetrically following complexation with anionic dyes prior 1980s. Nevertheless, this approach was found to be inappropriate for complex environmental matrices that also contained anionic surfactants. High pressure liquid chromatography

(HPLC) eventually supplanted a number of scientific tools and techniques that had been created over time. Cation exchange chromatography, thin layer chromatography, GC/MS techniques (based on thermal breakdown and Hofman degradation product detection), potentiometric measurement using specific electrodes, and infrared spectroscopy techniques were among them. Fortunately, extraction procedures for cationic compounds from sewage sludge have significantly improved. Traditionally, an acidic methanol extraction followed by a time consuming liquid–liquid extraction sequence was used. Supercritical fluid extraction has also been employed as an alternative. Solid phase microextraction (SPME) methods are effective at concentrations below 2 mmol per liter but are only suitable for batch sorption experiments or ecotoxicity tests due to the necessary equilibration of extraction fibers. A major analytical challenge is the strong sorption of QACs to surfaces, which complicates result interpretation, particularly in biodegradation studies. Systematic data has shown analyte loss due to sorption to reaction vessels, caps, and materials used in solid phase extraction (SPE) and SPME. Using aluminum foil has been shown to reduce losses of BAC-C12, BAC-16, and ATMAC-C16 to screw cap lids. Another obstacle to QAC analysis is their potential to form complexes with anionic compounds, such as anionic surfactants. Depending on the

analytical method, these complexes' altered hydrophobic nature can make QACs difficult to detect. Conversely, adding various counter anions during sample extraction has been shown to increase extraction efficiency and analytical response. For example, a zwitterionic detergent has been used as a matrix modifier in water samples. Others have employed octadecyl benzene sulfonate or the detergent Marlon A375 (a sodium alkylbenzene sulfonate) as hydrophobic ion pair complexing agents for sewage sludge extraction.

In HPLC, adding 0.1% (volume) trifluoroacetic acid (TFA) to methanol/water has been found to reduce peak tailing compared to the widely used formate, indicating superior ion pairing properties. Perfluoro heptanoic acid has also been shown to enhance BAC recoveries when added during the extraction of fortified soil samples.

Liquid chromatography (normal or reversed phase) with conductivity detection and post-column ion pair creation with phase separation and UV detection have been used for compound specific study of cationic surfactants. However, electrospray tandem mass spectrometry (ESI-MS/MS) has emerged as the primary analytical method due to the incomplete chromatographic separation of all homologues. For the purpose of identifying specific QACs and measuring their quantities in environmental samples, this technique is effective and selective. While LC-MS/MS offers a convenient way to analyze these nonvolatile, large

molecular mass cationic surfactants without derivatization, accurately determining their exact concentrations remains challenging due to their surface activity [45-46]. Ultrasonic assisted extraction (UAE) with acidic methanol has proven effective for extracting analytes from solid environmental matrices. For developing soil screening extraction procedures, several recent multi compound protocols for sewage sludge can serve as valuable references. These protocols have reported low limits of quantitation (LOQ) between 1 and 4 ng/g and demonstrated high versatility, with one study successfully quantifying 23 quaternary ammonium compound (QAC) homologues from Chinese sewage sludge.

More recently, a UAE procedure using an acetone:acetonitrile (1:1 v/v) mixture successfully recovered benzalkonium compounds (BACs) from contaminated or spiked soil samples. However, further method development is needed to establish a standard analytical procedure for QAC extraction from agricultural soils. Accelerated solvent extraction (ASE) also warrants exploration for soil samples, as it could significantly reduce solvent consumption [47, 48]. Currently, analytical methods for environmental samples, particularly regarding analyte extraction and separation, vary widely. This variation is so extensive that nearly all studies on QAC concentrations differ in their analytical details. Consequently, a standardized analytical protocol for sample screening, especially for soil

samples, is still lacking. At present, the preferred method is an ASE-SPE approach, which involves an ion pairing agent for sample extraction and cleanup, followed by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS).

ROLE OF QAC IN THE SELECTION OF ANTIBIOTIC RESISTANCE

There is mounting evidence that using quaternary ammonium compounds (QACs) as biocides against microbes will eventually develop antibiotic resistance in addition to decreasing biocide efficacy. Increased expression of energy driven drug efflux systems, like the NorA and MdeA multidrug efflux systems or the AcrAB pump, which can export both QACs and multiple antibiotics. Numerous QAC resistance genes that are extensively distributed in environmental and clinical bacteria have been identified. Energy dependent efflux pumps that target more than thirty lipophilic cationic molecules from at least twelve distinct chemical classes are encoded by these genes. For instance, QAC-C has been shown to confer resistance to several β -lactam antibiotics. Cross resistance between biocides and antibiotics can also result from cell wall modifications that reduce permeability [49, 50].

Beyond factors like contact time, temperature, and target organism, the concentration of biocides is particularly important for selecting antimicrobial resistant bacteria. Co-selection of antimicrobial resistance

primarily occurs when bioavailable and bioaccessible QAC concentrations do not exceed biocidal levels. While "in-use" concentrations typically far exceed the lethal dose, environmental concentrations of bioavailable and bioaccessible QACs are likely much lower, potentially allowing for the co-selection of antimicrobial resistance. For example, exposure of bacteria to sub-lethal QAC concentrations has been shown to select for antibiotic-resistant mutants. BAC-tolerant *Salmonella*, compared to the wild type, exhibited significantly decreased susceptibility to 12 of 24 tested antimicrobial compounds across eight classes. Long term exposure of microbial communities (developed from contaminated sediment) to sub-inhibitory BAC concentrations led to reduced diversity and an enrichment of *Pseudomonas* species. Concurrently, susceptibility to BACs and antibiotics like penicillin G, tetracycline, and ciprofloxacin decreased, linked to compound degradation/transformation and efflux pump activity [51, 52].

QAC resistance genes and class 1 integrons are often embedded in plasmids or transposons, facilitating their spread within bacterial populations. These mobile genetic elements can carry multi resistance and heavy metal resistance genes, as reported for QAC-A and QAC-B genes, respectively. They have also been associated with degradative pathways in pesticide polluted environments. Class 1 integrons containing QAC-genes are frequently

found on IncP-1 plasmids, which are broad-host-range plasmids widely distributed in environments such as soil, manure, sludge, digestates, and river sediments [53, 54]. Human diseases have also been found to have plasmids with QAC resistance genes. For example, a gentamicin and methicillin-resistant *Staphylococcus aureus* was found to have a plasmid that confers resistance to kanamycin, gentamicin, tobramycin, amikacin, benzalkonium chloride, acriflavin, ethidium bromide, and chlorhexidine. Three years of BAC exposure reduced phylogenetic diversity in comparison to an untreated control, according to a comparative metagenomic investigation of a microbial community from contaminated river silt. This was mainly because *Pseudomonas* species were enriched in BAC-fed communities. Additionally, it was found that applying sewage or manure containing antibiotics and heavy metals can enrich QAC resistance genes in soil, while biocide exposure enriched multidrug efflux pumps and selected for genes linked to mobile genetic elements.

CONCLUSION

A thorough understanding of the fate and ecotoxicological consequences of QACs applied to soils is essential for a trustworthy risk assessment for human and environmental health, despite mounting evidence that QACs can enhance antibiotic resistance in environmental microorganisms. The kinetics and reversibility of QAC movements into

and out of clay mineral interlayers must be evaluated in order to determine the involvement of clay minerals in the sequestration and persistence of QACs in soil.

Author(s) Contribution: Data collection and analysis, H. Zubair and H. S. Qaiser.; review and proof-reading, A. Mauwiz Aziz and M. A. Anas.

Funding: No funding involved.

Ethical Approval: Not applicable.

Conflict of Interest: Nil.

Consent for Publication: All authors approved the manuscript for publication.

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