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Current Opinion in Biotechnology

Anthropogenic antimicrobial micropollutants and their implications for agriculture

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Antibiotics and disinfectants have saved millions of human lives and cured uncountable animal diseases, but their activity is not limited to the site of application. Downstream, these chemicals become micropollutants, contaminating water at trace levels, resulting in adverse impacts on soil microbial communities and threatening crop health and productivity in agricultural settings and perpetuating the spread of antimicrobial resistance. Especially as resource scarcity drives increased reuse of water and other waste streams, considerable attention is needed to characterize the fate of antibiotics and disinfectants and to prevent or mitigate environmental and public health impacts. In this review, we hope to provide an overview of why increasing concentrations of micropollutants such as antibiotics are concerning in the environment, how they can pose health risks for humans, and how they can be countered using bioremediation strategies.

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Current Opinion in Biotechnology 2023, **80**:102902

This review comes from a themed issue on **Environmental Biotechnology**

Edited by **Jaime Martinez Urtaza** and **Luis De Leon**

For complete overview of the section, please refer to the article collection, “Environmental Biotechnology (2023)”

Available online 20 February 2023

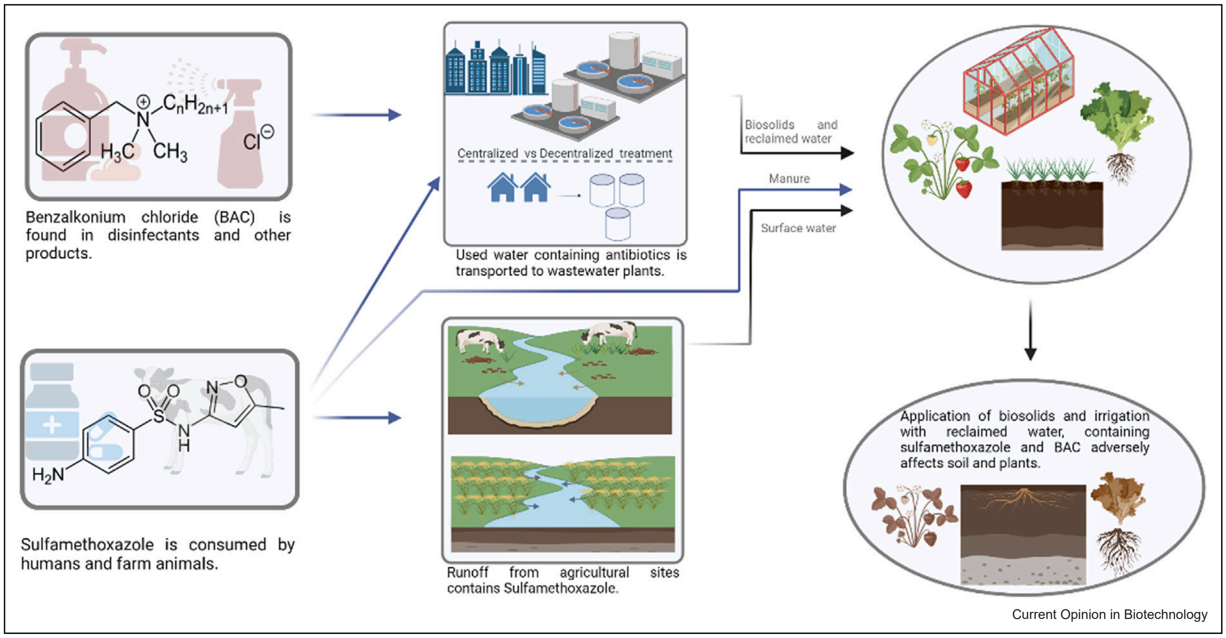
https://doi.org/10.1016/j.copbio.2023.102902

Introduction: antibiotics and disinfectants as micropollutants

We need antibiotics and disinfectants to battle infections and to grow healthy livestock, but widespread use has resulted in many serious problems such as emerging antimicrobial resistance, multidrug-resistant pathogens, and adverse effects on environmental microbial ecosystems [1]. In the United States alone, approximately 11 000 000 kg of antibiotics are sold and used for therapeutic and subtherapeutic purposes [2], and particularly in response to the COVID-19 pandemic, disinfectant use has skyrocketed [3]. At the same time, global development is driving changes in agricultural processes, including increased water reuse and land application of biosolids, in the name of sustainability and closing the loop on waste streams. Neither centralized nor decentralized wastewater treatment processes are designed to remove antibiotics or disinfectants, and these chemicals persist in both effluent waters and biosolids, ultimately resulting in contamination of agricultural soil (Figure 1). Understanding the fate of antibiotics and disinfectants is thus especially important, as a significant portion will end up where reclaimed water or biosolids are used for resource recovery [4]. In this review, we explore the fate, environmental impacts, and possible remediation strategies for sulfamethoxazole and benzalkonium chloride (BAC), as example antibiotics and disinfectants, respectively, focusing on their occurrence and implications in agricultural settings [5]. We chose sulfamethoxazole and BAC as they are among the most extensively used antibiotics and disinfectants, commonly found in nontarget environments, and relevant to agricultural settings such as agricultural runoff, biosolids/manure, and farmland soil (Figure 2).

Sulfonamides are bacteriostatic and inhibit the synthesis of folic acid [6]. Sulfamethoxazole is a sulfonamide antimicrobial, widely used as a pharmaceutical antibiotic for both human and animal bacterial infections. Approximately 18 000 000 kg of sulfamethoxazole enters the biosphere each year [7]. As antibiotics cannot be completely metabolized, sulfamethoxazole and its metabolites (e.g. N-acetyl-sulfamethoxazole) are excreted via urine and feces, entering hospital wastewater, municipal wastewater treatment plants, aquatic environments, agricultural runoff [8], and agricultural land as farmyard manure is applied as fertilizer [9]. A recent review [10] reported that sulfamethoxazole appeared in environmental matrices at the highest concentration (up to 3460.57 ng/L in groundwater) among commonly used antibiotics, including β-lactams, tetracycline, and fluoroquinolones. As a micropollutant, sulfamethoxazole directly negatively impacts human health and environmental quality and indirectly endangers lives via the introduction of antibiotic-resistant bacteria and associated genes to food production [1,11,12]. Sulfamethoxazole was added to the European Union watch list of pollutants that should be carefully monitored in surface water in 2020 in response to potential risks it posed in aquatic environments [13].

Figure 1



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Antibiotics and disinfectants travel from their point of use, either passing through municipal wastewater treatment facilities, decentralized treatment (such as graywater treatment or composting), or bypassing them entirely, ultimately contaminating agricultural soil via irrigation and fertilizer. Created with BioRender.com.

BAC, a subset of quaternary ammonium compounds (QAC), is a type of disinfectant and cationic surfactant. They have a positive charge on the nitrogen atom and the general formula of $[C_6H_5CH_2N(CH_3)_2R]Cl$, where R represents a mixture of alkyls ranging from C8 to C16 [14]. BACs are used extensively in both household and commercial products, eventually accumulating in municipal wastewater [15]. As a result of using biosolids on agricultural fields as fertilizers, BACs are introduced to soil environments. In addition, using reclaimed wastewater for irrigation, or even through direct discharge from inadequate treatment facilities, BACs may enter soil and aquatic systems. Owing to its alkyl chains and positive charge, BAC is more likely to be retained by soil rather than becoming bioavailable by leaching [14]. BAC is usually found in the environment in low concentrations, but high concentrations can also be found in effluents of facilities such as hospitals or industries such as hydraulic fracturing. Concerns exist surrounding antibiotic resistance and cross-resistance invoked by exposure to BACs even at low concentrations [15–17]. BACs can also be genotoxic in eukaryotic cells at concentrations as low as 1.0 mg/L, adding to its environmental and health risks [18].

Micropollutants such as sulfamethoxazole and BACs accumulating in environmental matrices such as water and soil raise concerns about ecological impacts and public health risks. They thus require careful monitoring, evaluation, and mitigation.

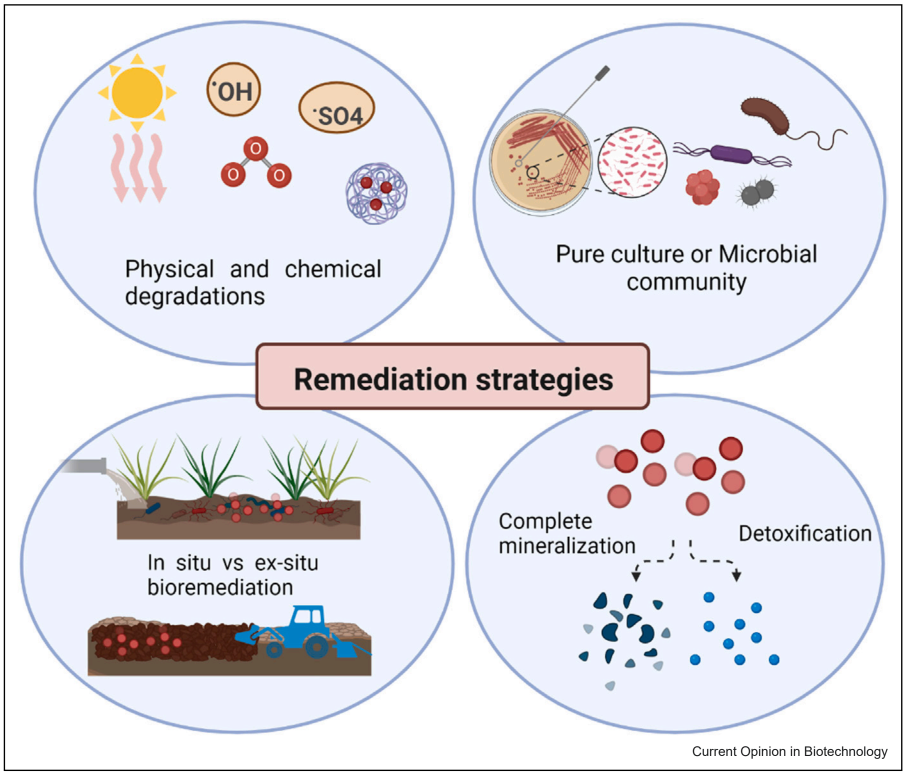
Impacts of antimicrobial micropollutants on soil microbiota and plants

Contamination in soil can change taxonomic and functional characteristics of microbial communities. Two major concerns about the impacts on soil microbiota are decreased productivity in terms of nutrient cycling and increased antimicrobial resistance.

Sulfamethoxazole contamination not only alters the phylogenetic profile of soil microbiota, but also changes their functional profile [12]. A study using composting material, including straw and cow manure supplemented with 25, 50, or 100 mg/kg sulfamethoxazole, monitored over 45 days, found that the protease, cellulase, and urease content in the control setting (no sulfamethoxazole) was significantly higher ($p < 0.05$) than all the sulfamethoxazole treatments. These results indicate that sulfamethoxazole has inhibitory effects on nutrient cycling.

Similarly, BAC pollution can impact nitrogen conversion [19]. A study examining two different soil samples (acidic forest soil and alkaline cultivated soil) spiked with 0.1 g of BAC and monitored for 60 days found that the relative abundance of *nifH* (which encodes nitrogenase ferritin) in acid forest soil increased by 91% and 79% in alkaline soil.

Figure 2



Remediation strategies range from abiotic methods to use of pure culture or microbial communities to biodegrade the antibiotics present in the environment. Strategies can be implemented *in situ* or *ex situ* and may or may not result in complete mineralization. Created with BioRender.com.

Also, in both soil samples, after 7, 15, and 60 days, the abundances of ammonia-oxidizing archaea (*AOA*) and bacteria (*AOB*), as indicated by their monooxygenase gene (*amoA*), were significantly higher compared with day 0. The higher abundance of *AOA* and *AOB* correlates with higher ammonia oxidation capacity of soil [20]. This suggests that the presence of BAC in soil amendments may inadvertently decrease available nitrogen. In the same vein, a three-year study on BAC-contaminated river sediments showed that these microbial communities were noticeably decreased in diversity compared with the control samples (no BAC exposure) [15].

Micropollutant exposure may also enrich antimicrobial resistance. Understanding this process is particularly important in the One Health framework, as resistance in environmental and agricultural settings is intrinsically linked to resistance in the clinic, and antimicrobial resistance is one of the most pressing global threats to public health [21]. BAC resistance in human pathogens in pure culture can be nonspecific resistance through morphological and cell surface properties (i.e. alterations in fatty acid composition) [19,22], as well as specific through efflux pumps (i.e. *mdrL*). Enrichment of both nonspecific antimicrobial resistance strategies and specific efflux pumps is problematic for public health, as these strategies may translate both to increased resistance against the biocide in question and other clinically or commercially relevant antimicrobials. As shown in a longitudinal study conducted in a pig farm, withdrawal of antimicrobial (apramycin or trimethoprim sulfamethoxazole) decreased multidrug-resistant and increased fully susceptible *Escherichia coli*, indicating the importance of responsible use of antibiotics such as sulfamethoxazole in agriculture [24].

Once in soil, micropollutants can affect plants both directly and indirectly via the microbiota. Inhibition of chlorophyll biosynthesis, hindering the rhizosphere microbiome and rhizosphere enzyme activity reduction are some effects of antibiotic accumulation in soil [23]. Sulfamethoxazole is no exception, acting as a competitive inhibitor of dihydropteroate synthase, which is an important enzyme in folate metabolism [20]. In strawberries (*Fragaria ananassa*), sulfamethoxazole first damages the roots and reduces the photosynthesis rate after being transported to the leaves [8]. There is also indirect damage caused by sulfamethoxazole; when photosynthesis is reduced, the excessive light energy affects the reactive oxygen species balance and damages membranes.

Although studies on QAC toxicity to terrestrial crops are scarce, it is reported that high QAC concentrations decrease root fresh weight, total root length, and photosynthetic pigment content, leading to severe inhibition of plant growth [25]. Based on evidence from multiple other organic pollutants, uptake of QAC by lettuce, cucumber, and spinach is likely. BAC has a molecular mass of approximately 300 Daltons and its solubility is greater than 500 g/L. It therefore has a high potential for uptake by plants [26]. BAC uptake by lettuce and toxicity effects such as reduced plant dry weight, necrosis, chlorosis, and wilting were observed [27].

Remediation and mitigation strategies

Remediation technologies can be categorized into physical, chemical, and biological approaches. Physical and chemical technologies such as advanced oxidation and flocculation are used despite their drawbacks of by-product toxicity and high cost [28]. Novel physical and chemical treatment methods include redox transformation of sulfamethoxazole by nanoscale zerovalent iron particles [29], reduction of pollutant bioavailability using environment-friendly biochar [30], and degradation of QACs, including BAC, using ozone/UV treatments [31]. Notably, many technology-intensive methods are designed for centralized treatment facilities rather than decentralized facilities or treatment at the point of use. Here, we focus our discussion of remediation on biodegradation-based strategies due to their cost-effectiveness and versatility.

Biocidal characteristics of sulfamethoxazole and BAC dictate that successful biodegradation relies on microorganisms with resistance to the micropollutants as well as production of degrading enzymes. Isolates from several genera biodegrade sulfamethoxazole, for example, *Rhodococcus*, *Archromobacter*, *Variovorax*, *Microbacterium*, *Arthrobacter*, *Orchrobacterum*, and *Paenarthrobacter* [23]. Biodegradation of BAC can be performed by strains in the genus *Pseudomonas*, which is known for its resistance to disinfectants and its ability to degrade various organic contaminants [32]. In addition, *Bacillus niabensis*, *Xanthomonas* sp., *Thalassospira* sp., and *Aeromonas* sp. can degrade QACs [7,15]. These degraders were enriched and isolated from traditional sources such as activated sludge [33,34] and surface water [35]. In addition, waste yeast biomass [36] (*Ogataea polymorpha*), white rot fungi (*Phanerochaete chrysosporium*) [37], and marine diatoms (*Chaetoceros muelleri*) [38] have been explored as resources to enhance treatment and expand application scenarios.

Complete mineralization of micropollutants is ideal but not always achievable. Notably, one strain of *Pseudomonas* completely mineralizes BAC, which is considered rare in the environment [14,39]. Advances in analytical approaches such as HPLC–MS, LC–MS/MS, and GC–MS [40,41] allow for identification of putative metabolites and proposal of pathways for sulfamethoxazole and BAC biodegradation [22,42], which can further assist the assessment of toxicity reduction. From a biotechnology application standpoint, it is of interest to decipher the 'microbial ingredients' to successful (either complete mineralization or biotransformation to low-toxicity products) biodegradation. Growing evidence indicates that microbial communities outperform pure cultures [23], motivating investigations into micropollutant-degrading communities with molecular biology methods. Ertekin et al. revealed *Pseudomonas* sp. strain, BIOMIG1 as a key BAC degrader and proposed a responsible gene cluster in BAC-degrading microbial communities developed from four different habitats (sewage, activated sludge, soil, and sea sediment) through 16S rRNA pyrosequencing and metagenomics sequencing. Oh et al. [42] identified a candidate amine oxidase gene for BAC dealkylation using metatranscriptomics and further verified the gene product activity from cell extracts. Qi et al. [44] fed microbial community samples from wastewater treatment plants with sulfamethoxazole and 3-amino-5-methylisoxazole (3A5MI, an intermediate of sulfamethoxazole degradation) to enrich for biodegrading groups of these two chemicals. DNA-stable isotope probing (SIP) along with 16S rRNA gene sequencing and other analytical approaches revealed key players in complete sulfamethoxazole biodegradation, where *Paenarthrobacter* and *Nocardioides* were the primary degraders of sulfamethoxazole and 3A5MI, respectively, and nondegraders *Acidovorax* and *Sphingobium* enhanced 3A5MI degradation. Considering that only 1% of bacteria and archaea can be cultivated, applying the above technologies is vital in studying and screening micropollutant-degrading microbial communities. This approach is more effective, and less labor-intensive compared with the traditional 'enrichment–isolation–verification' route.

Successful bioremediation of micropollutants is limited by poor bioavailability, as well as suboptimal environmental conditions [45]. *Sphingobacterium mizutaii* can achieve 93.87% degradation of 50 mg/L sulfamethoxazole in 7 days under optimal conditions (30.8°C, pH 7.2), while degradation as low as $< 40\%$ was observed under nonoptimal conditions [43]. Co-contamination might also be an important factor, as biodegradation of one BAC was inhibited in the presence of another BAC with different R-chains [18]. The limitations of operational conditions of bioremediation technologies (e.g. temperature, pH, and initial targeted micropollutant concentrations) can be challenging because of high energy and operation costs even in centralized treatment facilities where system control is possible. Owing to the characteristics of the routes of micropollutants entering agricultural settings (Figure 1), *in situ* bioremediation at the source, reservoir, or decentralized treatment facilities is desired for more effective mitigation. In this context, limited working conditions of bioremediation should be addressed since environmental conditions are usually not subject to control for *in situ* remediation. To this end, immobilization of micropollutant-degrading microbes on biochar [45], agriculture residues (bagasse) [46], and amendment with *in situ*-generated biogenic manganese oxides [40] to enhance biodegradation under wider environmental conditions or low pollutant concentrations were investigated. In addition to combining biological approaches with physical and chemical approaches, 'non-conventional' biological resources can also facilitate *in situ* bioremediation. For example, *Chaetoceros muelleri*, a marine diatom that can be applied as an aquaculture feed and agriculture fertilizer, was able to remove 38.9% sulfamethoxazole over 6.3 days [38]; *Ogataea (Hansenula) polymorpha* as a resource for BAC contamination bioremediation was evaluated in the context of recycling waste yeast biomass from bioethanol production [36]. These explorations, although not immediately effective or successful, broaden the possibilities of applying bioremediation technologies *in situ*, inspiring the development of fertilizers or feeds containing micropollutant-degrading microbes.

Conclusions and outlook

Antibiotics and disinfectants such as sulfamethoxazole and BAC are necessary, as is water reuse and resource recovery. However, when present as micropollutants, these compounds threaten soil and crop health by disrupting microbial communities and damaging plant tissues directly and indirectly and contribute to the global threat of antimicrobial resistance. While biological processes that are commonly employed in wastewater treatment can degrade micropollutants, they need to be engineered intentionally to target such chemicals individually or in mixtures. This lens needs to be applied not only to large-scale centralized facilities but also to decentralized facilities, which are increasingly adopted for on-site water reuse or composting [47]. Such facilities are considerably different from their municipal counterparts and require special considerations for risk assessment [48]. Incomplete mineralization and constraints on environmental and operational conditions are major challenges faced by bioremediation technologies, especially for *in situ* applications that benefit agricultural settings the most. Advanced analytical tools such as mass spectrometry and molecular biology tools such as metagenomics [49], metatranscriptomics, and SIP can be used to study processes occurring *in situ*, revealing potential problems, helping to quantify risk, and suggesting potential bio-based solutions. Combining physical, chemical, and biological approaches and exploring unconventional resources for micropollutant degraders enable new advancements in cost-effective and easy-to-apply bioremediation technologies for *in situ* contamination mitigation. Given the prospect of increasing production of antibiotics and disinfectants, as well as increasing resource reuse and agricultural demand for water and nutrients, we need to rethink and manage how these products are used, transported through environmental matrices, and treated to ensure that they remain effective and do not cause harm.

CRediT authorship contribution statement

AAM: Investigation, Visualization, Writing – original draft; **WS:** Writing – review & editing; **EMH:** Conceptualization, Writing – review & editing, Supervision.

Conflict of interest statement

The authors have no conflicts to declare.

Data availability

No data were used for the research described in the article.

Acknowledgements

WS and EMH are supported in part by the National Science Fundation Chemical, Bioengineering, Environmental, and Transport Systems Division, USA (grant ID: 2043156).

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