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Environmental fate and ecotoxicological impacts of antibiotic transformation products: Microbial reactivation, resistance selection, and ecosystem risks

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Abstract

Antibiotics and their transformation products (TPs) make up a critical class of emerging contaminants. While parent compounds are frequently monitored, their metabolic derivatives often bypass detection due to analytical gaps and a lack of systematic risk assessments. This review evaluates the persistence, bioactivity, and ecological impact of antibiotic TPs across ecosystems. Our review illustrates the biotic and abiotic pathways of TP formation, showing that these metabolites often retain or amplify the bioactivity of their parent compounds. We elucidate how subinhibitory concentrations of TPs induce physiological stress, quorum sensing disruption, biofilm formation, and fundamental biogeochemical cycling, and how chronic exposure to trace levels of TPs drives antimicrobial resistance. To address these risks, we advocate for integrating improved wastewater treatment with advanced analytical frameworks, including non-target screening and multiomics approaches. We propose the implementation of TP-specific risk assessments and green antibiotic design to mitigate the global escalation of such risks.

Keywords: Antibiotic transformation products; Emerging contaminants; Antimicrobial resistance; Ecotoxicity; Non-target screening; Green antibiotic design

1. Introduction

Antibiotics are fundamentally important in contemporary human and veterinary medicine, livestock production, and aquaculture, but their widespread and growing use creates multiple pathways through which antibiotic residues can enter natural and engineered environments. Estimated global antibiotic consumption increased from approximately 40.8 billion defined daily doses (DDDs) in 2016 to 49.3 billion DDDs in 2023 and is projected to reach approximately 75.1 billion DDDs by 2030 if

current trends continue.[1] As administered antibiotics are not always fully metabolized in humans or animals, this growing consumption has environmental implications. Both parent antibiotics and metabolites are excreted in urine and feces during or shortly after administration, entering municipal sewage, hospital discharges, manure, biosolids, aquaculture discharges, and agricultural runoff. Pharmaceutical manufacturing, inappropriate disposal of unused medicines, and leakage from waste-management systems can all result in multiple geographically heterogeneous emission pathways, leading to additional releases.

The environmental occurrence of pharmaceuticals is a global, not a local, phenomenon. The ubiquity of active pharmaceutical ingredients was demonstrated in a large-scale survey of 1,052 sampling points along 258 rivers in 104 countries, showing that pharmaceutical pollution occurs across regions with highly different socioeconomic and wastewater management conditions.[2] Thus, aquatic microbial communities are usually exposed to dynamic mixtures of parent antibiotics, metabolites, transformation products (TPs), other pharmaceuticals, nutrients, metals, disinfectants, and other anthropogenic contaminants, rather than a single antibiotic. Conventional municipal wastewater treatment plants (WWTPs) are designed to remove biodegradable organic matter, suspended solids, nutrients, and pathogens, rather than trace organic micropollutants. Hence, the removal of antibiotics may be incomplete and compound-specific, driven by a combination of biodegradation, sorption, transformation, re-transformation, and dilution rather than complete mineralization.[3] Therefore, a decrease in the measured concentration of a parent antibiotic during treatment cannot be interpreted by default as elimination of its overall biological or environmental hazard.

This limitation is particularly pertinent because conventional environmental assessments have generally focused on unchanged parent antibiotics. An antibiotic released into the environment represents only one element of a larger, dynamic chemical continuum. This continuum includes human and animal metabolites, microbial biotransformation products, photolysis products, hydrolysis products, oxidation products, conjugated metabolites, and TPs generated during treatment.[3,4] Transformation may occur during metabolism before excretion, in sewage networks and wastewater treatment processes, or later in surface water, groundwater, sediment, soil, manure, and other environmental matrices. The products may have substantially different physicochemical and biological properties from the parent molecules. Transformation may reduce antimicrobial activity or toxicity, but it does not necessarily result in detoxification since some TPs retain antimicrobial activity, display altered ecotoxicity, become more mobile or persistent, or interact differently with sediments, organic matter, microorganisms, and biological membranes.[3,4] Moreover, the lack of analytical standards and the large number of unknown or poorly characterized TPs may lead to underestimation of the overall chemical burden associated with antibiotic use when using conventional targeted monitoring.

Another important complication arises from the reversibility of certain transformation processes. Biological or chemical transformation of conjugated or other transformed antibiotic derivatives back to parent compounds may result in delayed or spatially displaced exposure. For example, studies on sulfonamide antibiotics have shown that transformation and conjugation processes in activated sludge can create reservoirs of antibiotic-related compounds that contribute to persistent environmental exposure.[5] Modeling and experimental assessments of biological wastewater treatment have also shown that the re-transformation of conjugated metabolites can impact apparent antibiotic-removal efficiencies and subsequent environmental exposure estimates.[6] Therefore, rather than assuming that the depletion of the parent molecule represents irreversible elimination, the environmental fate should be conceptualized as a parent–metabolite–TP continuum.

Antibiotics and their TPs can be disseminated across wastewater, freshwater, marine environments, soil, sediment, and biota after discharge, or through land application via effluent discharge, irrigation, flooding, agricultural runoff, sorption and desorption, sediment resuspension, and possibly trophic transfer. The environmental fate of TPs is affected by pH, redox conditions, solar radiation, temperature, dissolved and particulate organic matter, mineral surfaces, hydrological conditions, and the structure and activity of microbial communities. These factors collectively determine the persistence, mobility, bioavailability, transformation pathways, and composition of the contaminant mixtures to which environmental microorganisms and higher organisms are exposed.[4,7] Importantly, environmental concern is not limited to conventional acute ecotoxicity. Antibiotics, even at subinhibitory concentrations, can still exert biologically meaningful selective pressure on microorganisms, making antimicrobial resistance (AMR) an environmental risk endpoint that is not adequately represented by conventional ecotoxicological assessment alone.[8] Therefore, environmental risk assessments must incorporate microbial community responses and resistance selection in addition to standard test organism toxicity

assessment.

Recent experimental evidence strongly supports extending this assessment to TPs of parent antibiotics. Lakhey *et al.*[9] showed that several antibiotic TPs influenced microbial growth in wastewater-derived microbial communities at concentrations similar to the corresponding parent antibiotics. More crucially, the 7-day evolution experiments showed that TPs such as desmethyl ofloxacin, *N*-acetyl sulfamethoxazole, and desmethyl erythromycin enriched the class 1 integron-integrase gene *intI1* to levels comparable to or even higher than those produced by the corresponding parent antibiotics.[9] The *intI1* gene is commonly employed as a marker linked to anthropogenic pollution and the spread of mobile resistance determinants.[10] These results challenge the notion of antibiotic TPs as environmentally negligible residues and point toward the possibility that at least some TPs may be active ecological stressors able to contribute to the overall selective pressure for AMR. This is especially true in wastewater environments, where complex microbial communities, antibiotic-related compounds, resistance genes, and mobile genetic elements can all coexist in the same ecological compartment.

However, large knowledge gaps remain regarding the identity, occurrence, persistence, mobility, bioactivity, mixture effects, resistance-selection potential, and environmental reactivation of antibiotic TPs. Many environmental monitoring programs still focus on a relatively small number of target parent compounds. In contrast, the lack of reference standards, incomplete transformation libraries, and limitations of conventional targeted analytical methods hamper routine surveillance of metabolites and unknown TPs. Recent advances in liquid chromatography coupled with high-resolution mass spectrometry (LC–HRMS), suspect screening, non-target screening (NTS), effect-directed analysis, metagenomics, resistome profiling, and systems-biology approaches have provided new opportunities to characterize these neglected components of antibiotic pollution and associate chemical exposure with ecological and evolutionary responses.[4]

To address this, the main objective of this review is to critically evaluate the environmental relevance of antibiotic TPs throughout their environmental life cycle, from their formation during human or veterinary metabolism and wastewater treatment to their transport, persistence, re-transformation, ecotoxicity, and implications for AMR. Specifically, this review aims to: (i) identify the principal metabolic, microbial, photochemical, hydrolytic, oxidative, and treatment-related pathways responsible for TP formation; (ii) evaluate the occurrence, environmental distribution, mobility, persistence, bioavailability, and potential reactivation of parent antibiotics and their TPs across wastewater, surface water, groundwater, soil, sediment, and biota; (iii) critically assess evidence regarding the antimicrobial activity, ecotoxicity, mixture effects, microbial-community disruption, and AMR-selection potential of antibiotic TPs; (iv) evaluate recent analytical developments, particularly LC–HRMS-based suspect screening and NTS, effect-directed approaches, metagenomics, and resistome-analysis tools, for identifying and prioritizing environmentally relevant TPs; (v) identify major deficiencies in existing environmental monitoring and regulatory risk-assessment frameworks that predominantly consider parent antibiotics; and (vi) integrate these processes into a conceptual framework linking antibiotic use and release with environmental transformation, microbial exposure, ecological effects, resistance selection, and dissemination. This review synthesizes environmental chemistry, microbial ecology, ecotoxicology, analytical science, and AMR research from a One Health perspective to inform more holistic environmental surveillance, improved risk assessment, targeted wastewater-treatment strategies, and sustainable interventions that can address both parent antibiotics and their biologically relevant TPs.

2. Sources, transformation pathways, and environmental fate

The environmental behavior of antibiotics cannot be understood based solely on their release concentrations, as these compounds are continuously redistributed and transformed in interconnected human, animal, wastewater, soil, and aquatic compartments following their use. Therefore, parent antibiotics, human and veterinary metabolites, environmental TPs, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs) may coexist and move through partially overlapping pathways. Final environmental concentrations are determined by the magnitude and frequency of emission, treatment efficiency, molecular properties, transformation kinetics, sorption–desorption behavior, hydrology, and biological activity. In this section, we distinguish five interrelated components of antibiotic fate: (i) sources and environmental dissemination, (ii) biotransformation in humans and animals, (iii) microbial transformation after environmental release, (iv) abiotic transformation, and (v) persistence, mobility, and bioavailability.

2.1. Sources and environmental dissemination

Municipal wastewater is a major continuous pathway for human-use antibiotics and their metabolites to enter the environment. Following administration, portions of the parent drug and its metabolites are excreted in urine and

feces and transported through sewer networks to WWTPs. While some parent compounds can be substantially reduced through conventional treatment, removal efficiency is highly dependent on the compound and the process; consequently, treated effluents and sewage sludge remain important secondary sources of antibiotic-related residues. WWTPs are not simply endpoints but rather interfaces between human antibiotic consumption and surface waters, sediments, agricultural soils, and, in the case of reclaimed wastewater reuse, agroecosystems.

Hospital wastewater is a relatively concentrated but spatially localized contributor to the municipal waste load. Hospital effluents may contain not only therapeutic antibiotic residues and metabolites but also antibiotic-resistant clinical bacteria, ARGs, and mobile genetic elements. Metagenomic analysis of a hospital's wastewater treatment process showed that ARGs associated with clinically relevant antibiotic classes persisted through the treatment process, demonstrating that the chemical and biological dimensions of antimicrobial pollution can be released together.[11] However, the relative contribution of hospitals to an entire municipal catchment varies considerably by hospital size, intensity of antibiotic use, sewer configuration, dilution, and presence of other community sources. Thus, hospital wastewater should be regarded as a major hotspot rather than the main source as typically assumed. Importantly, human-derived antibiotic emissions to wastewater are not restricted to hospitals. Following antibiotic administration in the general population, unchanged parent compounds along with human metabolites and conjugated derivatives are excreted mainly through urine and feces and enter municipal sewer systems. This community-derived pathway represents a continuous source of antibiotic-related residues to WWTPs and should be distinguished from hospital wastewater, which represents a more localized and potentially concentrated source.

In pharmaceutical manufacturing, the point source is different, in which the release may not be related to therapeutic excretion. Manufacturing wastes may generate local concentrations of antibiotics far greater than those in municipal effluent. A published study of effluent from bulk-drug manufacture in India found ciprofloxacin concentrations as high as about 31 mg/L, illustrating the ability of industrial discharges to generate extremely high local exposure and resistance-selection pressure.[12] While these extreme concentrations should not be generalized to all pharmaceutical facilities, the finding demonstrates the need for source-specific monitoring and controls on manufacturing emissions.

Veterinary antibiotics create another pathway from treated animals to manure, urine, bedding material, and slurry. The persistence of antibiotics in manure, even after storage, composting, or anaerobic treatment, can result in their subsequent entry into agricultural soils when the manure is applied to land. Field-scale evidence has demonstrated the transfer of veterinary antibiotics from treated animals to manure and subsequently to agricultural soil.[13] Once in soil, residues may be sorbed, biochemically or chemically transformed, incorporated in runoff at the time of rainfall, transported via preferential flow paths, or leached to groundwater. When containing sorbed antibiotic residues or associated TPs, biosolids produced during municipal wastewater treatment represent a conceptually similar land-based pathway.

Unlike terrestrial livestock production, antibiotics can be introduced directly into the aquatic environment in aquaculture. Residues may arise from uneaten medicated feed, excretion of treated organisms, and dispersion from culture facilities. Detection of antibiotic residues in water and sediment associated with aquaculture systems suggests that the sediment compartment can act as an important sink following water-column exposure.[14] Sediment accumulation is especially significant for compounds with a high affinity for particles because benthic microbial communities may be exposed long after concentrations in the overlying water have decreased.

A further pathway for urban antibiotic emissions to reach agricultural environments is the reuse of treated municipal wastewater for irrigation. Reclaimed water may still contain residual parent antibiotics, metabolites, TPs, and resistance determinants after conventional treatment. The environmental fate of resistance determinants depends on irrigation frequency, treatment quality, soil properties, climatic conditions, and crop properties. Importantly, the transfer of resistance determinants from reclaimed water to soil and crops is not necessarily proportional to their abundance in irrigation water, as soil texture and ecological barriers can strongly constrain establishment. For example, Marano *et al.*[15] demonstrated that the behavior of ARGs along an effluent–irrigation water–soil–crop continuum was strongly controlled by the environmental context rather than by simple mass transfer.

Collectively, these pathways show that environmental dissemination is best conceptualized as a source–pathway–receptor network (Figure 1). Continuous sources, such as municipal wastewater, can maintain chronic low-level exposures, while industrial or agricultural releases can lead to spatially or temporally discrete exposures. Once residues enter receiving environments, transformation and partitioning determine the fate of the parent antibiotic, whether it disappears, remains bioavailable, or is replaced

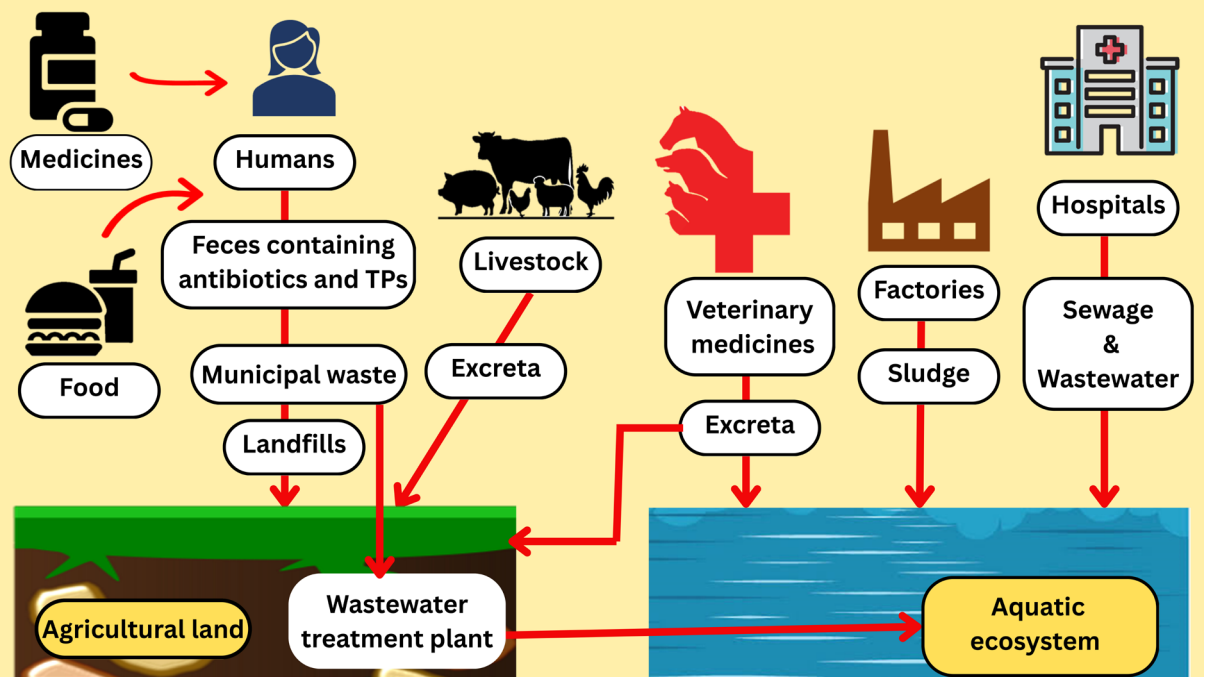


Figure 1. Major anthropogenic sources and environmental pathways for dissemination of parent antibiotics, metabolites, TPs, ARB, and ARGs. Figure created by the authors using Canva Pro and Adobe Photoshop.
Abbreviations: ARB: Antibiotic-resistant bacteria; ARG: Antibiotic resistance gene; TP: Transformation product.

by TPs with different environmental properties (Table 1).

2.2. Biotransformation in humans and animals

The chemical composition of antibiotics may already be altered substantially in humans and treated animals before they reach wastewater or manure. This alteration occurs through metabolism in humans and treated animals. Drug metabolism is generally described by phase I functionalization and phase II conjugation reactions, but these processes should not be seen as an obligatory sequential pathway. Depending on the antibiotic and host species, a compound may be excreted without significant change, undergo only phase I transformation, direct conjugation, or several parallel and sequential metabolic reactions.[16] Environmentally, this distinction is important, as excreta can contain mixtures of the parent drug and multiple metabolites, rather than a single residual compound.

2.2.1. Phase I reactions

Phase I metabolism generally involves oxidation, reduction, or hydrolysis. Oxidative transformations are typically catalyzed by cytochrome P450 enzymes and include hydroxylation, *N*- or *O*-dealkylation, heteroatom oxidation, and side-chain modification. Meanwhile, hydrolytic enzymes can cleave susceptible ester, amide, or related functional groups, whereas reductive metabolism is especially relevant in low-redox tissues and through intestinal microbial activity. These reactions may generate or reveal functional groups that enable subsequent conjugation, but they do not necessarily abrogate antimicrobial activity.

For instance, fluoroquinolone ciprofloxacin is an example of the multiple metabolites generated through phase I metabolism. Desethyl ciprofloxacin, oxociprofloxacin, and other metabolites have been identified after therapeutic administration, and both the parent and metabolites are excreted.[17] A particularly important veterinary example is enrofloxacin, which is metabolically converted to ciprofloxacin in cattle and other food-producing animals.[18] The metabolism of ciprofloxacin itself is of interest because the drug is an active fluoroquinolone antibiotic, and the metabolic transformation here does not represent a simple detoxification but the partial transformation of one pharmacologically active antimicrobial into another.

2.2.2. Phase II reactions

Phase II metabolism refers to the conjugation of the parent antibiotic or a phase I metabolite with an endogenous moiety, including glucuronidation, sulfation, acetylation, methylation, and conjugation with glutathione.

Table 1. Principal sources and dissemination pathways of antibiotics and associated contaminants

Source	Principal material released	Dominant dissemination route	Main environmental compartments affected	Representative evidence
Municipal wastewater/ WWTP effluent	Parent antibiotics, human metabolites, TPs; potentially ARB and ARGs	Sewer transport to treatment facilities, followed by treated effluent discharge	Rivers, lakes, sediment, and downstream water systems	Persistence/removal is compound- and treatment-dependent[15]
Hospital wastewater	Antibiotics, metabolites, ARB, ARGs, and mobile genetic elements	Treated hospital sewage before discharging into receiving surface waters	WWTPs and aquatic environments	ARGs persisted throughout a hospital wastewater treatment system[11]
Pharmaceutical manufacturing	Active pharmaceutical ingredients and synthesis-related residues	Direct environmental release via industrial effluent	Surface water, sediment, and downstream microbial communities	Extremely high local antibiotic concentrations have been documented[12]
Livestock production/ manure	Veterinary antibiotics, metabolites, and resistance determinants	Land application of treated manure leads to runoff and soil leaching	Agricultural soil, drainage water, groundwater, and surface water	Transfer from treated livestock to manure and soil demonstrated experimentally[13]
Aquaculture	Uneaten medicated feed and excreted antibiotics/metabolites	Direct release into culture water causes physical dispersion and sedimentation	Water column, sediment, and adjacent aquatic ecosystems	Antibiotics detected in water and mud associated with shrimp aquaculture[14]
Reclaimed-wastewater irrigation	Residual antibiotics, TPs, ARB, and ARGs	Wastewater irrigation introduces contaminants directly into soils and crops	Topsoil, rhizosphere, crops, and potentially groundwater	Resistance determinants tracked across irrigation water–soil–crop continuum[15]

Abbreviations: ARB: Antibiotic-resistant bacteria; ARG: Antibiotic resistance gene; TP: Transformation product; WWTP: Wastewater treatment plant.

Glucuronidation, sulfation, acetylation of suitable functional groups, and conjugation with glutathione are catalyzed by UDP-glucuronosyltransferases, sulfotransferases, *N*-acetyltransferases, and glutathione S-transferases, respectively. Conjugation tends to enhance polarity and favor elimination, but this tendency is not universally true. For example, acetylation can alter ionization and solubility differently from glucuronidation.

A well-characterized example of parallel metabolic pathways involves sulfamethoxazole. Studies of human pharmacokinetics identified unchanged sulfamethoxazole and N^4-acetyl sulfamethoxazole, an N^1-glucuronide, and a number of hydroxylated metabolites in urine.[19] In that study, the primary urinary component was N^4-acetyl sulfamethoxazole, indicating that estimates of environmental loading based only on unchanged sulfamethoxazole may underestimate the total amount of sulfamethoxazole-related material entering wastewater.

Metabolic transformation is of particular environmental relevance when metabolites still possess antibacterial activity or can regenerate the parent antibiotic. Conjugates within the sewer system are exposed to microbial enzymes and varying pH and redox conditions, and some may be deconjugated or further transformed during wastewater treatment. Therefore, the environmentally emitted material is better conceptualized as a parent–metabolite pool rather than as the unchanged parent antibiotic alone (Table 2).

2.3. Microbial transformation of antibiotics

Antibiotic transformation in wastewater, activated sludge, manure, soil, sediment, and natural waters is largely driven by microorganisms following excretion and environmental release. However, we should distinguish environmental microbial transformation from the purposeful use of microorganisms in industry for the synthesis of modified antibiotics. In the present context, the processes considered are biodegradation and biotransformation of antibiotic residues already present in environmental matrices.

Microbial transformation can be mediated by growth-linked metabolism, in which the contaminant is a source of carbon, nitrogen, sulfur, or energy for microbial growth, or by co-metabolism, in which enzymes produced for

Table 2. Representative human and veterinary metabolic transformations of antibiotics

Antibiotic	Host/context	Major transformation	Representative metabolite/product	Environmental significance	Reference
Sulfamethoxazole	Humans	*N*-acetylation, glucuronidation, and oxidation/hydroxylation	N^4-acetyl sulfamethoxazole, N^1-glucuronide, and hydroxylated metabolites	Excreta contain a substantial metabolite burden in addition to unchanged sulfamethoxazole	19
Ciprofloxacin	Humans	Modification/oxidation of the piperazinyl substituent	Desethyl-, oxo- and other ciprofloxacin metabolites	Parent compound and metabolites can enter wastewater simultaneously	17
Clarithromycin	Humans	Hydroxylation	14-(*R*)-hydroxy clarithromycin	The metabolite retains antimicrobial activity, demonstrating that metabolism need not equal biological inactivation	20
Enrofloxacin	Cattle/veterinary use	*N*-dealkylation/ metabolic conversion	Ciprofloxacin	Transformation produces another pharmacologically active fluoroquinolone that can be excreted	18

another primary substrate fortuitously transform the antibiotic. In complex communities, transformation can also be sequential, with a TP produced by one organism subsequently transformed or mineralized by another. Therefore, the outcome depends not only on the antibiotic structure but also on community composition, enzymatic capacity, redox conditions, and alternative substrate availability.[21]

Common microbial transformations include hydroxylation, demethylation, dealkylation, deamination, cleavage of functional groups, acetylation, conjugation/deconjugation, ring modification, and reduction or oxidation of moieties susceptible to these transformations. The enzymes involved in these reactions are monooxygenases, dioxygenases, cytochrome P450-type enzymes, transferases, hydrolases, laccases, and peroxidases. In wastewater biofilms and activated sludge, extracellular polymeric substances also influence antibiotic fate by providing sorption domains that alter local concentration and accessibility of compounds to cells and extracellular enzymes. Thus, apparent "removal" during biological treatment can be a combination of sorption, transformation, and true mineralization.

The redox conditions are of particular importance for microbial transformation of antibiotics. Aerobic and anaerobic communities can result in different transformation pathways and products, and a compound that is persistent under one condition may be transformed upon transition to another. Redox changes can increase overall transformation and affect the behavior of intermediate products, as shown in a sequential anaerobic–aerobic study of sulfamethoxazole.[22] This has practical implications for wastewater-treatment and digestion systems, manure, and sediment, in which oxygen availability can vary over short spatial scales.

Another important mechanism for microbial transformation of antibiotics is co-metabolism. Co-metabolism causes modification of molecules, which can either decrease or increase toxicity. The co-metabolic system provides additional energy sources for microorganisms and enhances their adaptability to the environment. Primary substrates may stimulate microbial growth, generate reducing equivalents, or promote enzyme expression, thereby enhancing the transformation of a contaminant that cannot support microbial growth by itself. However, the addition of readily degradable organic carbon does not always improve the removal of antibiotics, because the primary substrate may also compete for enzymes or electron acceptors. Therefore, generalized claims that glucose, acetate, or other carbon sources invariably enhance antibiotic degradation should be avoided unless verified for the specific compound and microbial system.[23]

In particular, biotransformation should not be confused with biodegradation that produces harmless end products. Achermann *et al.*[5] showed that sulfonamide transformation in activated sludge could lead to the formation of pterin-conjugated products, demonstrating that microbial processing can redistribute rather than simply remove sulfonamide-associated material.

Conversely, a recent study has shown complete microbial mineralization of sulfamethoxazole at environmentally low concentrations under suitable community conditions.[24] These contrasting observations suggest a continuum of microbial transformation from reversible conversion and TP accumulation to complete mineralization.

Thus, the environmental significance of microbial transformation depends on the depletion of the parent chemical and the properties of the resulting products. A TP may or may not lose its antibacterial activity, exhibit altered toxicity, become more mobile or persistent, or act as an intermediate for further degradation. Thus, transformation studies should ideally include monitoring of the parent compound depletion, identification of TPs, assessment of mass balance, evaluation through antimicrobial/effect assays, and, where possible, measurement of mineralization (Table 3).

2.4. Abiotic transformation

Abiotic transformation may also occur without the direct involvement of microbes through photochemical reactions, hydrolysis, mineral-mediated redox reactions, and treatment-induced oxidation or disinfection. Abiotic processes may dominate in environmental compartments with high exposure to light, where the molecular framework contains hydrolytically unstable groups, or where reactive minerals and oxidants are abundant. Similar to microbial transformation, the depletion of parent compound through abiotic transformation does not necessarily mean mineralization, since abiotic reactions often produce structurally related TPs (Figure 2).[25]

2.4.1. Photochemical transformation

Photolysis is an especially important antibiotic transformation process in sunlit surface waters. Direct photolysis refers to when an antibiotic absorbs solar radiation and subsequently undergoes chemical transformation from its excited state. Indirect photolysis occurs when other light-absorbing constituents (e.g., dissolved organic matter, nitrate, or other sensitizers) form reactive intermediates such as hydroxyl radicals, singlet oxygen, or triplet-state species that subsequently react with the antibiotic. The balance between direct and indirect photolytic pathways depends on the antibiotic absorption spectrum, water depth and turbidity, dissolved organic matter concentration, pH, and the concentrations of photosensitizers and radical scavengers.

Fluoroquinolones and sulfonamides have been investigated using high-resolution mass spectrometry (HRMS) under simulated sunlight, and the results showed that photolysis can generate multiple photoproducts through pathways such as oxidation, cleavage, and modification of heterocyclic or side-chain structures.[26] Thus, photochemical half-lives alone are insufficient for conducting meaningful environmental risk assessment. The identity, persistence, and biological activity of the resulting photoproducts should also be considered (Table 4).

Table 3. Representative environmentally relevant microbial transformation processes

Environmental system	Antibiotic	Dominant process	Key outcome	Environmental interpretation	Reference
Activated sludge	Sulfonamide antibiotics	Microbial biotransformation and conjugation	Formation of pterin-conjugated TPs	Parent compound depletion may conceal persistent/ reversible antibiotic-related material	5
Sequential anaerobic–aerobic treatment	Sulfamethoxazole	Redox-dependent sequential biotransformation	Different transformation behavior under anaerobic and aerobic conditions	Redox transitions can determine whether intermediates accumulate or undergo further degradation	22
Activated-sludge microbial communities	Sulfamethoxazole	Community-mediated catabolism	Transformation followed by mineralization at environmentally low concentrations	Complete biodegradation is possible but depends on microbial ecological interactions	24
Biological wastewater matrices in general	Multiple antibiotic classes	Sorption + enzymatic transformation + co-metabolism	Partial removal, TP formation, or mineralization	Treatment performance must distinguish true degradation from redistribution to sludge or intermediate products	22

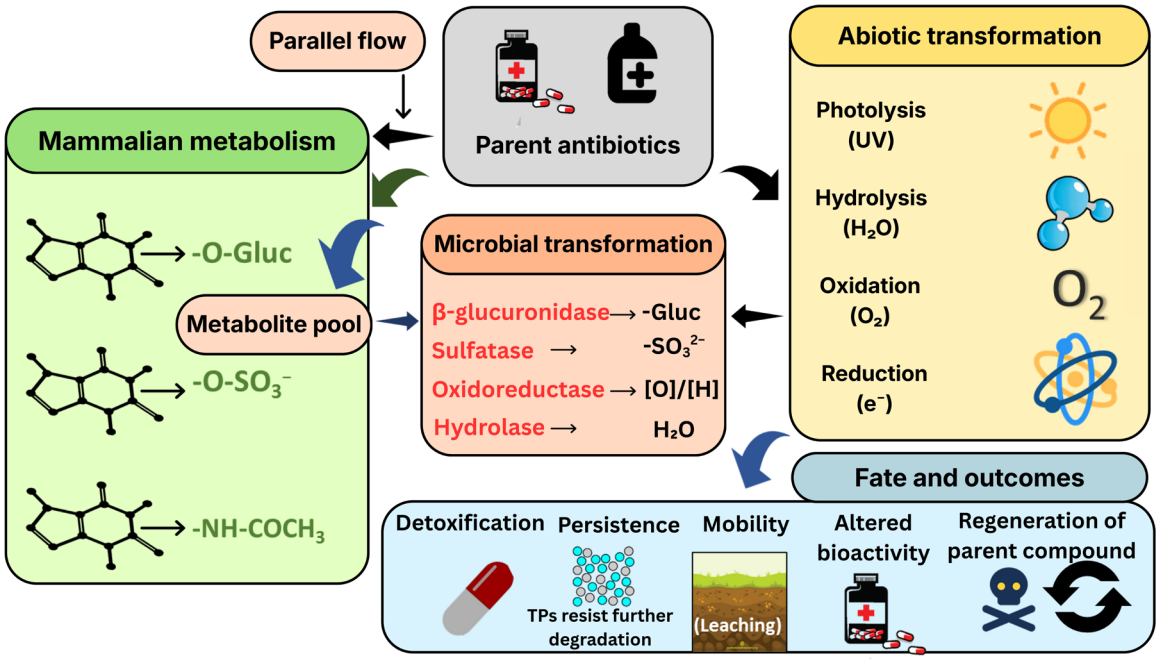


Figure 2. Integrated pathways of antibiotic transformation by mammalian metabolism, microbial biotransformation, and abiotic processes. These parallel or sequential pathways lead to a range of environmental outcomes: detoxification, persistence, altered mobility, retained bioactivity, or regeneration of the parent compound. Figure created by the authors using Canva Pro and Adobe Photoshop.

2.4.2. Hydrolysis

Hydrolysis is a significant transformation process for antibiotics with chemically labile functional groups. β-lactam antibiotics are particularly susceptible to degradation in aqueous systems by cleavage or rearrangement of the β-lactam ring. Hydrolytic rate and product distribution are strongly affected by pH, temperature, ionic composition, and catalytic metal species. A detailed analysis of amoxicillin revealed various pH-dependent hydrolysis products, demonstrating that transformed structures persist even when the parent antibiotic was difficult to detect.[27] Thus, low measured concentrations of hydrolytically unstable antibiotics may reflect rapid conversion rather than a lack of environmental loading (Table 4).

2.4.3. Mineral-mediated redox reactions

The strong complex-forming ability of tetracycline greatly limits the efficiency of conventional wastewater treatment. Kabanov *et al.*[28] reported that tetracycline forms highly stable soluble complexes with iron, especially incompletely oxidized Fe(II), which shifts the hydroxide precipitation pH thresholds and can completely inhibit precipitation. Therefore, adding iron-based coagulants to wastewater with high tetracycline concentration did not remove the drug, but increased the concentration of soluble iron-organo complexes, thereby allowing these persistent contaminants to pass through the conventional treatment barriers.[28] Natural minerals may act as reactive surfaces rather than as passive sorbents. For example, manganese and iron oxides can catalyze electron-transfer reactions with susceptible antibiotics. The reactivity of oxytetracycline with hydrous manganese oxide suggests that mineral-associated oxidation can play a role in antibiotic attenuation in soils and sediments. Oxytetracycline is rapidly degraded through an oxidative process promoted by hydrous manganese oxide (MnO_2), accompanied by the generation of Mn^{2+} ions. The reaction rate is strongly dependent on pH—the reaction rate increases significantly as pH decreases. While reactivity across tetracyclines varies (with chlortetracycline degrading the fastest and rolitetracycline the slowest), MnO_2 reactivity toward oxytetracycline slows down over time, following a retarded rate equation. In summary, natural manganese oxides in soils and sediments are likely to drive substantial degradation of tetracycline antibiotics; however, overall degradation rates depend heavily on solution conditions and reaction duration.[29] Reaction rates depend on pH, mineral surface characteristics, dissolved ligands, and chemical speciation of the antibiotic. Thus, sorption and transformation can occur simultaneously at mineral-water interfaces (Table 4).

2.4.4. Treatment-induced transformation

Engineered oxidation and disinfection processes provide an additional abiotic pathway. Chlorine, ozone, ultraviolet irradiation, and advanced oxidation processes can reduce parent-antibiotic concentrations but may form TPs before complete mineralization. Effect-directed analysis after chlorination of ciprofloxacin and azithromycin revealed that some chlorination products still possessed detectable antibacterial or toxicological effects.[30] This finding is important because risk reduction based on the loss of the parent compound alone may overestimate the risk (Table 4).

2.5. Persistence, mobility, and bioavailability

The environmental risk of an antibiotic-related compound after release and transformation depends on its total concentration and on its persistence, mobility, and biologically available fraction. These properties are interconnected and differ greatly among antibiotic classes and between individual parent compounds and TPs. Whether the compound remains dissolved, becomes sorbed, undergoes transformation, or is available for uptake depends on the interaction between the molecular charge, acid dissociation constant, hydrophobicity, polarity, and functional groups of the compound, and environmental pH, organic carbon content, clay minerals, metal ions, ionic strength, moisture, and microbial activity.

2.5.1. Sorption and desorption

Antibiotics in soil and sediment can sorb to solid phases via hydrophobic partitioning, electrostatic attraction, cation exchange, hydrogen bonding, van der Waals interactions, cation bridging, metal complexation, and surface complexation. Many antibiotics are ionizable, and their sorption cannot be predicted from hydrophobicity alone. Environmental pH controls the relative abundance of cationic, neutral, zwitterionic, and anionic species, while soil organic carbon, clay mineralogy, and cation-exchange capacity determine the abundance and characteristics of available binding sites. Sorption onto activated sludge and mineral surfaces should not be considered exclusively as a partitioning process, because solid-phase association can modify antibiotic speciation, accessibility, and subsequent transformation mechanisms. Partial adsorption of antibiotics onto activated sludge or clays significantly alters their environmental oxidation mechanisms by physically altering reactant accessibility, chemical speciation, and degradation pathways.

Although strong sorption reduces immediate dissolved-phase mobility in general, it does not necessarily lower long-term risk. Sorbed residues may remain available to sediment- or soil-associated microorganisms, may be

Table 4. Major abiotic transformation pathways and representative antibiotic examples

Abiotic pathway	Principal driver	Representative antibiotic(s)	Major transformation consequence	Environmental relevance	Reference
Direct/indirect photolysis	Solar radiation and photochemically generated reactive species	Fluoroquinolones and sulfonamides	Oxidation, cleavage, and multiple photoproducts	Can dominate attenuation in sunlit surface waters while producing TPs	26
Hydrolysis	Water; strongly influenced by pH and temperature	Amoxicillin and other β-lactams	β-lactam ring transformation and multiple hydrolysis products	Rapid hydrolysis can make the parent compound difficult to detect despite environmental release	27
Mineral-mediated oxidation	Reactive Fe(II), Mn/Fe mineral surfaces	Tetracycline, Oxytetracycline	Electron-transfer-driven transformation	Links antibiotic sorption and degradation in soils and sediments	28,29
Chlorination/treatment oxidation	Chemical disinfectants or oxidants	Ciprofloxacin and azithromycin	Formation of treatment-related TPs	Some TPs may retain antibacterial or toxicological activity	30

Abbreviation: TP: Transformation product.

released following changes in pH or ionic composition, or may be transformed on particle surfaces. Desorption can also show hysteresis, in which the path and amount of release are not a direct reversal of the original adsorption process.

The disparate behavior of commonly detected antibiotics indicates that their environmental fate cannot be generalized by therapeutic class alone. Sorption of ofloxacin and trimethoprim was strong under environmentally relevant soil-to-water conditions,[31] while sulfamethoxazole and lincomycin showed weaker interactions and thus higher potential mobility. In a previous study, the tested soil systems retained more than 99.9% and 99.5% of ofloxacin and trimethoprim, respectively, while sulfamethoxazole and lincomycin showed considerably higher desorption and mobility.[31]

2.5.2. Persistence and degradation kinetics

Persistence should be considered an interplay of biotic and abiotic transformations, rather than a single intrinsic property. Temperature is a common factor that influences chemical reaction and microbial growth rates. However, higher temperature does not always accelerate degradation, as microbial communities may be inhibited above their optimal temperature range. pH can also affect the physiology of the microbes and the speciation of the antibiotics, and therefore increase or decrease the transformation rate depending on the antibiotic compound and matrix. Microbial pathways that are redox-dependent are regulated by oxygen, whereas photolysis in surface environments is regulated by sunlight.

Organic matter exhibits multifaceted and occasionally conflicting effects on antibiotic fate. It can improve sorption and decrease concentrations of freely dissolved molecules, provide substrates that stimulate co-metabolic activity, generate complex metal ions involved in catalytic transformation, quench reactive photochemical species, or compete with antibiotics for oxidants and enzymatic pathways. Therefore, blanket statements about persistence in the environment must be treated with caution; generalizing changes in antibiotic degradation rate due to high organic carbon content is not appropriate without matrix- and compound-specific evidence.

Transformation products may possess different persistence than their parent antibiotics, as a relatively small structural change can affect charge, polarity, steric interactions, photoreactivity, and susceptibility to microbial enzymes. Therefore, environmental persistence should be considered a dynamic parent–TP system and not based on the half-life of the parent molecule alone.

2.5.3. Mobility

The mobile fraction of an antibiotic or TP is governed by the competing dynamics between retention and transport processes. Compounds with low sorption potential can migrate via pore water to deeper soil horizons or drainage systems, whereas compounds with high sorption potential are more likely to remain in the application zone or be transported with eroded particles and suspended sediments. Preferential flow through macropores can also bypass the highly reactive surface horizons and allow compounds to move more rapidly than predicted from equilibrium sorption coefficients.

Transformation can increase or decrease antibiotic mobility in environments. Ionization, polarity, and consequently the distribution coefficient between solid and aqueous phases can be influenced by oxidation, hydrolysis, dealkylation, or conjugate cleavage. This phenomenon is a major reason why models developed for the parent antibiotic cannot be directly applied to its metabolites or environmental TPs.[32]

2.5.4. Bioavailability and biological exposure

The bioavailability of a contaminant is defined as the contaminant fraction available for uptake by organisms or microorganisms, enabling it to interact with biological targets. As such, total environmental concentration and bioavailable concentration are not interchangeable. Strong sorption may reduce the freely dissolved fraction but leave a desorbable reservoir, and dissolved organic matter may reduce cellular uptake by complexation or modify mobility to increase exposure under certain chemical conditions.

Experiments have demonstrated that plants are able to take up antibiotic residues from the soil. Soil retention is not necessarily a barrier to biological transfer, as reported by Kang *et al*.[33] regarding the uptake of several veterinary antibiotics by vegetable crops grown in soils amended with manure. Bioavailability is also affected by the chemical environment: organic acids can alter the speciation of tetracyclines and increase their availability to bacteria, which is crucial for uptake and resistance selection.[34]

For TPs, predicting bioavailability based on the properties of the parent compound is particularly uncertain. Depending on the structural change involved, a TP may become more polar and mobile, less membrane-permeable, more strongly ionized, or more strongly sorbed. Hence, assumptions that TPs are intrinsically more soluble or bioavailable than their parent compounds should not be generalized. Rather, mobility and bioavailability should be assessed on a chemical-by-chemical basis using experimentally measured physicochemical properties, environmental partitioning, and biological-effect data.

Persistence, mobility, and bioavailability collectively establish a coupled framework of environmental exposure. Strong persistence and low mobility favor long-term accumulation in soil or sediment, while moderate persistence and high mobility favor transport to groundwater and surface water. High bioavailability may amplify the biological effects even at relatively low total concentrations. These properties are particularly important for antibiotic TPs, because transformation can reduce the concentration of the parent compound and produce TPs with different environmental distributions and biological effects at the same time. Therefore, environmental risk assessments should account for both parent antibiotics and environmentally relevant TPs, rather than using the depletion of the parent compound as the sole indicator of attenuation (Table 5).

3. Environmental distribution across ecosystems

After emission and transformation, antibiotics and their TPs are not confined to the emission compartment but distributed among connected terrestrial and aquatic compartments. They occur in soil, water, sediment, groundwater, and biota due to the combined effects of source strength, hydrological connectivity, sorption–desorption, degradation and transformation, chemical speciation, biological uptake, and environmental management practices. Importantly, TP distribution cannot necessarily be predicted from parent antibiotic distribution, as transformation can alter molecular charge, polarity, solubility, persistence, and affinity for organic matter or mineral surfaces. Therefore, the joint consideration of parent compounds and environmentally relevant TPs is required to accurately identify reservoirs, transport pathways, and potential exposure compartments.

3.1. Soil and agricultural systems

Agricultural soils are an important receiving compartment for veterinary and human-use antibiotics because residues can be repeatedly introduced via livestock manure, slurry, sewage sludge or biosolids, reclaimed-water irrigation, and in some settings, direct agricultural use. After application, parent antibiotics and metabolites may remain in surface soils, degrade, sorb to organic or mineral particles, percolate through the soil profile, leach to drainage systems, or be taken up by plants. Thus, measured concentrations in agricultural soil reflect repeated inputs, degradation, transformation, and redistribution, not the original amount applied.

Livestock play a particularly important role because antibiotics administered to food-producing animals may be excreted as parent compounds, metabolites, or reversible TPs. These compounds may undergo further biological and chemical transformations after the manure is stored and applied to agricultural land. Field investigations on dairy farms have found sulfonamides, tetracyclines and their epimers or isomers, and other veterinary antimicrobials in manure-associated farm environments. The distribution

Table 5. Key controls on persistence, mobility, and bioavailability of antibiotics and transformation products

Fate-controlling property/ process	Representative example	Observed/expected behavior	Environmental implication	Reference
Strong soil sorption	Ofloxacin	>99.9% retention under the tested soil conditions	Low immediate aqueous mobility but potential persistence in the solid phase	31
Strong sorption with limited desorption	Trimethoprim	>99.5% retained in the tested soils	Reduced pore-water transport; possible prolonged soil-associated reservoir	31
Weaker soil interaction	Sulfamethoxazole	Greater desorption than ofloxacin or trimethoprim	Higher potential for dissolved-phase transport and leaching	31
Plant uptake from contaminated soil	Several veterinary antibiotics in manure-amended systems	Residues transferred from soil into crop tissues	Demonstrates that sorbed soil residues may remain biologically accessible	33
Modification of microbial bioavailability by solution chemistry	Tetracycline	Organic acids altered accessibility to bacteria	Local chemical conditions can modify exposure and resistance-selection potential	34

of compounds from different antibiotic classes differs markedly, indicating that repeated agricultural use does not necessarily lead to equal soil accumulation of all antibiotics.[35]

Another important example of compound-specific partitioning is the behavior of sewage sludge and biosolids. During wastewater treatment, antibiotics with a high affinity for solids can be transferred from the aqueous phase to the sludge. Experimental work with aerobically digested biosolids demonstrated strong sorption of ciprofloxacin, tetracycline, doxycycline, and clindamycin, while sulfamethazine and sulfamethoxazole exhibited substantially weaker sorption.[36] Thus, wastewater treatment may redistribute some of the antibiotics from the aqueous phase to the solid phase rather than eliminate them. Consequently, subsequent land application of biosolids can transport sorbed residues to agricultural soils.

Once in the soil, the relative distribution between the solid phases and the soil solution is controlled by both the compound properties and the soil characteristics. Antibiotics are often ionizable molecules, and sorption can therefore be highly pH-dependent due to the different amounts of cationic, zwitterionic, neutral, and anionic species. Retention and transport are also affected by organic carbon, clay mineralogy, cation-exchange capacity, ionic strength, and dissolved organic matter. Strong sorption may limit vertical mobility in the short term but at the same time may create a stable solid-phase reservoir, whereas weakly sorbing compounds may remain in pore water with a higher potential to be leached or transported by drainage and runoff.

Transformation products add another level of complexity to environmental distribution of antibiotics. In a mechanistic soil study, sulfadiazine was simultaneously sorbed and transformed to form 4-hydroxysulfadiazine and other products whose formation and persistence were dependent on soil type and depth.[37] The results indicate that TPs may have different fate characteristics from the administered parent antibiotic and that behavior in the subsoil may differ from that in the cultivated surface layer. Therefore, if only the parent antibiotic is monitored, the total chemical burden of antibiotics in agricultural soils may be underestimated.

Runoff and erosion are important lateral transport pathways for antibiotic distribution across ecosystems following manure or biosolid application, particularly if rain occurs shortly after application. Conversely, dissolved residues with low soil affinity may migrate vertically through the soil profile. Thus, contaminant transport is influenced by the interactions among rainfall or irrigation intensity, soil permeability, preferential flow, antibiotic sorption, and the timing and method of waste application. This phenomenon explains why highly sorbing tetracyclines and fluoroquinolones tend to associate with solids, while more mobile compounds or TPs can be transported more widely through soil–water pathways.

Agricultural distribution also occurs beyond the soil itself. Plant roots can absorb antibiotic residues that are sufficiently available in soil solution. Kang *et al.*[33] demonstrated that several veterinary antibiotics were absorbed by vegetable crops in soils amended with manure, with the extent of uptake influenced by the antibiotic compound, crop species, soil type, and manure treatment. Thus, plant uptake should be interpreted as evidence of the transfer from the soil compartment to biological matrices, but not as evidence that all antibiotic residues accumulated in soil are equally bioavailable.

A related pathway for antibiotic inputs to soils and agricultural systems is reclaimed-wastewater irrigation. Repeated irrigation can introduce low concentrations of antibiotic residues to surface soil and modify soil moisture, dissolved organic carbon content, and microbial conditions that affect subsequent transformation. The resulting distribution is therefore dynamic: some residues stay in the upper soil layer, some are degraded or transformed, some are associated with particles, and some are taken up by plants or transported to drainage systems or groundwater. This source–soil–plant–water continuum is particularly significant in areas where reclaimed wastewater is used over long periods (Table 6).

3.2. Aquatic ecosystems

Aquatic ecosystems are exposed to antibiotics through multiple pathways, such as municipal and hospital wastewater, agricultural drainage, livestock runoff, aquaculture, pharmaceutical manufacturing, and urban stormwater and groundwater discharge. Consequently, mixtures of parent antibiotics, metabolites, and environmental TPs from multiple sources are found in rivers, lakes, reservoirs, estuaries, and coastal environments. Wastewater discharge, hydrological flow, seasonal antibiotic consumption, rainfall, dilution, photochemical transformation, and sediment–water exchange often result in spatially and temporally heterogeneous antibiotic concentrations.

Dissolved antibiotic transport via the water column is the most direct pathway by which antibiotics are transported to aquatic ecosystems. Wastewater-derived residues can be transported downstream over long distances, although concentrations may decrease due to dilution, photolysis, microbial transformation, hydrolysis, and transfer to suspended particles or sediment. For

Table 6. Representative occurrence and distribution pathways of antibiotics and transformation products in soil and agricultural systems

Environmental matrix/pathway	Representative antibiotic/TP	Key observation on distribution	Environmental significance	Reference
Dairy manure and manured agricultural soils	Sulfonamides, tetracyclines and their epimers/isomers, lincomycin	Antibiotics occurred in farm waste streams and soil, but their accumulation and transport differed markedly among compounds	Demonstrates compound-specific behavior following repeated veterinary use	35
Aerobically digested biosolids	Ciprofloxacin, tetracycline, doxycycline, clindamycin, sulfamethazine, and sulfamethoxazole	Ciprofloxacin, tetracycline, doxycycline, and clindamycin sorbed strongly, whereas sulfonamides showed weaker sorption	WWTPs can redistribute antibiotics into sludge that may later be applied to land	36
Agricultural soil	Sulfadiazine transforms into 4-hydroxysulfadiazine and other TPs	Parent compound and TPs exhibited different sorption and transformation behavior across soil layers	Parent-only monitoring may underestimate antibiotic-related residues	37
Contaminant transfer from manure-amended soil into crops	Chlortetracycline, sulfamethazine, tylosin, monensin, and virginiamycin	Antibiotic uptake was observed in vegetable crops, with strong compound- and crop-specific differences	Shows transfer from soil into biological/agricultural compartments	33

Abbreviations: TP: Transformation product; WWTP: Wastewater treatment plant.

example, fluoroquinolone measurements in raw sewage, treated wastewater, and receiving waters revealed that wastewater discharge can lead to a direct continuum between human antibiotic use and downstream aquatic occurrence.[38]

However, measuring only dissolved antibiotic concentrations provides an incomplete picture of aquatic distribution. Many antibiotics are ionizable and can bind to suspended particulates, organic matter, and sediment surfaces. In particular, fluoroquinolones and tetracyclines can bind strongly to particles through electrostatic interactions, cation bridging, and complexation with mineral surfaces. Therefore, particle-associated residues may be transported with suspended matter and later deposited in river, lake, estuarine, or coastal sediments.

Thus, sediments can act as both sinks and secondary sources of antibiotics in aquatic ecosystems. Generally, fine-grained sediments have a larger reactive surface area and often contain more organic matter than coarse sands, which favors the retention of compounds with a strong solid-phase affinity. However, sediment accumulation does not necessarily mean final immobilization of antibiotic residues. Changes in pH, ionic composition, redox conditions, hydrodynamic disturbance, or sediment resuspension may alter partitioning and remobilize a fraction of the residues to pore water or the overlying water column.

Estuarine and coastal systems in particular are highly complex, as they receive contaminant loads exported from entire upstream catchments. The partitioning of antibiotics changes with salinity gradients, fluctuating suspended-particle concentrations, sedimentation, and the mixing between freshwater and seawater. Marine-field evidence from Laizhou Bay showed the presence of sulfonamide, fluoroquinolone, macrolide antibiotics, and trimethoprim in offshore water and aquatic organisms, indicating transfer from the water column into marine food-web compartments.[39]

Some TPs are sufficiently persistent to co-occur with their parent antibiotics and should therefore be explicitly considered in aquatic monitoring. In a recent river monitoring, anhydroerythromycin A, a well-known TP of erythromycin, was detected along with azithromycin, clarithromycin, roxithromycin, and erythromycin in different hydrological periods, indicating that parent compounds and TPs can coexist in receiving waters.[40]

Aquatic distribution may also serve as a conduit among surface water, subsurface groundwater, and sediment. In the Chenhu Lake system, 22 antibiotics and four sulfonamide metabolites were simultaneously detected in lake water,

sediments, and groundwater. Important groups detected were fluoroquinolones, tetracyclines, and N^4-acetylated sulfonamides. Groundwater antibiotic concentrations were related to the distance from the lake, showing evidence of hydrological connectivity between these compartments.[41]

Seasonality is another major source of variability in antibiotic residue concentrations. During high-flow conditions, dilution can decrease dissolved antibiotic concentrations even when the total contaminant loads are increasing, while rainfall can wash agricultural residues into surface water. During dry periods, lower river flows may increase the relative contribution from wastewater effluent. Temperature and solar radiation have a combined effect on biological transformation and photochemical degradation. Thus, differences between seasons should not be automatically interpreted as changes in antibiotic usage alone (Table 7).

3.3. Groundwater and drinking water concerns

Groundwater represents a distinct environmental compartment, as contaminant inputs are typically indirect and attenuation processes differ from those in sunlit surface waters. Antibiotics and TPs can enter aquifers via contaminated surface-water infiltration, agricultural irrigation, manure or biosolid application, leakage of wastewater infrastructure, landfill leachate, artificial recharge, and industrial discharges. Surface water–groundwater exchange and riverbank filtration can also carry residues from rivers and lakes to connected aquifers.

The mobility and persistence of an antibiotic during transport through the unsaturated zone strongly determine whether it reaches the groundwater. Compounds that sorb strongly may be retained in surface soils, whereas weakly sorbing or sufficiently persistent compounds may migrate with percolating water. Antibiotic transformation may alter this behavior: A TP that is more polar or less strongly sorbed than its parent may move differently through the soil profile. Additionally, a standard transformation may yield products that are more persistent or more easily degraded.

The Chenhu Lake study offers direct evidence for the transport of antibiotics and their metabolites across linked surface-water and groundwater environments. Lake water, sediment, and groundwater were analyzed to determine the concentrations of 22 antibiotics and 4 sulfonamide metabolites. Antibiotic concentrations in groundwater generally decreased with increasing distance from the lake.[41] This result indicates that hydrological connectivity should be considered while identifying contaminant sources of antibiotics in aquifers.

Groundwater contamination associated with pharmaceutical manufacturing is an important, exceptional, and high-exposure scenario. Fick *et al.*[42] studied the surface water, groundwater, and drinking-water wells in a large pharmaceutical production area in India and found pharmaceutical contamination in all the

Table 7. Representative distribution of parent antibiotics and transformation products in aquatic ecosystems

Aquatic compartment	Representative compound(s)	Key observation	Interpretation	Reference
Wastewater discharge introduces contaminants into receiving surface waters	Ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin	Fluoroquinolones were detected across sewage-treatment and receiving-water compartments	Illustrates direct wastewater–river connectivity	38
Coastal water and marine food web	Sulfonamides, fluoroquinolones, macrolides, and trimethoprim	Antibiotics occurred in offshore water and multiple marine organisms	Demonstrates redistribution from water into biological compartments	39
Urban river	Erythromycin and anhydroerythromycin A	Parent macrolides and the erythromycin TP were monitored together across hydrological periods	Confirms that persistent TPs can coexist with parent antibiotics in surface water	40
Lake water–sediment–groundwater continuum	22 antibiotics and four sulfonamide metabolites, including N^4-acetylated sulfonamides	Compounds occurred across interconnected water and sediment compartments	Shows cross-compartment transport and the need to monitor metabolites in addition to parents	41

Abbreviation: TP: Transformation product.

wells studied. Ciprofloxacin and several other drugs were detected at levels much higher than typically observed in studies of municipal drinking water. These values must be explicitly stated as an industrial-pollution hotspot and must not be used to represent concentrations in groundwater or drinking water in general. This distinction is important when interpreting the results from the study. Effluent from the treatment plant contained a reported concentration of about 14 mg/L ciprofloxacin, whereas concentrations of up to about 6.5 mg/L ciprofloxacin were found in highly contaminated lake waters near the industrial area. Consequently, these values should not be reported as normal groundwater or drinking water concentrations. The associated wells had pharmaceutical contamination, and the environmental setting was a severe manufacturing discharge scenario.[42]

A later study of the same pharmaceutical-producing region found fluoroquinolones and plasmid-mediated quinolone-resistance genes in the sediment, water, soil, and well-water samples, also demonstrating that heavily polluted industrial environments can create linked chemical and microbiological pollution pathways.[43] This evidence illustrates a hotspot scenario but needs to be clearly separated from background or municipal drinking-water exposure.

At the other end of the exposure spectrum, trace concentrations of antibiotics have also been detected along the urban water cycle. Watkinson *et al.*[44] traced antibiotics from wastewater through an urban watershed to drinking-water systems, showing that some antibiotic residues can survive through successive environmental and treatment compartments. Their work shows the importance of considering the entire wastewater–surface water–drinking water continuum instead of examining each compartment separately.

However, detection in finished drinking water does not imply that concentrations are comparable to those measured at industrial hotspots. Twenty-one drugs spanning 14 antibiotic classes were detected in the tap, bottled, and barreled drinking water in Shanghai. Municipal tap-water samples contained only florfenicol and thiamphenicol at medians of about 8.9 and 6.4 ng/L, respectively, while most of the tested antibiotics were not detected in finished water.[45] This presents a useful contrast to environments contaminated by manufacturing, highlighting the significance of reporting concentrations in their environmental context.

The importance of TPs in groundwater and drinking water studies is particularly noteworthy, as parent compound-only analytical methods may fail to detect chemicals formed during soil passage, aquifer transformation, or drinking water treatment. Detection of N^4-acetylated sulfonamide metabolites in coupled lake–groundwater systems demonstrates the mobility of metabolites out of the source compartment.[41] Furthermore, finished-water monitoring should differentiate between compounds present in the source water and those formed during treatment (TPs), as chlorination, oxidation, and other treatment processes can generate additional products.

Groundwater also differs from surface water in terms of the environment where antibiotics are transformed. Photolysis does not occur in the aquifer, as there is no direct solar irradiation. Oxygen availability, organic carbon content, mineralogy, and microbial activity vary widely with aquifer type and depth. Consequently, some compounds may be more persistent than they are in illuminated surface waters, while others can be susceptible to biodegradation, hydrolysis, redox reactions, or interactions with mineral surfaces. Therefore, the presence of antibiotics in groundwater should be considered from a hydrogeological point of view, not only as concentrations measured in a single well.

Therefore, the main concern addressed in this review from a drinking-water perspective is not that all detected antibiotics necessarily pose an immediate toxicological hazard, but that drinking-water sources are the ultimate receptor of upstream environmental transport. Monitoring should include source-water characterization, wastewater or agricultural impact identification, parent/TP profiling, treatment performance evaluation, and spatial trends analysis in distribution systems. This approach provides a more scientifically defensible assessment than mixing extreme industrial cases and trace municipal drinking-water detections in a single concentration range (Table 8).

4. Ecotoxicological effects on microbial communities and ecosystem functions

Antibiotics and their TPs impose profound selective and disruptive pressures on environmental microbiota far beyond their intended therapeutic endpoints. Once they are released in aquatic and terrestrial ecosystems, these compounds represent chronic environmental stressors, causing cellular physiological distress, inducing DNA repair mechanisms such as the SOS response, and promoting resistance selection within biofilms through subinhibitory exposure. In addition to the toxic effects on individual cells, antibiotic residues perturb cell-to-cell signaling mechanisms such as quorum sensing, alter microbial community structures, and inhibit keystone taxa that are essential for natural attenuation. Thus, changes in microbial biomass, diversity, and metabolic activity cascade

Table 8. Representative occurrence of antibiotics and transformation products in groundwater and drinking-water systems

Environmental setting	Representative compounds	Key finding	Interpretation	Reference
Lake-connected groundwater	22 antibiotics and 4 sulfonamide metabolites	Parent compounds and metabolites were detected across lake water, groundwater, and sediment	Demonstrates surface-water–groundwater connectivity and metabolite transport	41
Pharmaceutical-manufacturing hotspot in India	Ciprofloxacin, enoxacin, and other pharmaceuticals	Extremely elevated contamination occurred in industrial effluent and nearby surface/groundwater systems	Exceptional point-source scenario; values must not be generalized to ordinary drinking water	42
Manufacturing-affected wells and surrounding matrices	Fluoroquinolones and plasmid-mediated quinolone-resistance genes	Chemical and resistance determinants were identified across water, sediment, soil, and well-water compartments	Illustrates multicompartment dissemination around a severe industrial source	43
Urban wastewater–drinking-water continuum	Multiple human-use antibiotics	Antibiotics were transported through wastewater, receiving waters, and drinking-water systems	Demonstrates connectivity between wastewater emissions and downstream water supplies	44
Finished drinking water in Shanghai	Florfenicol and thiamphenicol	Median tap-water concentrations of florfenicol and thiamphenicol were approximately 8.9 ng/L and 6.4 ng/L, respectively; most investigated antibiotics were not detected	Provides realistic trace-level drinking-water context distinct from industrial hotspots	45

to macroscopic ecological changes, affecting key nutrient processes such as nitrification, denitrification, organic phosphorus compensation, and overall biogeochemical cycling (Figure 3).

4.1. Subinhibitory concentrations and physiological stress

Antibiotics can induce oxidative damage in bacteria, leading to the production of reactive oxygen species (ROS). This causes DNA, protein, and lipid damage, which eventually results in cell death. For example, interaction of aminoglycosides with ribosomes can cause mistranslation and abnormal folding of membrane proteins, leading to production of toxic peptides and subsequent activation of the bacterial envelope stress response, oxidative stress generation, and finally cell death. Regardless of their molecular targets, the major classes of bactericidal antibiotics such as quinolones, β-lactams, and aminoglycosides can induce mitochondrial dysfunction in various mammalian cell types (Figure 3).[46]

The SOS response is a conserved bacterial stress response triggered primarily by DNA damage. The SOS response triggers and coordinates DNA repair pathways, arrests bacterial cell division, and can induce prophage activation. TPs are also associated with biofilm formation. Biofilm-associated bacteria are less sensitive to antibiotics than free-living (planktonic) cells. With varying antibiotic concentrations throughout a biofilm, microbial cells are often exposed to subinhibitory concentrations and may develop resistance.[47] Therefore, biofilms provide the ideal environment for resistance selection. Because the extracellular polymeric substance matrix limits liquid diffusion, bacteria within a biofilm are often exposed to low levels of TPs. Instead of killing the bacteria, these low levels of TPs induce a selective pressure that favors the survival of resistant strains. Due to their structural resemblance to bacterial autoinducers, some TPs can bind directly to quorum sensing receptors, either triggering a false response or blocking the true signal. This phenomenon can lead to a decreased biological functioning capacity in bacteria.

4.2. Community assembly and functional shifts

Microbes are principally responsible for the transformation and assimilation of excess nitrogen in urban streams (e.g., denitrification, nitrogen fixation, and organic matter decomposition). If antibiotics alter benthic microbial communities, this may result in significant changes to these critical nitrogen processing functions. Excessive reactive nitrogen in streams can cause eutrophication, harmful algal blooms, oxygen depletion, and acidification. Photolysis and microbial degradation, which enable the rapid mineralization of organic phosphorus, constitute the crucial mechanism for phosphorus compensation during

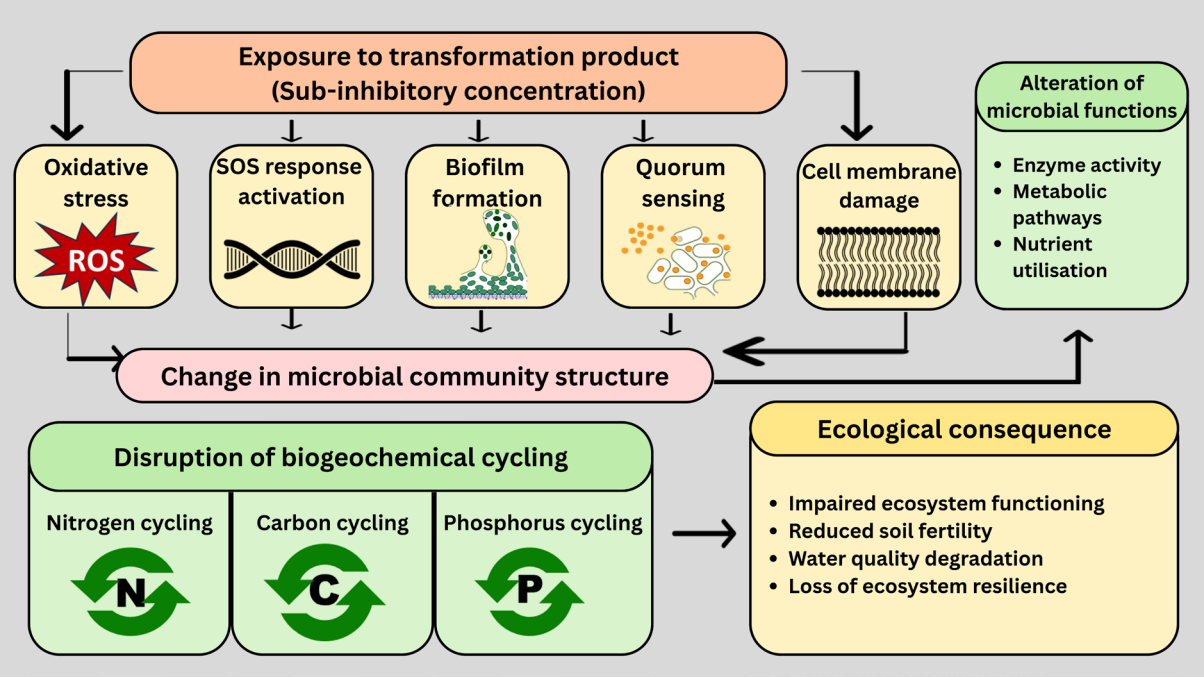


Figure 3. Conceptual pathways linking exposure to parent antibiotics and transformation products to responses of microbial communities and changes in ecosystem functions
Abbreviation: ROS: Reactive oxygen species.

algal bloom outbreaks in shallow lakes. During algal bloom outbreaks, rapid biological uptake of dissolved inorganic phosphorus decreases the concentration of inorganic phosphorus in the water column rapidly. Algae-derived organic phosphorus becomes an important reservoir of phosphorus, and its migration and transformation subsequently affect phosphorus compensation during algal growth. Previous studies have shown that 40–90% of algae-derived organic phosphorus can be mineralized in the water column, releasing dissolved inorganic phosphorus and supplying sufficient nutrients for the persistent occurrence of blooms.[48]

In one study, ^{13}C-labeled sulfamethoxazole was added to sediment samples from Zhushan Bay, West Shore, and Gonghu Bay in Taihu Lake to explore the interplay of bacterial and eukaryotic communities during a 30-day incubation period. The cumulative sulfamethoxazole mineralization on day 30 ranged from 5.2% to 19.3%, which was the highest in West Shore and the lowest in Gonghu Bay. The bacterial community showed larger within-group interactions than between-group interactions, and the positive interactions decreased during incubation. However, the eukaryotic community exhibited larger between-group interactions than within-group interactions, and the positive interactions increased during incubation. Fifty genera (including 46 bacterial and 4 eukaryotic genera) were identified as the keystone taxa due to their dominance in the co-occurrence network and tolerance to sulfamethoxazole. The cumulative relative abundance of these keystone taxa significantly increased during incubation and was consistent with the sulfamethoxazole mineralization rate. These taxa cooperated closely and played vital roles in co-occurrence networks and microbial community interactions, signifying their crucial role in sulfamethoxazole mineralization.[49]

Some antibiotic TPs also revert to their parent compounds, or form even more toxic compounds through a process called reactivation. For example, when ingested orally by humans, approximately 85% of sulfamethoxazole is excreted in urine as free and conjugated metabolites. Microorganisms can then reconvert the conjugated antibiotic metabolites to their parent forms; thus, this constitutes a non-negligible source of antibiotics. Very little is known about metabolite reactivation; hence, further research advancements are required to understand their true potential.[50] A recent study reported that tetracycline and sulfonamide TPs often showed higher mutagenicity and carcinogenicity levels, respectively, than their parent compounds.[4] Another study revealed the transformation of cephalosporin antibiotics at the MnO_2 surface mediated by

iodide, where the presence of iodide can either accelerate or decelerate the oxidation of cephalosporin antibiotics by MnO_2, thereby significantly affecting their environmental fate due to the formation of reactive iodine species. Toxicity assessments indicate that the iodinated TPs exhibit higher toxicity than the parent compound and its non-iodinated products.[51]

4.3. Impacts on biogeochemical cycling

Studies show that the structure and function of microbial communities in soil and sediment are modified by antibiotic exposure, but the resulting impact on biogeochemical processes is poorly understood. Only a few studies have investigated the effects of antibiotics on microbial nitrogen cycling. In one study, among 19 antibiotics and antimicrobials investigated, fewer than half (9) showed nitrification inhibitory effects, and the minimum inhibitory concentration (MIC) ranged from 200 μg/kg to 200 mg/kg. Among the remaining antibiotics, the following five had no observable effect on nitrification: chlortetracycline, difloxacin, monensin, ivermectin, and chloramphenicol. The effects of 18 different antibiotics on denitrification were also investigated, and the results varied considerably among the different solid matrices and concentrations tested. Inhibition was reported in soil, sediment, and/or groundwater treated with the following 12 antibiotics: bacitracin, amoxicillin, clarithromycin, chlortetracycline, erythromycin, flumequine, gentamicin, narasin, sulfadiazine, sulfamethazine, sulfamethoxazole, and vancomycin.[52]

In another study, the impact of a shock load of roxithromycin on waste activated sludge anaerobic digestion was investigated. Roxithromycin inhibited acidogenesis and methanogenic activity in waste activated sludge anaerobic digestion, leading to decreased methane production.[53]

5. Transformation products and antimicrobial resistance

Antimicrobial resistance is a major global health challenge. Most estimates of AMR burden address clinical infections, but the importance of environmental compartments as critical interfaces where antimicrobial residues, ARB, ARGs, mobile genetic elements, and diverse microbial communities can coexist is increasingly recognized.[54]

Antibiotic resistance may arise from de novo genetic mutation or acquisition of resistance determinants through horizontal gene transfer (HGT) mechanisms such as conjugation, transformation, and transduction. Once acquired, resistance determinants can then be inherited vertically during bacterial replication. Therefore, vertical transmission should be distinguished from the mechanisms by which novel resistance initially emerges within a bacterial population. Environmental antibiotics and their TPs may affect these processes mainly through selective pressure that enhances the relative fitness and persistence of resistant organisms compared to susceptible competitors.

Antibiotic exposure need not achieve the MIC to impact microbial evolution. Experimental competition studies have shown selection of resistant bacteria at concentrations far below the MIC, giving rise to the concept of the minimum selective concentration.[55] Hence, environmental risk cannot be evaluated based on microbial growth inhibition concentrations alone. Even subinhibitory concentrations can enrich pre-existing resistant populations or prolong the persistence of resistance determinants with prolonged exposure.

A study simulated an aerobic activated sludge process treating livestock wastewater containing enrofloxacin and heavy metals (Cu^{2+} and Zn^{2+}) to evaluate the development of antibiotic resistance using metagenomic and metatranscriptomic approaches. It was found that ARG profiles under co-exposure to enrofloxacin and heavy metals more closely resembled those under heavy metal exposure alone than under enrofloxacin exposure alone. Zn^{2+} exposure resulted in the highest absolute ARG abundance, nearly double that of the control group. Both enrofloxacin and heavy metals significantly altered the abundance and phylogenetic composition of ARB. Exposure to Zn^{2+} enhanced the relative abundance and expression level of both metal resistance gene-carrying ARB and the ARG-carrying plasmids.[56]

5.1. Resistance selection by antibiotic transformation products

An antibiotic might interact with bacterial targets as a direct result of its transformation. Structural modification may decrease, retain, or even increase antimicrobial activity depending upon the functional group modified and whether the TP retains affinity for the biological target of origin. Thus, some antibiotic TPs retain biological activity even when the parent antibiotic concentration has declined significantly. This distinction is particularly important for environmental exposure, as parent antibiotics and multiple TPs are frequently present at the same time. Such a situation may lead to microbial communities being susceptible to the collective selective pressure of an antibiotic-related chemical mixture rather than only the parent compound. Thus, risk assessments based only on parent-antibiotic concentrations may underestimate the total selective pressure exerted by a discharged antimicrobial agent.

Some sulfamethoxazole TPs exhibit residual antibacterial activity. Majewsky *et al.*[57] showed that modification of the *para*-position of sulfamethoxazole did not uniformly abolish antibacterial activity, and some TPs retained biologically relevant effects. Such residual activity provides a mechanistic basis for resistance selection concerns, although antimicrobial activity should not be taken as direct evidence of AMR selection. We now have direct experimental evidence that antibiotic TPs promote AMR selection in complex microbial communities. Lakhey *et al.*[9] systematically compared parent antibiotics and their TPs with bacterial communities from wastewater. Some TPs exhibited growth effects at similar concentrations as their parent compounds, and moxifloxacin sulfate and descladinose roxithromycin were more potent in growth inhibition than their respective parents. More importantly, desmethyl ofloxacin, *N*-acetyl sulfamethoxazole, and desmethyl erythromycin enriched the class 1 integron-integrase gene *intI1* to comparable or higher levels than their respective parent antibiotics during seven-day evolution experiments.[9] A study investigated the impact of three typical sulfonamide metabolites, namely 4-nitro-sulfamethoxazole, N^4-acetylated sulfamethoxazole, and N^4-acetylated sulfadiazine, on the risk of ARG transmission. The results revealed that the TPs significantly enhanced the risk of conjugative transfer of the RP4 plasmid at clinically and environmentally relevant concentrations (10 ng/L to 100 μg/L), with a maximum increase of up to 73-fold. These three metabolites' capabilities to enhance the conjugative transfer of ARGs are more pronounced than the parent sulfonamides.[58]

5.2. Mechanisms linking transformation product exposure with antimicrobial resistance

Resistance selection is the main mechanism by which biologically active TPs promote AMR. If two types of susceptible and resistant microbes are present, then an antimicrobial compound that is more inhibitory to the susceptible organisms may provide a relative fitness advantage to resistant populations. Therefore, repeated exposure may lead to an enrichment of resistant organisms even when the absolute concentration of the compound is too low to completely eradicate the susceptible population. Importantly, selection at concentrations well below the classical MICs of the parent antibiotics has been experimentally shown, providing a critical conceptual basis for the evaluation of active TPs.[55]

Other stress-mediated mechanisms may also be involved under certain exposure conditions. Antibiotic stress can change DNA-repair systems, stress-response pathways, membrane permeability, efflux activity, and genetic exchange rates. These processes depend on the antibiotic compound, its concentration, and the organism. Hence, the assertion that antibiotic TPs generally cause resistance via ROS overproduction should be reevaluated. While stress-induced mutagenesis and ROS may be involved in response to some antimicrobial exposures, they do not represent universal mechanisms of resistance across all antibiotic TPs.

Once ARGs are present, HGT provides another important pathway for environmental dissemination of AMR. Mobile genetic elements such as plasmids, integrons, and transposons can mediate movement of resistance determinants between bacterial taxa. Class 1 integrons are particularly significant because they can capture and express gene cassettes, including resistance determinants. Therefore, the *intI1* gene, encoding the class 1 integron-integrase, is often used as an indicator of anthropogenic pollution and as a marker associated with environments enriched in mobile resistance determinants.[10] However, *intI1* is not a resistance gene for a particular antibiotic, and an increase in *intI1* should be taken as evidence of the increased abundance of class 1 integron-associated genetic potential rather than proof that a specific clinical resistance phenotype has been generated.

5.3. Direct evidence for antimicrobial resistance selection by transformation products

Most evidence on antibiotic TPs has so far been associated with their occurrence, persistence, toxicity, or residual antimicrobial activity, while direct measurements of resistance selection have been scarce. This represents an important distinction, because evidence of bacterial growth inhibition by a TP does not necessarily reflect resistance selection. Therefore, the wastewater-community experiments of Lakhey *et al.*[9] are an important step forward, as parent compounds and TPs were directly compared within the same experimental framework. All three antibiotics, desmethyl ofloxacin, *N*-acetyl sulfamethoxazole, and desmethyl erythromycin, induced enrichment of *intI1* in seven-day exposure experiments, and responses were comparable to or greater than those of the corresponding parent antibiotics. These results demonstrate that biological transformation does not necessarily reduce resistance-selection potential. *N*-acetyl sulfamethoxazole, a major human metabolite of sulfamethoxazole, is of particular environmental relevance due to its widespread detection in WWTP effluents and receiving surface waters. *N*-acetyl sulfamethoxazole concentrations have been reported in wastewater effluent within the μg/L range, indicating that environmental exposure to this metabolite is plausible.[57] Additionally, desmethyl ofloxacin has been studied in wastewater and receiving-water monitoring programs along with ofloxacin

and other fluoroquinolone-related compounds. The work showed that fluoroquinolone metabolites and TPs can be found in wastewater–surface-water pathways along with parent compounds.[58] These findings provide credence to the transition from a parent compound-based model of AMR risk to a cumulative parent + TP approach. According to this theory, antibiotic transformation does not always imply the removal of AMR selective pressure, but rather a shift in its chemical makeup (Table 9).

5.4. Implications for environmental antimicrobial resistance risk assessment

The evidence reviewed above has several implications for environmental AMR risk assessment. First, depletion of a parent antibiotic should not be equated with removal of resistance selection pressure. Second, TP evaluation should distinguish among chemical occurrence, residual antimicrobial activity, and experimentally demonstrated resistance selection, which represent different levels of evidence. Third, resistance endpoints should be determined at environmentally relevant concentrations and, where possible, in complex microbial communities rather than only in a single laboratory strain.

Existing AMR-specific environmental assessment approaches are increasingly acknowledging that safe environmental concentrations should consider selection thresholds beyond traditional ecotoxicity endpoints.[8] However, despite their persistence, mobility, and potential residual biological activity, TPs are still underrepresented in routine regulatory monitoring.

An improved framework should therefore integrate (i) measured concentrations of parent antibiotics and major TPs, (ii) TP-specific antimicrobial activity, (iii) minimum selective concentrations or comparable community-level selection endpoints, (iv) changes in ARGs and mobile genetic elements, (v) re-transformation of metabolites to active parent compounds, and (vi) mixture effects generated by simultaneous exposure to parent compounds and multiple TPs. Such an approach would present a more realistic estimate of the total environmental selective pressure exerted by the use and discharge of antibiotics. Available evidence suggests that antibiotic transformation does not necessarily reflect AMR-related detoxification. While some TPs lose their antimicrobial activity and others retain their biological activity, a smaller but increasingly

Table 9. Representative antibiotic transformation products with demonstrated or mechanistically relevant potential to contribute to antimicrobial-resistance selection

Parent antibiotic	TP	TP type/transformation	AMR-related experimental observation	Environmental relevance	Reference
Ofloxacin	Desmethyl ofloxacin	*N*-demethylated metabolite/TP	Seven-day exposure of wastewater-derived microbial communities enriched the *intI1* gene/class 1 integrons to concentrations equal to or above the response triggered by the parent antibiotic	Contributed to the literature on metabolites/TPs of ofloxacin in wastewater and receiving waters	9
Sulfamethoxazole	*N*-acetyl sulfamethoxazole	N^4-acetylated human metabolite	In wastewater microbial communities, *intI1* was enriched on exposure, and responses were comparable to or greater than the parent in the experimental system	Detected in WWTP effluent and surface water environments; may also revert to sulfamethoxazole	9,57
Erythromycin	Desmethyl erythromycin	Demethylated metabolite/TP	After seven days of exposure, *intI1* was enriched to equal or greater concentrations than those triggered by the corresponding parent antibiotic	Biological activity can persist in wastewater-derived chemical mixtures from macrolide metabolites and TPs	9

Abbreviations: AMR: Antimicrobial resistance; *intI1*: class 1 integron-integrase gene; TP: Transformation product; WWTP: Wastewater treatment plant.

important group has now been shown experimentally to select for AMR-associated genetic markers. Thus, it is important to include these compounds in environmental risk monitoring and assessment to determine whether wastewater treatment and environmental transformation effectively reduce AMR selection or merely alter the chemical form of selective pressure.

6. Bioaccumulation and trophic transfer

Bioaccumulation refers to the net increase of a chemical in an organism via uptake from all relevant environmental sources (water, soil or sediment, diet, and irrigation water for terrestrial plants), considering elimination, metabolism, and growth dilution. It should therefore be distinguished from biosorption, which describes association with biological surfaces, and from bioconcentration, which refers specifically to chemical accumulation directly from the surrounding medium, especially water, without dietary exposure. Trophic transfer is the transfer of a chemical from prey or food to consumers in a diet. When chemical concentrations increase systematically with trophic level, the process is called trophic magnification; when concentrations decrease with increasing trophic position, it is called trophic dilution.

These distinctions are particularly important for antibiotics and their TPs, as most antibiotics are ionizable rather than persistent, neutral hydrophobic pollutants. Therefore, octanol/water partition coefficients alone cannot reliably predict biological accumulation. Uptake and retention depend on acid dissociation constant, environmental and physiological pH, pH-dependent distribution coefficient, molecular size, polarity, protein and mineral binding, membrane permeability, transporter activity, metabolic transformation, and excretion rates. These properties can be further modified by antibiotic transformation so that a TP can accumulate to a greater, similar, or lower degree than its parent compound.

Organisms can also serve as transformation sites rather than simply as passive sinks. Parent antibiotics may be susceptible to oxidation, reduction, hydrolysis, or conjugation following uptake, forming organism-derived biotransformation products that may be retained in specific tissues, excreted, or transferred via food webs. Hence, evaluation of bioaccumulation based on the parent antibiotic alone may lead to an underestimation of the overall antibiotic-related residue load.[59]

6.1. Uptake by primary producers

Primary producers represent the main entry point for antibiotic residues in terrestrial and aquatic food webs. In agricultural systems, crops may be exposed via reclaimed-wastewater irrigation, manure-amended soil, biosolid application, and contaminated soil pore water. In aquatic systems, dissolved antibiotics can be taken up directly by phytoplankton and other photosynthetic microorganisms from surrounding water. These organisms are the foundation for many food webs, and accumulation at this level may determine the initial chemical burden available for subsequent trophic transfer.

6.1.1. Uptake by terrestrial plants

Absorption of an antibiotic or TP by terrestrial plants is typically initiated in the roots via the fraction dissolved in soil pore water. Strong sorption of compounds to soil organic matter, clay minerals, or metal ions may reduce the freely dissolved fraction available for uptake, whereas relatively mobile compounds may reach the root surface more easily. Uptake is therefore dependent on both the total concentration in soil and the fraction that remains bioavailable.

Contaminants can enter plant root tissues through apoplastic and symplastic pathways upon contact with the root surface, and can subsequently be transported by the transpiration stream through the xylem. The efficiency of root-to-shoot translocation varies greatly between compounds. The relative abundance of charged and neutral species of ionizable antibiotics is pH-dependent. Generally, neutral species penetrate the lipid membrane more easily, whereas ionization upon entering a compartment of different pH can favor intracellular retention. However, this "ion-trapping" behavior should be considered one of the contributing mechanisms rather than a universal explanation for antibiotic uptake.

Plant species and tissue type are also important factors influencing antibiotic uptake. Large differences in uptake between plants can occur even when they are exposed to the same external concentration, due to root architecture, transpiration rate, lipid content, rhizosphere conditions, and metabolic capacity. Controlled experiments with vegetables grown in manure-amended soils have demonstrated measurable uptake of veterinary antibiotics, but the magnitude of accumulation varies greatly among antibiotic–crop combinations.[33]

Additionally, the irrigation route can change plant exposure to antibiotics. Bhalsod *et al.*[60] compared overhead and soil-surface irrigation of greenhouse lettuce with pharmaceutical-contaminated water. The study showed that overhead irrigation produced substantially greater residues in shoots than soil-surface irrigation because contaminants could contact foliage directly. Root concentration factors for soil-surface irrigation also varied highly among compounds; e.g., sulfamethoxazole

accumulated in the roots to a much greater extent than tylosin.[60] These results show that the contamination of plants cannot be predicted from the concentrations in the irrigation water alone because the application method, root uptake, foliar deposition, and subsequent translocation all contribute to the final residue burden.

Crucially, chemical uptake does not mean that the parent compound remains unchanged in plant tissues. Plants have metabolic systems that can oxidize, hydrolyze, and conjugate parent compounds; thus, pharmaceutical residues can be converted to phytometabolites after uptake. Therefore, plants should be considered both exposure receptors and potential transformation compartments for antibiotic TPs, since parent-only analysis may not detect conjugated or otherwise transformed residues retained within plant tissues. Accordingly, the assumption that TPs generally bind to "specific plasma membrane receptors" should be rejected. The evidence available supports a multifactorial uptake model controlled primarily by chemical speciation, bioavailability, membrane transport, plant physiology, and metabolism rather than a general receptor-mediated mechanism.

6.1.2. Uptake by aquatic primary producers

Aquatic primary producers serve as a similar entry point to aquatic food webs. Phytoplankton are consistently exposed to dissolved antibiotics and TPs and can accumulate compounds through direct cellular uptake and association with cell surfaces. The uptake rate depends on the antibiotic form, the pH of the surrounding environment, the amount of dissolved organic matter, cell composition, the rate of cell growth, and the amount of free chemicals in the water column.

Phytoplankton biomass may also affect contaminant fate at the ecosystem scale. Tang *et al.*[61] revealed the predominant role of the phytoplankton biological pump in spatiotemporal bioaccumulation and trophic transfer of antibiotics in the Pearl River. Bioaccumulation factors in phytoplankton were dependent on bloom conditions, and higher phytoplankton biomass was negatively correlated with overall antibiotic bioaccumulation and trophic magnification in that system. Thus, primary producers are not simply passive accumulators of contaminants; variation in biomass, community structure, and chemical bioavailability may influence the quantity of antibiotics entering higher trophic levels.

The evidence suggests that primary-producer uptake should be viewed as a dynamic equilibrium between external exposure, sorption, cellular uptake, transformation, growth dilution, and elimination. For antibiotic TPs, these processes are still much less characterized than for their parent compounds and represent an important research gap.

6.2. Trophic transfer

Trophic transfer is the ingestion and uptake of accumulated parent antibiotics or TPs by a predator or consumer through contaminated food. Biomagnification or dilution of a compound along a food web is a function that reflects the balance of dietary intake, assimilation efficiency, uptake from ambient water, metabolic transformation, tissue distribution, excretion, and organismal growth. Thus, the occurrence of a compound at several trophic levels does not mean biomagnification.

Contaminant data are often integrated with stable-isotope analysis, particularly the estimation of trophic position from $\delta^{15}N$, to calculate trophic magnification factors (TMFs). TMF > 1 suggests increasing concentration with trophic position, while TMF < 1 suggests trophic dilution. Biota-sediment accumulation factors, bioaccumulation factors, and concentration ratios in specific tissues provide complementary information but should not be used interchangeably with TMFs.

Like many persistent neutral organic pollutants, antibiotics generally do not biomagnify through food webs. The ionizable character, relatively rapid elimination of some compounds, active transport, and metabolism can make antibiotic trophodynamics highly specific to each compound. For example, fluoroquinolones were found to have relatively high bioaccumulation potential in a study of aquatic and terrestrial food webs in the Yellow River Delta. However, the total antibiotic measurement showed trophic dilution in the aquatic food web but trophic magnification in the terrestrial food web.[62] In the two systems, the physicochemical properties were also differently related to the trophic behaviors, which shows that conclusions drawn from the aquatic food webs cannot be extrapolated to the terrestrial systems.

TPs introduce an additional level of complexity, as free and conjugated forms of the same antibiotic may have different trophic behaviors. In an estuarine food web, Wu *et al.*[50] studied both free and conjugated antibiotics. Conjugated forms accounted for 23.8–76.9% of the total antibiotic load in biological samples, indicating that the organismal residues may be significantly underestimated if only free parent compounds are considered. Trophic dilution for the free form of anhydroerythromycin and for the conjugated forms of trimethoprim and ciprofloxacin, and trophic amplification for free trimethoprim and conjugated ofloxacin were also observed.[50]

The results are particularly relevant for environmental risk assessment of TPs, as they suggest that transformation

or conjugation may alter trophodynamics and not necessarily result in a decrease in exposure. Hence, the trophic behavior of the parent compound cannot be confidently extrapolated to the corresponding TP. Antibiotics can also be metabolized by aquatic organisms after uptake. Zhu *et al.*[63] studied seven antibiotics in juvenile sea cucumbers (*Apostichopus japonicus*) and identified seven biotransformation products. Some biotransformation products were highly localized in the digestive tract, indicating the ability of aquatic consumers to bioaccumulate and biotransform antibiotic residues. The kinetic bioconcentration factor of ofloxacin reached a maximum value of 1,719.7 L/kg within the experimental system.[63] This underscores the requirement for tissue-specific analysis, as whole-body or muscle-only measures may not detect organs with high transformation and accumulation.

Studies in the field have shown that tissue distribution can be highly variable in aquatic organisms. Zhou *et al.*[64] found 24 of the 41 studied antibiotics in the biota of the food web in Lake Taihu. Concentrations were typically in the order of fish plasma and muscle < liver and bile, with invertebrates and plankton also showing substantial residues. Fluoroquinolones had higher potential for bioaccumulation than some other antibiotic classes; however, antibiotics generally showed trophic dilution in that food web.[64]

These studies collectively suggest that trophic transfer of antibiotics and TPs does not show universal trends of amplification or dilution. The direction and magnitude of trophic transfer depend on antibiotic class, chemical form, food web structure, habitat, organism physiology, and tissue-specific metabolism. Therefore, when analytical methods are available, future studies should quantify parent compounds, free TPs, and conjugated residues simultaneously.

6.3. Ecological and organismal consequences of bioaccumulation

The ecological relevance of bioaccumulation is distinct from impacts of mere detection of antibiotics in an environmental matrix. Accumulation leads to higher internal exposure and can maintain concentrations at biologically relevant levels in tissues even when concentrations in the surrounding water or soil are relatively low. Internal exposure may thus affect physiological processes, microbiomes, and potential transfer of residues to consumers. But bioaccumulation alone should not be interpreted as an indication of toxicity, since biological effect depends on tissue concentration, exposure length, chemical activity, and organism sensitivity.

6.3.1. Tissue-specific internal exposure

Antibiotic accumulation is often heterogeneous between tissues due to differences in blood supply, protein content, transport systems, pH, metabolic capacity, and excretory function between organs. For some antibiotics, the liver, bile, and gastrointestinal tissues may have higher concentrations than edible muscle. This distinction is environmentally relevant because tissues involved in metabolism may both accumulate parent compounds and produce additional TPs.

An excellent example of this process is the sea cucumber study mentioned above, where antibiotic biotransformation products were found primarily in the digestive tract, suggesting that the digestive system is a significant transformation site.[63] Similarly, the liver and bile of fish from Taihu Lake contained higher antibiotic burdens than their muscle and plasma.[64] Thus, monitoring only edible muscle may be appropriate for dietary exposure assessment but may grossly underestimate whole-organism exposure and internal transformation.

6.3.2. Consequences for plants and associated microbiomes

In terrestrial food webs, accumulation in crop roots and shoots directly exposes plant-associated microbial communities to antibiotic residues. These communities occupy the rhizosphere, root endosphere, phyllosphere, and internal tissues of plants and provide functions related to nutrient acquisition, stress tolerance, and plant development. Application of manure can simultaneously introduce antibiotic residues, ARB, and ARGs to agricultural soils, thereby establishing an exposure pathway from soil to plant-associated microbial communities. Zhang *et al.*[65] demonstrated the transfer of resistance determinants from manure-amended soils to lettuce-associated microbiomes and alterations in rhizosphere and phyllosphere resistomes. This observation is relevant to bioaccumulation, as the same soil–plant continuum can play a role in chemical uptake and microbiological transfer. But these processes are not to be mistaken for one another. An example of chemical bioaccumulation is the uptake of an antibiotic molecule into plant tissues. An example of biological dissemination is the movement of ARB or ARGs from soil into the plant microbiome. Although their coexistence may increase ecological relevance, the mechanisms should be discussed separately. Therefore, the detailed mechanisms of resistance selection and HGT are described in Section 5 and are not repeated here.

6.3.3. Consequences for aquatic organisms and food webs

Accumulation in plankton, benthic organisms, invertebrates, and fish offers a pathway for the spatial and trophic redistribution of antibiotic-related residues. Organisms can transport accumulated compounds away from the original contamination site through migration, predation, and excretion. Prey consumption can expose predators to both parent antibiotics and TPs. However, existing food-web studies show that this process often leads to trophic dilution rather than classical biomagnification. In the Beibu Gulf, several antibiotics (e.g., enrofloxacin, ciprofloxacin, ofloxacin, norfloxacin, anhydroerythromycin, and trimethoprim) were biodiluted through the food web studied.[66] Other systems and chemical forms, however, show trophic amplification, indicating that trophodynamics must be evaluated on a compound- and ecosystem-specific basis rather than generalized to antibiotics as a single class. From an ecological standpoint, the internal residues may prolong exposure longer than the elevated dissolved environmental concentrations. Antibiotic-related chemicals may be retained in biological compartments through tissue retention, repeated dietary uptake, and recycling through excretion or decomposition. This recycling may be especially complex for TPs, as organisms can ingest TPs in the environment, internally transform parent antibiotics and release additional metabolites back into water, sediment or soil.

6.3.4. Dietary exposure and One Health relevance

Bioaccumulation in edible crops, fish, and shellfish is a potential dietary pathway linking environmental antibiotic contamination to humans and other consumers. However, the presence of a residue in edible tissue should not be automatically equated with a significant risk to human health. Human health risk depends on residue concentrations, consumption rate, body weight, toxicological or microbiological threshold, and exposure to other sources. For example, although Zhou *et al.*[64] identified several antibiotic residues in the Lake Taihu food web, the calculated daily intakes based on the consumption of fish, crab, and prawns were below the corresponding acceptable daily intakes, and the hazard quotients were substantially less than 1, based on the assumptions made in that study. Therefore, the study demonstrated a measurable pathway of dietary exposure, but not a significant direct toxicological risk for the population under study. However, dietary exposure to conjugated residues and TPs is less well characterized. Routine residue monitoring usually focuses on a limited number of parent antibiotics, and conjugated or organism-derived TPs are often not measured. The finding that conjugated forms could account for a large proportion of the total antibiotic-related load in food-web organisms[50] implies that parent-only monitoring may provide an incomplete picture of chemical exposure. Future bioaccumulation assessments should therefore consider (i) freely dissolved environmental concentrations; (ii) parent antibiotics and major environmental TPs; (iii) conjugated and organism-generated metabolites; (iv) tissue-specific accumulation; (v) bioconcentration and bioaccumulation factors; (vi) stable-isotope-based trophic position and TMFs; and (vii) depuration and metabolic transformation. This integrated approach would allow better distinction among simple environmental occurrence, organismal accumulation, trophic transfer, and true trophic magnification.

Generally, available evidence suggests that the bioaccumulation and trophic transfer of antibiotic residues are highly compound-, organism-, and ecosystem-specific. Transformation may change the chemical form taken up by an organism, and organisms may generate further TPs themselves. Furthermore, conjugated products may display trophic behavior different from that of their corresponding free forms. Therefore, environmental assessments should extend beyond measuring parent-only concentration and encompass the total parent–TP–conjugate load across primary producers, consumers, and higher trophic levels.

7. Analytical and detection methods

Reliable characterization of antibiotic TPs is analytically challenging due to the presence of complex mixtures of parent antibiotics, metabolites, TPs, and unrelated organic micropollutants in environmental samples over broad ranges of polarity and concentration. Many environmentally relevant TPs are present at trace levels, lack commercially available reference standards, and may have physicochemical properties that differ significantly from their parent compounds. Thus, no single analytical technique can characterize the entire parent–TP–biological-response continuum. Current analytical strategies can be generally classified into targeted analysis, suspect screening, and NTS.[67] Targeted approaches allow sensitive and accurate quantification of known compounds for which analytical standards are available. Suspect screening utilizes HRMS data to search for anticipated compounds based on known metabolic or environmental transformation pathways, even without reference standards. NTS is not limited to the search for predefined compound lists; instead, it attempts to detect, prioritize, and structurally characterize unrecognized chemical features.[67] This differentiation is especially relevant for antibiotic TPs, where a targeted analysis of the parents alone can significantly underestimate the total antibiotic-related chemical load. Recent HRMS-based studies illustrate the

advantage of these complementary approaches. Hu *et al.*[68] developed an integrated suspect and NTS workflow designed for antibiotic TPs in WWTP effluents, showing that TP occurrence and associated environmental risks may be underestimated when monitoring focuses only on parent antibiotics. Similarly, Yu *et al.*[69] employed a molecular network-based NTS to analyze pharmaceutical wastewater, identify antimicrobial TPs, and reconstruct previously unidentified transformation relationships. These studies highlight the increasing need for the integration of quantitative targeted analysis and exploratory HRMS workflows in comprehensive environmental surveillance (**Table 10**).

7.1. Advanced chemical detection methods

7.1.1. Targeted liquid chromatography with tandem mass spectrometry analysis

Liquid chromatography with tandem mass spectrometry (LC–MS/MS), especially triple-quadrupole instruments working in selected- or multiple-reaction-monitoring modes, is still one of the main techniques for quantification of known antibiotics and TPs. Chromatographic separation decreases matrix interference, and the high analytical selectivity and sensitivity are obtained with precursor-to-product ion transitions. When authentic reference standards and isotope-labeled internal standards are available, LC–MS/MS can reliably quantify concentrations in wastewater, surface water, sediment extracts, soil extracts, and biological matrices. The main strength of targeted analysis lies in its quantitative robustness; however, it is primarily limited by the requirement that compounds be predefined by the analyst. Thus, unknown TPs, unexpected metabolites, and products for which appropriate transitions or standards have not been established may go undetected. Senta *et al.*[70] showed the need to extend the targeted methods to other parent compounds in a one-year study of macrolides in municipal wastewater. Parent antibiotics were measured along with metabolites, synthesis by-products, and TPs, and the results showed that these related compounds contributed substantially to the overall macrolide mass balance.[70] This study shows the necessity of incorporating major known TPs into validated multiresidue methods, provided that authentic standards are available.

7.1.2. Liquid chromatography coupled with high-resolution mass spectrometry for suspect and non-target screening

Liquid chromatography coupled to HRMS is currently the most informative analytical platform for polar and moderately polar antibiotic-related compounds for the discovery of unknown or incompletely characterized TPs. Quadrupole time-of-flight and Orbitrap-based instruments generate accurate-mass full-scan data along with isotopic patterns and tandem mass spectra that can be used to assist in the assignment of molecular formulas and structural elucidation.

Instrument performance cannot be described by a single universal resolving power or sensitivity value, as these parameters are dependent upon instrument architecture, acquisition settings, ionization efficiency, analyte chemistry, and sample matrix. Instead, the key advantage of HRMS is the combination of high mass accuracy, high resolving power, and information-rich full-scan/MS/MS acquisition, which allows retrospective interrogation of stored data for compounds not part of the original analytical target list.

In suspect screening, candidate structures are based on the knowledge of parent antibiotics, metabolic reactions, biodegradation pathways, photochemical transformation, hydrolysis, and treatment processes. Candidate features can then be assessed based on exact mass, isotopic pattern, retention behavior, and diagnostic MS/MS fragments. Features detected in NTS are prioritized without a predefined chemical identity and subsequently annotated using spectral libraries, in silico fragmentation, molecular networking, homologous analysis of series, or transformation relationships.

Essentially, an accurate molecular mass does not define chemical identity. Structural annotations should have an explicit confidence level assigned to them. The most widely used framework by Schymanski *et al.*[71] distinguishes between confirmed structures supported by authentic reference standards and probable, tentative, and molecular formula-level annotations. This is especially relevant for antibiotic TPs, for which structural isomers can have similar elemental compositions but may vary widely in persistence in the environment and biological activity.

Hu *et al.*[68] applied a suspect screening and NTS strategy using HRMS to investigate antibiotic TPs in WWTP effluent. Yu *et al.*[69] integrated molecular networking to connect related antimicrobial structures in pharmaceutical wastewater. Collectively, these approaches demonstrate the potential of HRMS to advance environmental monitoring from a list of compounds to a more transformation-aware chemical characterization.

7.1.3. Sample preparation and analytical quality assurance

Sample preparation is an important factor influencing the analytical reliability, as antibiotic TPs often occur at concentrations significantly lower than the major matrix components. In aqueous samples, particulate material

is generally removed, and analytes are concentrated by filtration or centrifugation, before solid-phase extraction (SPE) is performed. Soil, sediment, sludge, and biological tissues must be extracted in a matrix-specific manner and cleaned up further to reduce organic matter, lipids, proteins, and other compounds that can suppress or enhance ionization. Special consideration should be given to the analysis of TPs, since a sample preparation method that works well for a parent antibiotic may not recover a more polar, ionic, or less stable TP equally well. Thus, if standards are available, TP-specific recovery should be part of the method validation. Analytical quality assurance should include procedural, solvent, and (if possible) matrix blanks, along with replicate samples, matrix spikes, recovery tests, calibration checks, and matrix effect evaluations. Isotope-labeled internal standards are preferred for quantitative liquid chromatography–mass spectrometry because they can compensate for extraction losses and ionization suppression or enhancement; if isotope-labeled standards are unavailable, semi-quantitative estimates should be clearly identified as such and reported with a lower confidence level than fully quantified TPs.[72]

7.1.4. Capillary electrophoresis

Another separation technique for ionizable antibiotics is capillary electrophoresis (CE), which is based on their electrophoretic mobility. Advantages of CE include low solvent and reagent consumption, relatively simple instrumentation, and high separation efficiency. Because many antibiotics have acidic or basic functional groups, CE can be particularly useful for targeted analysis after optimization of buffer composition, pH, electric field, and sample-preconcentration conditions. For example, Moreno-González *et al.*[73] developed a method based on capillary zone electrophoresis with offline and online preconcentration for trace determination of tetracyclines in water. Nevertheless, CE with conventional optical detection typically provides less structural information than HRMS; thus, it is less suitable as a single method for the discovery of unknown TPs. Coupling CE to mass spectrometry can improve the ability to identify compounds, but it adds instrumental complexity at the same time.

7.1.5. Immunochemical screening

Immunoassays, including enzyme-linked immunosorbent assays (ELISA), fluorescence-polarization immunoassays, and lateral-flow/immune-chromatographic assays, offer rapid and relatively inexpensive screening of selected classes of antibiotics. The main advantages are high throughput and relatively low instrumentation requirements. However, antibody cross-reactivity is of particular concern when considering TPs. An antibody raised against a parent antibiotic may also cross-react with structurally related metabolites or degradation products. Such cross-reactivity may be advantageous in screening total antibiotic-related residues, but it may not allow unequivocal identification and accurate compound-specific quantification. Kumar *et al.*[74] adopted ELISA methods for tetracycline and tylosin analysis in environmental waters and reported that transformed or structurally related tetracycline compounds could contribute to immunoassay responses that did not match liquid chromatography–mass spectrometry measurements. ELISA should thus be considered a screening or class-response method, and positive or mechanistically important findings should be confirmed by chromatographic–mass-spectrometric analysis wherever possible.

7.2. Multiomics approaches

Chemical analysis identifies the compounds to which an environmental community is exposed but does not determine which microorganisms carry resistance determinants, which genes are actively expressed, or which cellular pathways respond to exposure. Thus, multiomics approaches are especially useful for studying the relationships among antibiotics, TPs, microbial communities, and AMR, as they provide this complementary biological information.

7.2.1. Metagenomics

Shotgun metagenomics analyzes the entire genomic DNA of a microbial community without cultivation and can simultaneously characterize microbial taxonomy, AMR genes (ARGs), mobile genetic elements, and other functional genes. This is essential because a large proportion of environmental microorganisms cannot be easily characterized using routine culture-dependent techniques. Li *et al.*[75] analyzed metagenomes from 50 samples spanning 10 environmental categories and found 260 ARG subtypes belonging to 18 ARG classes. The abundance of ARGs was strongly correlated with the degree of anthropogenic influence, illustrating the power of metagenomics to characterize resistomes across complex environmental systems.[75] Metagenomics may also be used to support investigations of antibiotic transformation by identifying microorganisms and metabolic genes that may be associated with degradation pathways. However, the detection of a gene simply implies genetic potential, not actual expression or biochemical activity. Therefore, metagenomic results should not be interpreted as evidence of a given TP being produced or a detected ARG being functionally expressed.

7.2.2. Metatranscriptomics

Metatranscriptomics overcomes part of this limitation by examining community RNA and identifying genes that are actively transcribed under the environmental conditions at the time of sampling. When combined with metagenomics, it is possible to distinguish between genetic potential and transcriptional activity, and to investigate which microbial populations are actively expressing resistance or metabolic functions. Liu *et al.*[76] integrated metagenomic and metatranscriptomic analyses of activated sludge, demonstrating that integration of DNA- and RNA-based data provides information on the diversity and activity of ARGs and their microbial hosts. Likewise, analogous studies of hospital wastewater have demonstrated that ARG transcription can vary over time and can capture information not captured by DNA abundance alone.[77]

7.2.3. Proteomics and metabolomics

Proteomics provides evidence that genes identified by genomic or transcriptomic analysis are translated into proteins; thus, it can help to characterize enzymes associated with resistance, stress responses, and contaminant transformation. Characterization of low-molecular-weight metabolites can reveal changes in cellular metabolic pathways triggered by antibiotic or TP exposure. Metabolomics is conceptually useful for antibiotic–TP research, as metabolic fingerprints can be combined with HRMS-based contaminant measurements to investigate whether altered microbial metabolism is related to chemical exposure. However, correlations between omics profiles and environmental concentrations should not be presumed to be causal. Mechanistic relationships still need to be established from controlled exposure experiments, time-resolved analyses, or complementary functional assays.[78] A major development is the use of omics data not just to catalog ARGs, but also to rank their potential relevance to biology or public health. Zhang *et al.*[79] proposed an omics-based framework for ARG risk assessment based on human-associated enrichment, genetic mobility, and occurrence in pathogenic hosts (Table 10).

7.2.4. Integrated multiomics and risk prioritization

Multiomic approaches illustrate how metagenomic data can shift from determining ARG abundance to a risk-based interpretation. Thus, the optimal analytical design for antibiotic TP studies is to combine chemical and biological measurements. LC–HRMS can identify parent compounds and TPs; quantitative LC–MS/MS can determine concentrations of confirmed analytes; metagenomics can characterize ARG abundance and potential hosts; metatranscriptomics can determine which resistance or transformation genes are active; and metabolomic or proteomic measurements can provide additional evidence of functional biological responses. An integrated workflow combining targeted chemical analysis, HRMS-based TP discovery, and multiomics characterization of microbial responses is summarized in Figure 4.

7.3. Environmental risk assessment frameworks

Analytical detection is most informative when chemical occurrence data are interpreted in the light of environmental risk. A typical approach in ecological risk assessment is to compare environmental exposure to a predicted no-effect concentration (PNEC). Where an environmental concentration is directly measured, the risk quotient should be expressed as:

$$\text{Risk quotient (RQ)} = \text{MEC/PNEC} \quad (1)$$

where *MEC* is the measured environmental concentration.

When monitoring data are unavailable, and exposure is estimated from usage, emissions, dilution, or fate modeling, the corresponding relationship is:

$$\text{Risk quotient (RQ)} = \text{PEC/PNEC} \quad (2)$$

where *PEC* is the predicted environmental concentration.

An RQ ≥ 1 is typically considered a potential concern that requires further evaluation. However, an RQ < 1 does not rule out the possibility of an environmental effect. The conclusion depends on the endpoint used to derive the PNEC, the uncertainty factors used, the exposure duration, the mixture composition, and the completeness of the chemical monitoring.

This limitation is most relevant to antibiotic TPs. Chronic ecotoxicity data are often missing for TPs, and there is a temptation to use the PNEC of the parent antibiotic for the respective TP. Such substitution should be approached with caution because transformation can change antimicrobial potency, toxicity, mobility, and bioavailability. Antibiotics require consideration of resistance-selection endpoints in addition to traditional ecotoxicological endpoints. Bengtsson-Palme and Larsson[80] suggested using PNECs intended to limit ARB selection, on the basis that environmentally relevant thresholds for resistance selection may differ from those based on traditional toxicity tests alone.

Therefore, there may be two conceptually different PNECs: an ecotoxicological PNEC for the protection of organisms and ecosystem functions, and an AMR-related PNEC to lower the probability of resistance selection. For active antibiotic TPs, both endpoints should be considered if sufficient data are available. In risk assessment, analytical uncertainty should also be considered. A TP that is only tentatively detected by NTS should not be assigned

Table 10. Examples of analytical and molecular methods for detection of antibiotics, transformation products, and related antimicrobial-resistance responses

Method/approach	Primary information obtained	Representative application	Major advantages	Principal limitations	Reference
Targeted LC–MS/MS	Quantitative concentrations of predefined parents and known TPs	Macrolide antibiotics, metabolites, synthesis by-products, and TPs monitored through municipal wastewater treatment	High selectivity and robust quantification; suitable for routine monitoring when standards are available	Restricted to predefined analytes; authentic TP standards frequently unavailable	70
LC–HRMS suspect screening	Detection and tentative/confirmed identification of expected TPs	Antibiotic TPs in WWTP effluents	Accurate mass, isotope, and MS/MS information; broad compound coverage; retrospective data analysis	Identification confidence varies; quantitative standards often absent	68
LC–HRMS NTS/molecular networking	Discovery of previously unrecognized TPs and structurally related compound families	Antimicrobial TPs discovered in pharmaceutical wastewater	Does not require a complete predefined target list; useful for transformation-pathway elucidation	Complex data processing; risk of false annotations; confirmation frequently requires standards	69
Capillary zone electrophoresis	Separation and targeted determination of charged/ionizable antibiotics	Trace tetracycline determination in water following sample preconcentration	Low solvent consumption and high separation efficiency; relatively economical	Lower structural information without MS; sensitivity may require preconcentration	73
ELISA/immunoassay	Rapid screening of selected antibiotic classes or structurally related residues	Tetracycline and tylosin screening in environmental waters	Rapid, inexpensive, and suitable for large sample numbers	Cross-reactivity can prevent distinction between parent compounds and structurally related TPs; chromatographic–mass-spectrometric confirmation may be required	74
Shotgun metagenomics	Microbial composition, ARGs, mobile elements, and genetic functional potential	ARG profiling across multiple environmental compartments	Culture-independent; broad resistome characterization	Gene detection does not demonstrate expression or phenotype	75
Metagenomics + metatranscriptomics	ARG abundance together with transcriptional activity and potential microbial hosts	Activated-sludge resistome characterization	Distinguishes genetic potential from active transcription	RNA is less stable; sampling and normalization are demanding; transcription does not necessarily equal protein activity	76
Omics-based ARG risk framework	Prioritization of ARGs according to biological/public-health relevance	Risk ranking based on human enrichment, mobility, and pathogenic hosts	Moves beyond simple ARG abundance toward risk-oriented interpretation	Depends on completeness and quality of genomic databases and host assignments	79

Abbreviations: ARG: Antibiotic resistance gene; ELISA: Enzyme-linked immunosorbent assay; LC–HRMS: Liquid chromatography coupled with high-resolution mass spectrometry; LC–MS/MS: Liquid chromatography with tandem mass spectrometry; MS: Mass spectrometry; TP: Transformation product; WWTP: Wastewater treatment plant.

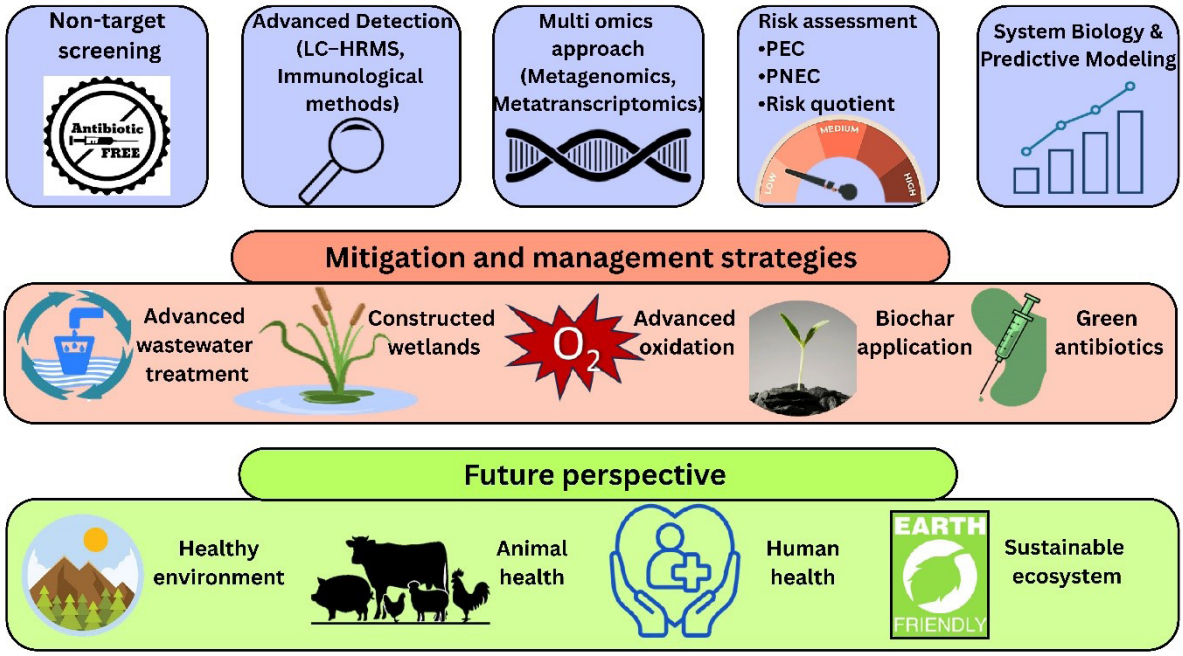


Figure 4. Integrated antibiotic risk management framework including advanced detection, multiomics assessment, forecasting, mitigation techniques, and future One Health perspectives for sustainable environmental protection. Figure created by the authors using Canva Pro and Adobe Photoshop.

the same quantitative certainty as a compound that is confirmed with an authentic standard. Thus, a practical workflow should be: (i) discovery and prioritization using HRMS; (ii) structural confirmation where possible; (iii) quantitative measurement by validated targeted methods; (iv) ecotoxicological and AMR-related effect characterization; and (v) risk characterization using measured or predicted exposure.

Compound-by-compound RQs may be used as an initial screening tool for complex mixtures of environmental contaminants but may not adequately address additive, synergistic, or antagonistic effects. Thus, parent antibiotics and biologically active TPs should increasingly be assessed as associated chemical mixtures rather than isolated contaminants. A complete analytical framework for antibiotic TPs should include chemical identification, quantification, biological-response characterization, and risk assessment. Targeted LC–MS/MS is still needed for reliable quantification, while LC–HRMS suspect and NTS may reveal previously undescribed TPs. This chemical information can be used in conjunction with multiomics approaches to identify microbial community composition, resistance determinants, and active functional responses. Combining these datasets provides a stronger basis for evaluating whether environmental transformation actually reduces antibiotic-related risk or simply alters the chemical form in which ecological and AMR-related hazards are maintained.

8. Mitigation and sustainable management strategies

This section critically evaluates intervention strategies that may reduce the environmental release, persistence, and biological effects of parent antibiotics and their TPs, while simultaneously limiting the dissemination and selection of antibiotic resistance. Because transformation and detoxification are not necessarily coupled, a beneficial mitigation strategy cannot be evaluated based on the depletion of the parent antibiotic alone. Ideally, treatment reduces the total antibiotic-related chemical load, prevents the accumulation of biologically active TPs, reduces ARB and ARGs, minimizes resistance-selection pressure, and is technically, economically, and environmentally sustainable. Mitigation should therefore be considered across the entire antibiotic life cycle and not just as an end-of-pipe wastewater treatment issue. Here, a hierarchical approach is suggested, comprising (i) source prevention and environmentally informed pharmaceutical design; (ii) nature-based and sorption-assisted treatment; (iii) advanced oxidative treatment for persistent residues; and (iv) integrated monitoring and regulatory measures. Each intervention's applicability is influenced by wastewater

or environmental matrix, contaminant concentration, antibiotic class, TP profile, treatment goal, energy and material requirements, and the possibility of unwanted secondary effects.

8.1. Source prevention and life-cycle mitigation

The most sustainable solution is to prevent the entry of avoidable antibiotic emissions into wastewater, manure, soil, or receiving waters at source. Source interventions could include appropriate antimicrobial stewardship, improved collection and disposal of unused medicines, reductions in unnecessary releases from hospitals and manufacturing facilities, optimized management of manure containing antibiotics, and improved treatment at high-emission point sources. These measures supplement downstream treatment; they do not replace it. Controlling sources is a particularly important stage, since once antibiotics enter wastewater or the natural environment, they can undergo multiple transformations, leading to chemical mixtures that are more difficult to identify and remove than the original parent compounds. Therefore, treatment technologies that are effective against one antibiotic may be less effective against its more polar, persistent, or mobile TPs.[81]

8.1.1. Benign-by-design pharmaceuticals

One upstream approach that is gaining increasing attention is the "benign by design" (BbD) concept. BbD seeks to include environmental fate in molecular design so that an active pharmaceutical ingredient retains the physicochemical and pharmacological properties needed for therapeutic efficacy but degrades rapidly, and preferably completely, after its intended use.[82] However, producing a chemically unstable pharmaceutical is insufficient. Sufficient stability is required during manufacture, storage, administration, and pharmacological action. Environmental degradability should instead be considered an additional design criterion in pharmaceutical development.

Structural determinants of pharmaceutical activity and environmental biodegradability are not necessarily mutually exclusive design requirements; Puhlmann *et al.*[82] showed that it is possible to design active pharmaceutical ingredients with improved environmental mineralization profiles. However, applying BbD in antibiotic manufacturing is particularly challenging, since structural changes must preserve target specificity, pharmacokinetics, safety, and clinical efficacy, while avoiding metabolites or environmental TPs that possess antimicrobial activity.

Glucose-containing derivatives of persistent pharmaceuticals have been frequently cited as proof of concept for environmentally informed molecular design. However, early examples like ifosfamide derivatives concern antineoplastic rather than antibiotic drugs, and therefore should not be presented as direct evidence of environmentally degradable antibiotics. Rather, these studies show the wider possibility of integrating biodegradability into the molecular design of pharmaceuticals.[83] Therefore, for antibiotics, future BbD development should evaluate therapeutic activity and environmental properties simultaneously, including biodegradation, TP formation, residual antimicrobial activity, and resistance-selection potential. This approach could prevent environmental persistence at the design stage rather than depend exclusively on intensive downstream treatment.

8.2. Nature-based and sorption-assisted mitigation

8.2.1. Constructed wetlands

Constructed wetlands (CWs) are man-made ecosystems that use water, porous substrates, vegetation, and diverse microbial communities to treat contaminated water through interactions among physical, chemical, and biological processes. Antibiotics may be attenuated by sedimentation, filtration, sorption to substrates and organic matter, microbial biodegradation, plant-associated transformation, photochemical reactions in exposed-water zones and, to a lesser extent for some compounds, plant uptake. Thus, removal efficiency is strongly compound- and system-specific and not attributable to a single pathway.

As such, the notion that ARGs are removed mainly by "plant absorption and root biofilm breakdown" should be rejected. ARG behavior in CWs is controlled by complex interactions among microbial community restructuring, bacterial cell retention, predation, sedimentation, substrate adsorption, rhizosphere interactions, and changes in environmental conditions. A major review by Liu *et al.*[84] concluded that CWs can remove antibiotics and ARGs, but removal performance is dependent on wetland configuration, substrate, vegetation, hydraulic retention time, microbial communities, and contaminant characteristics.

The distinction between the removal of an antibiotic and the removal of resistance determinants is also important. A reduction in dissolved antibiotic concentration does not necessarily lead to an equivalent reduction in ARG abundance. Changes in the microbial community during treatment could decrease some resistance determinants and increase others. Thus, CW performance should preferably be evaluated using multiple endpoints rather than a single chemical endpoint, including parent-antibiotic concentrations, major TPs, ARB, intracellular

and extracellular ARGs, and appropriate microbial-community indicators.

Hybrid treatment systems can provide performance benefits by integrating complementary mechanisms. For example, a recent evaluation of tertiary-treatment processes identified ultraviolet treatment and CW polishing as potentially providing complementary reductions in AMR and pathogenicity-related indicators; however, responses varied across microbial endpoints.[85] This demonstrates the need to evaluate treatment technologies as combined barriers instead of assuming consistent ARG removal.

CWs are low-energy-consuming treatment options with potential for additional ecosystem services, but they require land area and efficiency can vary seasonally and with hydraulic loading, vegetation status, and influent composition. Thus, their greatest value may be as polishing systems or as components of treatment trains, rather than as a universal replacement for conventional treatment.

8.2.2. Biochar-based remediation

Biochar is a carbonaceous porous material produced by thermal conversion of biomass in conditions with limited oxygen. The ability to mitigate antibiotic contamination is governed by surface area, pore structure, aromaticity, mineral composition, surface charge, and oxygenated functional groups, which are largely controlled by the feedstock and pyrolysis conditions.

Biochar can interact with antibiotics by pore filling, hydrophobic interactions, π-π interactions, electrostatic attraction, hydrogen bonding, and surface complexation. Therefore, biochar can decrease the freely dissolved or bioavailable fraction of selected antibiotics in water or soil. However, it should be distinguished from destruction: the transfer of an antibiotic from water to a solid sorbent does not, per se, eliminate the chemical, and the long-term stability, regeneration, or disposal of spent biochar must subsequently be considered.

Biochar could also influence microbial communities and ARG dynamics. Chen *et al.*[86] showed that the abundance of several ARGs in antibiotic-contaminated soil and in the phyllosphere of *Brassica chinensis* decreased after biochar amendment; however, these responses depend on biochar characteristics, soil conditions, and microbial ecology. Consequently, biochar amendment should not be characterized as universally reducing ARGs. A combination of biochar and antibiotic-degrading microorganisms could be a more active remediation strategy, as adsorption may concentrate contaminants near microbial cells, while the inoculated organism promotes biodegradation. Zhang *et al.*[87] managed to immobilize about 85.5% of copper and 64.4% of zinc, and removed about 41.9% oxytetracycline and 40.7% enrofloxacin in a co-contaminated system by combining waste-fungus-chaff biochar with *Herbaspirillum huttiense*. Several ARGs and their potential microbial hosts were also affected by the treatment. Specifically, the treatment decreased the abundance of most ARGs, including tetracycline resistance genes such as *tet(PB)*, *tetH*, *tetR*, *tetS*, *tetT*, and *tetM*, as well as aminoglycoside resistance genes and heavy-metal resistance genes such as *czcA*. The treatment also altered the potential microbial hosts of these genes, which were predominantly affiliated with the phylum Proteobacteria and the genera *Brevundimonas* and *Rhodanobacter*. These results highlight a key principle that biochar-based remediation can provide combined benefits of adsorption, microbial habitat, and biodegradation. However, performance should be assessed in terms of contaminant mass balance and TP analysis. Removal from the dissolved phase should not be interpreted as degradation unless mineralization or TPs have been identified.

8.3. Advanced oxidation processes

Advanced oxidation processes (AOPs) are used to degrade persistent organic pollutants via the action of highly reactive oxidizing species, such as hydroxyl radicals (•OH), sulfate radicals ($SO_4^{\bullet -}$), or other ROS, depending on the treatment system. These processes include ozonation, UV/hydrogen peroxide, Fenton and photo-Fenton reactions, sulfate-radical-based oxidation, photocatalysis, and electrochemical oxidation. The main advantage of AOPs is the potential to attack antibiotic structures that are resistant to conventional biological treatments. However, stating that these processes necessarily convert the contaminants fully into harmless inorganic substances "without secondary pollution" is inaccurate. Complete mineralization typically requires much higher treatment intensity than the depletion of the parent compound, and partial oxidation can produce TPs with biological activities or toxicities that differ from those of the parent antibiotic.[88,89]

8.3.1. Ozonation

Ozone can either react directly with the electron-rich functional groups of antibiotic molecules or decompose in water to generate secondary reactive species. The significance and effectiveness of these pathways are very much dependent on pH, water, and antibiotic structure. Ozonation can rapidly reduce parent-antibiotic concentrations, but subsequent identification of oxidation products and evaluation of their toxicity are required before the treatment can be considered a full risk removal. For example, Park *et al.*[89] studied the ozonation of oxytetracycline and doxycycline and characterized

the degradation pathways and toxicity of the products obtained. This combined chemical and effect-based assessment provides a more appropriate measure of treatment performance than a simple measurement of the removal of parent compounds.

8.3.2. Fenton and photo-Fenton processes

Fenton chemistry is generally favored in acidic conditions since it involves the reaction of ferrous iron with hydrogen peroxide to produce highly reactive oxidizing species. Photo-Fenton processes also use light to increase catalyst regeneration and radical generation. Their effectiveness is dependent on pH, iron and peroxide concentration, water matrix, radical scavenger, and contaminant loading. Although high antibiotic-removal efficiencies are often achieved on a laboratory scale, large-scale implementation has to take into account the reagent consumption, sludge generation in conventional homogeneous Fenton systems, energy demands, catalyst recovery, and the performance in complex real wastewater environments. These operational factors are important when comparing laboratory-scale degradation efficiency with overall sustainability.[90]

8.3.3. Photocatalytic and sulfate-radical-based oxidation

In photocatalytic treatment, irradiation of a semiconductor catalyst produces electron–hole pairs, which induce redox reactions and generate reactive species that can attack antibiotic functional groups. Sulfate-radical-based AOPs employ activated persulfate or peroxymonosulfate to generate $SO_4^{\bullet-}$ and related oxidants. Michael-Kordatou *et al.*[91] demonstrated the degradation of erythromycin using UV-C-activated persulfate while also characterizing TPs, phytotoxicity, and inactivation of erythromycin-resistant *Escherichia coli*. The process led to rapid degradation of erythromycin, complete inactivation of the resistant *E. coli*, and reduced phytotoxicity under the tested conditions.[91] Importantly, the study assessed chemical, microbial, and toxicity endpoints collectively, providing a useful model for assessing antibiotic-treatment technologies. Future AOP studies should therefore not only assess degradation kinetics but also mineralization, identity and persistence of the TPs, residual antimicrobial activity, ecotoxicity, inactivation of ARB, fate of ARGs, and energy or chemical demand. A treatment that removes the parent antibiotic but produces persistent bioactive TPs cannot be unconditionally considered an environmental improvement.

8.4. Integrated treatment and sustainability criteria

Because the contaminants are fundamentally different in terms of chemical structure, environmental behavior, and biological form, no single treatment technology is expected to achieve optimal removal of all parent antibiotics, TPs, ARB, and ARGs. Combining treatments with biological, sorptive, nature-based, and oxidative barriers may therefore be more resilient than a single process. Conventional biological treatment can remove readily biodegradable materials; sorptive or nature-based polishing can remove residual contaminants; and advanced oxidation can target persistent dissolved chemicals. However, the combination of processes must be based on complementary mechanisms instead of maximizing the number of treatment steps. Sustainability of a mitigation technology should be taken into account along with the chemical removal efficiency. Important criteria include energy demand, chemical consumption, greenhouse-gas emissions, land requirements, production and disposal of residual sludge or spent sorbent, catalyst lifetime, operating cost, scalability, and performance under real wastewater conditions. These considerations are essential because a treatment that achieves near-complete parent-antibiotic removal at a substantially high energy or material cost may not be the most sustainable systems-level intervention (Table 11).

8.5. Monitoring and regulatory gaps

We cannot effectively evaluate mitigation without monitoring the endpoints that fully reflect the environmental hazards. Traditional assessment based solely on removal of parent antibiotics might overestimate treatment success, as a decrease in the parent compound might parallel the formation of persistent or biologically active TPs. Therefore, treatment monitoring must combine targeted analysis of parent antibiotics with suspect screening or NTS of TPs, effect-based testing, and AMR-related biological endpoints.

This subsection describes the specific roles of whole-genome sequencing, metagenomics, or other analytical techniques discussed in Section 7 as verification tools for mitigation performance. For example, metagenomics can be used to determine whether a treatment reduces or restructures resistomes, and HRMS can be used to determine whether the depletion of the parent antibiotic is associated with the formation of TPs.

Regulatory environmental risk assessment still heavily relies on individual parent compounds and traditional toxicity endpoints. Several authors have suggested that pharmaceutical environmental risk assessment would benefit from improved management of chronic effects, environmental persistence, mixture exposure, and further ecologically relevant endpoints.[92] For antibiotics in particular, the selection of resistance represents an

Table 11. Comparative role of major mitigation methods for antibiotics, transformation products, and antimicrobial resistance

Mitigation strategy	Primary mechanism	Major strengths	Important limitations/risks	Priority endpoints for performance assessment	References
Source control	Prevention/reduction of environmental release	Avoids contamination before dilution and transformation occur	Depends on prescription, manufacturing, waste-management, and agricultural practices	Emission load, consumption/use patterns, effluent concentrations	81
BbD	Molecular design for environmental degradation/ mineralization after therapeutic use	Addresses persistence at the product-design stage	Must retain efficacy, safety, stability, and pharmacokinetic performance; antibiotic-specific evidence remains limited	Biodegradation, mineralization, TP formation, antimicrobial activity of TPs	82
CWs	Sorption, filtration, biodegradation, rhizosphere processes, sedimentation, and photochemical processes	Low-energy nature-based treatment; useful for polishing	Land requirement; seasonal/ process variability; ARG behavior can be complex	Parent compounds, TPs, ARB, ARGs, and microbial indicators	83
Biochar-based remediation	Adsorption and possible microbial-habitat enhancement	Applicable to aqueous and soil remediation; properties can be engineered	Adsorption is not destruction; effectiveness depends strongly on biochar and matrix; spent biochar material requires management	Dissolved and sorbed residues, desorption, TPs, and ARG abundance	86,87
Ozonation/AOPs	Chemical oxidation through ozone and/or reactive radical species	Effective against many persistent dissolved antibiotics	Incomplete mineralization and formation of potentially bioactive TPs; chemical/ energy demand	Parent compound removal, TOC/ mineralization, TPs, antimicrobial activity, and ecotoxicity	89
UV/persulfate and related AOPs	Generation of sulfate and other reactive radicals	Can simultaneously address antibiotics and microbial contaminants	Matrix effects, energy/ reagent consumption, and TP formation	Antibiotic, TPs, ARB, ARGs, and effect-based endpoints	91

Abbreviations: AOP: Advanced oxidation process; ARB: Antibiotic-resistant bacteria; ARG: Antibiotic resistance gene; BbD: Benign by design; CW: Constructed wetland; TOC: Total organic carbon; TP: Transformation product.

additional hazard that can be induced at concentrations lower than those associated with conventional ecotoxicity endpoints.

Another major regulatory gap involves the assessment of TPs. Löffler *et al.*[4] reviewed antimicrobial TPs in aquatic environments and highlighted that the data on occurrence, ecotoxicological risk, and AMR-selection potential are still considerably more limited than the data for parent compounds. Therefore, a treatment or regulatory framework that only considers the parent antibiotic may underestimate residual risks after metabolism, wastewater treatment, or environmental transformation.

Additionally, mixture exposure should be more explicitly considered. Antibiotics, TPs, and unrelated pharmaceuticals coexist in environmental systems, but conventional assessments usually assess substances individually. Experimental and conceptual work on mixtures of pharmaceuticals indicates that combined effects can differ from predictions based on assessment of isolated chemicals and that both additive and interaction-based effects may be environmentally relevant.[93,94] Thus, the regulatory goal should move from demonstrating the removal of individual parent compounds to demonstrating real reduction in environmental hazards. The integrated assessment of antibiotic-containing waste streams should ideally include (i) parent-antibiotic concentrations; (ii) major TPs and metabolites; (iii) residual antimicrobial activity; (iv) ecotoxicity; (v) concentrations of ARB and clinically or environmentally relevant ARGs; (vi) indicators

of horizontal gene-transfer potential; and (vii) resistance-selection thresholds where appropriate.

Sustainable mitigation will thus require coordinated intervention in pharmaceutical design, clinical and veterinary use, manufacturing, wastewater and manure management, environmental monitoring, and regulatory assessment in a One Health framework. The central goal is not merely to transfer antibiotics from one environmental compartment to another or to chemically modify the parent compound, but to achieve a quantifiable reduction in total biological and ecological hazards.

8.6. Proposed integrated mitigation framework

The above evidence summary suggests a four-stage framework for managing antibiotic and TP pollution:

(i) Prevention at source (Stage 1): Reduce avoidable antibiotic emissions by responsible use, waste collection, manufacturing controls, manure management, and environmentally informed pharmaceutical design.
(ii) Treatment and containment (Stage 2): Use biological, nature-based, or sorptive processes suitable for the contaminant matrix and source characteristics.
(iii) Advanced polishing (Stage 3): Apply oxidation or other advanced treatments if persistent parent compounds or bioactive TPs are present after conventional treatment.
(iv) Verification and risk management (Stage 4): Measure parent compounds, TPs, biological activity, ecotoxicological endpoints, and AMR indicators to confirm that treatment reduces overall environmental hazard rather than simply altering contaminant form.

This hierarchical approach helps to clarify the purpose of mitigation in the current review, which is to connect the environmental fate and biological effects described in the previous sections with practical interventions that can break the pathway from antibiotic use and TP formation to ecosystem exposure and AMR selection.

9. Knowledge gaps and future research directions

While antibiotic TPs are increasingly recognized as relevant environmental contaminants, large uncertainties remain regarding their identification, occurrence, persistence, biological activity, contribution to AMR, trophic behavior, and fate during treatment processes. Research still focuses on parent antibiotics and the environmental relevance of their metabolites, while TPs are still poorly understood. Hence, future studies should progress from a parent-compound depletion approach to an integrated parent–TP framework that encompasses chemical transformation, environmental exposure, ecological effects, and AMR risks. Key research priorities include improved TP identification using advanced suspect screening and NTS, HRMS, expanded spectral libraries, and authentic analytical standards. These key research priorities are acknowledged, as recent studies have shown a much wider diversity of antibiotic-related compounds than previously recognized.[68,69] Long-term studies with environmentally realistic concentrations, chronic exposure scenarios, and parent–TP mixtures are needed, as conventional short-term laboratory tests often do not capture ecological adaptation, community-level responses, and resistance selection. In addition, TP-specific toxicity data and AMR-selection thresholds remain scarce, despite evidence showing some TPs can retain, or even exceed, the biological activity of their parent antibiotics.[8] Recent findings showing comparable AMR selection pressure induced by antibiotic TPs underline that parent-compound removal cannot be considered a reliable indicator for reduced environmental risk.[9] Integrated HRMS and multiomics approaches should also be applied to elucidate re-transformation pathways, bioaccumulation potential, trophic transfer behavior, and interactions between chemical transformation and microbial responses in future studies. Evaluation of treatment technologies should not only focus on the removal of parent antibiotics, but also on TP formation, mineralization, residual antimicrobial activity, ecotoxicity, and AMR-related endpoints, as treatment-generated intermediates may still present biological hazards.[30] Finally, regulatory structures should be shifted to parent–TP mixture assessment by assigning priority to TPs based on occurrence, persistence, mobility, biological activity, and resistance-selection potential. A coordinated One Health research strategy is needed, integrating field monitoring, advanced analytical techniques, mechanistic biological studies, fate modeling, and realistic treatment validation to confirm whether antibiotic transformation really reduces environmental hazards or just transforms them into less recognized chemical forms. Such an integrated approach will provide the scientific basis needed for effective monitoring, sustainable antibiotic management, and future environmental risk assessment.

10. Conclusion

This review demonstrates that monitoring parent compounds alone is insufficient to adequately assess the environmental relevance of antibiotics. Following human or veterinary use, antibiotics undergo metabolic, microbial, photochemical, hydrolytic, and treatment-related transformations. These transformations generate a wide range of TPs that may exhibit physicochemical and biological properties substantially different from those of

the parent antibiotics.

A key conclusion of this review is that TPs are widespread in wastewater, surface water, groundwater, soil, sediment, agricultural systems, and biota. Their persistence, mobility, and bioavailability are strongly dependent on environmental conditions. Some TPs can re-transform and regenerate active parent antibiotics. Essentially, transformation does not always equate to detoxification. Some TPs maintain antimicrobial activity, exhibit ecotoxicological effects, and, as recent evidence suggests, exert selective pressure for AMR similar to that of their parent compounds.

The review also emphasizes that bioaccumulation and trophic transfer are highly compound- and ecosystem-specific, and free, conjugated, and organism-generated TPs may have different trophic behaviors. Therefore, advancements in analytical techniques, especially LC–HRMS-based suspect screening, NTS, and multiomics approaches, are of critical importance to identify previously overlooked TPs and to correlate chemical exposure with microbial and resistance-related responses.

Presently, waste management strategies and regulatory frameworks are largely parent-compound oriented and may therefore underestimate residual environmental and AMR-related risks. Effective mitigation strategies include source control, advanced treatment approaches utilizing environmentally informed pharmaceutical LC–HRMS and nature-based technologies, robust monitoring, effect-based assessment, and AMR endpoints. Future environmental management should integrate the parent–TP–AMR framework from a One Health perspective. This approach is key to understanding whether antibiotic transformation and treatment actually reduce environmental hazard or merely alter the chemical form in which ecological and resistance-selection pressures persist.

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Author contributions

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Availability of data

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References

1. Klein EY, Impalli I, Poleon S, *et al*. Global trends in antibiotic consumption during 2016–2023 and future projections through 2030. *Proc Natl Acad Sci USA*. 2024;121(49):e2411919121.

   doi: 10.1073/pnas.2411919121

2. Wilkinson JL, Boxall ABA, Kolpin DW, *et al*. Pharmaceutical pollution of the world's rivers. *Proc Natl Acad Sci USA*. 2022;119(8):e2113947119.

   doi: 10.1073/pnas.2113947119

3. Göbel A, Thomsen A, McArdell CS, Joss A, Giger W. Occurrence and Sorption Behavior of Sulfonamides, Macrolides, and Trimethoprim in Activated Sludge Treatment. *Environ Sci Technol*. 2005;39(11):3981-3989.

   doi: 10.1021/es048550a

4. Löffler P, Escher BI, Baduel C, Virta MP, Lai FY. Antimicrobial transformation products in the aquatic environment: global occurrence, ecotoxicological risks, and potential of antibiotic resistance. *Environ Sci Technol*. 2023;57(26):9474-9494.

   doi: 10.1021/acs.est.2c09854

5. Achermann S, Bianco V, Mansfeldt CB, *et al*. Biotransformation of Sulfonamide Antibiotics in Activated Sludge: The Formation of Pterin-Conjugates Leads to Sustained Risk. *Environ Sci Technol*. 2018;52(11):6265-6274.

   doi: 10.1021/acs.est.7b06716

6. Polesel F, Andersen HR, Trapp S, Plósz BGy. Removal of Antibiotics in Biological Wastewater Treatment Systems—A Critical Assessment Using the Activated Sludge Modeling Framework for Xenobiotics (ASM-X). *Environ Sci Technol*. 2016;50(19):10316-10334.

   doi: 10.1021/acs.est.6b01899

7. Haddad T, Baginska E, Kümmerer K. Transformation products of antibiotic and cytostatic drugs in the aquatic cycle that result from effluent treatment and abiotic/biotic reactions in the environment: An increasing challenge

calling for higher emphasis on measures at the beginning of the pipe. *Water Res*. 2015;72:75-126.

doi: 10.1016/j.watres.2014.12.042

8. Murray AK, Stanton I, Gaze WH, Snape J. Dawning of a new ERA: Environmental Risk Assessment of antibiotics and their potential to select for antimicrobial resistance. *Water Res*. 2021;200:117233.

doi: 10.1016/j.watres.2021.117233

9. Lakhey P, Hayes A, Murray AK, *et al*. Antibiotic transformation products exert selective pressure for antimicrobial resistance comparable to parent compounds. *Nat Water*. 2026;4(7):871-881.

doi: 10.1038/s44221-026-00663-4

10. Gillings MR, Gaze WH, Pruden A, Smalla K, Tiedje JM, Zhu YG. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *ISME J*. 2015;9(6):1269-1279.

doi: 10.1038/ismej.2014.226

11. Petrovich ML, Zilberman A, Kaplan A, *et al*. Microbial and viral communities and their antibiotic resistance genes throughout a hospital wastewater treatment system. *Front Microbiol*. 2020;11:153.

doi: 10.3389/fmicb.2020.00153

12. Larsson DJ, De Pedro C, Paxeus N. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. *J Hazard Mater*. 2007;148(3):751-755.

doi: 10.1016/j.jhazmat.2007.07.008

13. De Liguoro M, Cibin V, Capolongo F, Halling-Sørensen B, Montesissa C. Use of oxytetracycline and tylosin in intensive calf farming: evaluation of transfer to manure and soil. *Chemosphere*. 2003;52(1):203-212.

doi: 10.1016/S0045-6535(03)00284-4

14. Le TX, Munekage Y. Residues of selected antibiotics in water and mud from shrimp ponds in mangrove areas in Viet Nam. *Mar Pollut Bull*. 2004;49(11-12):922-929.

doi: 10.1016/j.marpolbul.2004.06.016

15. Marano RB, Zolti A, Jurkevitch E, Cytryn E. Antibiotic resistance and class 1 integron gene dynamics along effluent, reclaimed wastewater irrigated soil, crop continua: elucidating potential risks and ecological constraints. *Water Res*. 2019;164:114906.

doi: 10.1016/j.watres.2019.114906

16. Montone CM, Giannelli Moneta B, Laganà A, Piovesana S, Taglioni E, Cavaliere C. Transformation products of antibacterial drugs in environmental water: Identification approaches based on liquid chromatography-high resolution mass spectrometry. *J Pharm Biomed Anal*. 2024;238:115818.

doi: 10.1016/j.jpba.2023.115818

17. Bergan T, Thorsteinsson SB, Rohwedder R, Scholl H. Elimination of ciprofloxacin and three major metabolites and consequences of reduced renal function. *Chemotherapy*. 1989;35(6):393-405.

doi: 10.1159/000238702

18. Fu LX, Jiang ZG, Ding HZ, Liu YH. Population pharmacokinetics of enrofloxacin and its active metabolite ciprofloxacin in ill cows. *J Vet Pharmacol Ther*. 2008;31(3):240-245.

doi: 10.1111/j.1365-2885.2008.00945.x

19. Vree TB, Van Der Ven AJAM, Koopmans PP, Van Ewijk-Beneken Kolmer EWJ, Verwey-van Wissen CPWGM. Pharmacokinetics of Sulfamethoxazole with its Hydroxy Metabolites and N4-Acetyl-, N1-Glucuronide Conjugates in Healthy Human Volunteers. *Clin Drug Invest*. 1995;9(1):43-53.

doi: 10.2165/00044011-199509010-00008

20. Chu SY, Sennello LT, Bunnell ST, Varga LL, Wilson DS, Sonders RC. Pharmacokinetics of clarithromycin, a new macrolide, after single ascending oral doses. *Antimicrob Agents Chemother*. 1992;36(11):2447-2453.

doi: 10.1128/AAC.36.11.2447

21. Smitha MS, Singh S, Singh R. Microbial biotransformation: a process for chemical alterations. *J Bacteriol Mycol Open Access*. 2017;4(2):47-51.

doi: 10.15406/jbmoa.2017.04.00085

22. Akay C, Ulrich N, Rocha U, Ding C, Adrian L. Sequential anaerobic–aerobic treatment enhances sulfamethoxazole removal: from batch cultures to observations in a large-scale wastewater treatment plant. *Environ Sci Technol*. 2024;58(28):12609-12620.

doi: 10.1021/acs.est.4c00368

23. Zhao K, Si T, Liu S, Liu G, Li D, Li F. Co-metabolism of microorganisms: A study revealing the mechanism of antibiotic removal, progress of biodegradation transformation pathways. *Sci Total Environ*. 2024;954:176561.

doi: 10.1016/j.scitotenv.2024.176561

24. Lopez Gordillo AP, Trueba-Santiso A, Lema JM, Schäffer A, Smith KEC. Sulfamethoxazole is Metabolized and Mineralized at Extremely Low Concentrations. *Environ Sci Technol*. 2024;58(22):9723-9730.

doi: 10.1021/acs.est.4c02191

25. Reis AC, Kolvenbach BA, Nunes OC, Corvini PF. Biodegradation of antibiotics: The new resistance determinants–part I. *N Biotechnol*. 2020;54:34-51.

doi: 10.1016/j.nbt.2019.08.002

26. Baena-Nogueras RM, González-Mazo E, Lara-Martín PA. Photolysis of Antibiotics under Simulated Sunlight Irradiation: Identification of Photoproducts by High-Resolution Mass Spectrometry. *Environ Sci Technol*.

2017;51(6):3148-3156.

doi: 10.1021/acs.est.6b03038

27. Hirte K, Seiwert B, Schüürmann G, Reemtsma T. New hydrolysis products of the beta-lactam antibiotic amoxicillin, their pH-dependent formation and search in municipal wastewater. *Water Res*. 2016;88:880-888.

doi: 10.1016/j.watres.2015.11.028

28. Kabanov MA, Ivantsova NA, Kuzin EN, Murzina ED, Korobov AYu. Evaluation of the Influence of Drug Complex Formation on the Efficiency of Water Conditioning with Reagents for Tetracycline. *Pharm Chem J*. 2022;55(11):1245-1249.

doi: 10.1007/s11094-022-02565-7

29. Yu X, Zhang X, Yang P, Liu B, Liu Z, Li X. Iron oxide-driven antibiotic reduction: From interfacial reactions to multi-system synergistic mechanisms. *Agric Environ Sustain*. 2026;1(2):100016.

doi: 10.1016/j.ages.2026.100016

30. Jaén-Gil A, Farré MJ, Sànchez-Melsió A, Serra-Compte A, Barceló D, Rodríguez-Mozaz S. Effect-Based Identification of Hazardous Antibiotic Transformation Products after Water Chlorination. *Environ Sci Technol*. 2020;54(14):9062-9073.

doi: 10.1021/acs.est.0c00944

31. Franklin AM, Williams C, Andrews DM, Watson JE. Sorption and desorption behavior of four antibiotics at concentrations simulating wastewater reuse in agricultural and forested soils. *Chemosphere*. 2022;289:133038.

doi: 10.1016/j.chemosphere.2021.133038

32. Gbadegesin LA, Tang X, Liu C, Cheng J. Transport of veterinary antibiotics in farmland soil: Effects of dissolved organic matter. *Int J Environ Res Public Health*. 2022;19(3):1702.

doi: 10.3390/ijerph19031702

33. Kang DH, Gupta S, Rosen C, *et al*. Antibiotic Uptake by Vegetable Crops from Manure-Applied Soils. *J Agric Food Chem*. 2013;61(42):9992-10001.

doi: 10.1021/jf404045m

34. Zhang Y, Boyd SA, Teppen BJ, Tiedje JM, Li H. Organic acids enhance bioavailability of tetracycline in water to Escherichia coli for uptake and expression of antibiotic resistance. *Water Res*. 2014;65:98-106.

doi: 10.1016/j.watres.2014.07.021

35. Watanabe N, Bergamaschi BA, Loftin KA, Meyer MT, Harter T. Use and environmental occurrence of antibiotics in freestall dairy farms with manured forage fields. *Environ Sci Technol*. 2010;44(17):6591-6600.

doi: 10.1021/es100834s

36. Wu C, Spongberg AL, Witter JD. Sorption and biodegradation of selected antibiotics in biosolids. *J Environ Sci Health A*. 2009;44(5):454-461.

doi: 10.1080/10934520902719779

37. Kasteel R, Mboh CM, Unold M, Groeneweg J, Vanderborght J, Vereecken H. Transformation and Sorption of the Veterinary Antibiotic Sulfadiazine in Two Soils: A Short-Term Batch Study. *Environ Sci Technol*. 2010;44(12):4651-4657.

doi: 10.1021/es100141m

38. Tong C, Zhuo X, Guo Y. Occurrence and Risk Assessment of Four Typical Fluoroquinolone Antibiotics in Raw and Treated Sewage and in Receiving Waters in Hangzhou, China. *J Agric Food Chem*. 2011;59(13):7303-7309.

doi: 10.1021/jf2013937

39. Liu S, Zhao H, Lehmler HJ, Cai X, Chen J. Antibiotic pollution in marine food webs in Laizhou Bay, North China: trophodynamics and human exposure implication. *Environ Sci Technol*. 2017;51(4):2392-2400.

doi: 10.1021/acs.est.6b04556

40. Yang C, Yan S, Zhang B, *et al*. Spatiotemporal distribution of the planktonic microbiome and antibiotic resistance genes in a typical urban river contaminated by macrolide antibiotics. *Environ Res*. 2024;262:119808.

doi: 10.1016/j.envres.2024.119808

41. Ma N, Tong L, Li Y, Yang C, Tan Q, He J. Distribution of antibiotics in lake water-groundwater-Sediment system in Chenhu Lake area. *Environ Res*. 2022;204:112343.

doi: 10.1016/j.envres.2021.112343

42. Fick J, Söderström H, Lindberg RH, Phan C, Tysklind M, Larsson DGJ. Contamination of surface, ground, and drinking water from pharmaceutical production. *Environ Toxicol Chem*. 2009;28(12):2522-2527.

doi: 10.1897/09-073.1

43. Rutgersson C, Fick J, Marathe N, *et al*. Fluoroquinolones and qnr Genes in Sediment, Water, Soil, and Human Fecal Flora in an Environment Polluted by Manufacturing Discharges. *Environ Sci Technol*. 2014;48(14):7825-7832.

doi: 10.1021/es501452a

44. Watkinson AJ, Murby EJ, Kolpin DW, Costanzo SD. The occurrence of antibiotics in an urban watershed: from wastewater to drinking water. *Sci Total Environ*. 2009;407(8):2711-2723.

doi: 10.1016/j.scitotenv.2008.11.059

45. Wang H, Wang N, Wang B, *et al*. Antibiotics in Drinking Water in Shanghai and Their Contribution to Antibiotic Exposure of School Children. *Environ Sci Technol*. 2016;50(5):2692-2699.

doi: 10.1021/acs.est.5b05749

46. Guillouzo A, Guguen-Guillouzo C. Antibiotics-induced oxidative stress. *Curr Opin Toxicol*. 2020;20-21:23-28.

doi: 10.1016/j.cotox.2020.03.004

47. Algburi A, Comito N, Kashtanov D, Dicks LM, Chikindas ML. Control of biofilm formation: antibiotics and beyond. *Appl Environ Microbiol*. 2017;83(3):e02508-16.

doi: 10.1128/AEM.02508-16

48. Wang Y, Wang M, Jiang Y, Song C, Zhang Y, Liu G. Photolysis triggers multiple microbial responses: New insights of phosphorus compensation for algal blooms. *J Hazard Mater*. 2025;490:137812.

doi: 10.1016/j.jhazmat.2025.137812

49. Zhang H, Zhou J, Wang K, Li Y, Niu L. Interaction patterns and keystone taxa of bacterial and eukaryotic communities during sulfamethoxazole mineralization in lake sediment. *Sci Total Environ*. 2024;924:171597.

doi: 10.1016/j.scitotenv.2024.171597

50. Wu NN, Liu S, Xu R, *et al*. New insight into the bioaccumulation and trophic transfer of free and conjugated antibiotics in an estuarine food web based on multimedia fate and model simulation. *J Hazard Mater*. 2024;465:133088.

doi: 10.1016/j.jhazmat.2023.133088

51. Qin H, Chen M, Shi Y, Li R, Yan J, Huang Y. Structure-dependent transformation of cephalosporin antibiotics at MnO2 surface mediated by iodide and formation of highly toxic iodinated products. *Water Res*. 2026;295:125515.

doi: 10.1016/j.watres.2026.125515

52. DeVries SL, Zhang P. Antibiotics and the Terrestrial Nitrogen Cycle: A Review. *Curr Pollut Rep*. 2016;2(1):51-67.

doi: 10.1007/s40726-016-0027-3

53. Ni BJ, Zeng S, Wei W, Dai X, Sun J. Impact of roxithromycin on waste activated sludge anaerobic digestion: methane production, carbon transformation and antibiotic resistance genes. *Sci Total Environ*. 2020;703:134899.

doi: 10.1016/j.scitotenv.2019.134899

54. Kim TJ. Bacterial Persister Cells as Evolutionary Catalysts of Antibiotic Resistance: Mechanisms, Clinical Implications, and Therapeutic Strategies. *Antibiotics*. 2026;15(6):526.

doi: 10.3390/antibiotics15060526

55. Gullberg E, Cao S, Berg OG, *et al*. Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. *PLoS Pathog*. 2011;7(7):e1002158.

doi: 10.1371/journal.ppat.1002158

56. Huang J, Zhang J, Liang H, *et al*. Antibiotics or Heavy Metals in Livestock Wastewater: Which One Is the Main Driver for the Development and Spread of Antibiotic Resistance under Coexposure? *Environ Sci Technol*. 2026;60(8):6510-6524.

doi: 10.1021/acs.est.5c06042

57. Majewsky M, Wagner D, Delay M, Bräse S, Yargeau V, Horn H. Antibacterial Activity of Sulfamethoxazole Transformation Products (TPs): General Relevance for Sulfonamide TPs Modified at the para Position. *Chem Res Toxicol*. 2014;27(10):1821-1828.

doi: 10.1021/tx500267x

58. Zhang Y, Rosado-García FM, Peña YP, *et al*. Sulfonamide metabolites enhance resistance transmission via conjugative transfer pathways. *J Hazard Mater*. 2025;491:137932.

doi: 10.1016/j.jhazmat.2025.137932

59. Geng J, Liu X, Wang J, Li S. Accumulation and risk assessment of antibiotics in edible plants grown in contaminated farmlands: A review. *Sci Total Environ*. 2022;853:158616.

doi: 10.1016/j.scitotenv.2022.158616

60. Bhalsod GD, Chuang YH, Jeon S, *et al*. Uptake and Accumulation of Pharmaceuticals in Overhead- and Surface-Irrigated Greenhouse Lettuce. *J Agric Food Chem*. 2018;66(4):822-830.

doi: 10.1021/acs.jafc.7b04355

61. Tang J, Zhang C, Jia Y, Fang J, Mai BX. Phytoplankton Biological Pump Controls the Spatiotemporal Bioaccumulation and Trophic Transfer of Antibiotics in a Large Subtropical River. *Environ Sci Technol*. 2023;57(37):14002-14014.

doi: 10.1021/acs.est.3c03478

62. Hu T, Zhang J, Xu X, *et al*. Bioaccumulation and trophic transfer of antibiotics in the aquatic and terrestrial food webs of the Yellow River Delta. *Chemosphere*. 2023;323:138211.

doi: 10.1016/j.chemosphere.2023.138211

63. Zhu M, Wang Z, Chen J, Xie H, Zhao H, Yuan X. Bioaccumulation, Biotransformation, and Multicompartmental Toxicokinetic Model of Antibiotics in Sea Cucumber (Apostichopus japonicus). *Environ Sci Technol*. 2020;54(20):13175-13185.

doi: 10.1021/acs.est.0c04421

64. Zhou LJ, Wang WX, Lv YJ, Mao ZG, Chen C, Wu QL. Tissue concentrations, trophic transfer and human risks of antibiotics in freshwater food web in Lake Taihu, China. *Ecotoxicol Environ Saf*. 2020;197:110626.

doi: 10.1016/j.ecoenv.2020.110626

65. Zhang YJ, Hu HW, Chen QL, *et al*. Transfer of antibiotic resistance from manure-amended soils to vegetable microbiomes. *Environ Int*. 2019;130:104912.

doi: 10.1016/j.envint.2019.104912

66. Wu Q, Pan CG, Wang YH, Xiao SK, Yu KF. Antibiotics in a subtropical food web from the Beibu Gulf, South China: Occurrence, bioaccumulation and trophic transfer. *Sci Total Environ*. 2021;751:141718.

doi: 10.1016/j.scitotenv.2020.141718

67. Feng Y, Zhang X, Lai S, *et al*. Nontarget screening uncovers the overlooked impact of antibiotic transformation products on riverine resistomes. *J Hazard Mater*. 2026;505:141425.

doi: 10.1016/j.jhazmat.2026.141425

68. Hu J, Lyu Y, Chen H, Li S, Sun W. Suspect and Nontarget Screening Reveal the Underestimated Risks of Antibiotic Transformation Products in Wastewater Treatment Plant Effluents. *Environ Sci Technol*. 2023;57(45):17439-17451.

doi: 10.1021/acs.est.3c05008

69. Yu N, Deng Y, Wang X, *et al*. Nontarget Discovery of Antimicrobial Transformation Products in Wastewater Based on Molecular Networks. *Environ Sci Technol*. 2023;57(22):8335-8346.

doi: 10.1021/acs.est.2c07774

70. Senta I, Kostanjevecki P, Krizman-Matasic I, Terzic S, Ahel M. Occurrence and Behavior of Macrolide Antibiotics in Municipal Wastewater Treatment: Possible Importance of Metabolites, Synthesis Byproducts, and Transformation Products. *Environ Sci Technol*. 2019;53(13):7463-7472.

doi: 10.1021/acs.est.9b01420

71. Schymanski EL, Jeon J, Gulde R, *et al*. Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Environ Sci Technol*. 2014;48(4):2097-2098.

doi: 10.1021/es5002105

72. Oka M, Miyoshi S, Isshiki T. Quantitative deviations arising from internal standards other than stable-isotope-labeled internal standards in LC–MS/MS. *Talanta Open*. 2026;13:100637.

doi: 10.1016/j.talo.2026.100637

73. Moreno-González D, Lupión-Enríquez I, García-Campaña AM. Trace determination of tetracyclines in water samples by capillary zone electrophoresis combining off-line and on-line sample preconcentration. *Electrophoresis*. 2016;37(9):1212-1219.

doi: 10.1002/elps.201500440

74. Kumar K, Thompson A, Singh AK, Chander Y, Gupta SC. Enzyme-Linked Immunosorbent Assay for Ultratrace Determination of Antibiotics in Aqueous Samples. *J Environ Qual*. 2004;33(1):250-256.

doi: 10.2134/jeq2004.2500

75. Li B, Yang Y, Ma L, *et al*. Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. *ISME J*. 2015;9(11):2490-2502.

doi: 10.1038/ismej.2015.59

76. Liu Z, Klümper U, Liu Y, *et al*. Metagenomic and metatranscriptomic analyses reveal activity and hosts of antibiotic resistance genes in activated sludge. *Environ Int*. 2019;129:208-220.

doi: 10.1016/j.envint.2019.05.036

77. Rowe WPM, Baker-Austin C, Verner-Jeffreys DW, *et al*. Overexpression of antibiotic resistance genes in hospital effluents over time. *J Antimicrob Chemother*. 2017;72(6):1617-1623.

doi: 10.1093/jac/dkx017

78. Xie M, Wang J, Wang F, *et al*. A Review of Genomic, Transcriptomic, and Proteomic Applications in Edible Fungi Biology: Current Status and Future Directions. *J Fungi*. 2025;11(6):422.

doi: 10.3390/jof11060422

79. Zhang AN, Gaston JM, Dai CL, *et al*. An omics-based framework for assessing the health risk of antimicrobial resistance genes. *Nat Commun*. 2021;12(1):4765.

doi: 10.1038/s41467-021-25096-3

80. Bengtsson-Palme J, Larsson DGJ. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. *Environ Int*. 2016;86:140-149.

doi: 10.1016/j.envint.2015.10.015

81. Zhu G, Su JQ, Lv M, *et al*. Tackling pharmaceutical pollution under the One Health framework. *Innovation*. Published online July 14, 2026:101507.

doi: 10.1016/j.xinn.2026.101507

82. Puhlmann N, Mols R, Olsson O, Slootweg JC, Kümmerer K. Towards the design of active pharmaceutical ingredients mineralizing readily in the environment. *Green Chem*. 2021;23(14):5006-5023.

doi: 10.1039/d1gc01048d

83. Leder C, Rastogi T, Kümmerer K. Putting benign by design into practice-novel concepts for green and sustainable pharmacy: Designing green drug derivatives by non-targeted synthesis and screening for biodegradability. *Sustain Chem Pharm*. 2015;2:31-36.

doi: 10.1016/j.scp.2015.07.001

84. Liu X, Guo X, Liu Y, *et al*. A review on removing antibiotics and antibiotic resistance genes from wastewater by constructed wetlands: Performance and microbial response. *Environ Pollut*. 2019;254:112996.

doi: 10.1016/j.envpol.2019.112996

85. Jia S, Gao X, Zhang Y, *et al*. Tertiary Wastewater Treatment Processes Can Be a Double-Edged Sword for Water Quality Improvement in View of Mitigating Antimicrobial Resistance and Pathogenicity. *Environ Sci Technol*. 2023;57(1):509-519.

doi: 10.1021/acs.est.2c06168

86. Chen QL, Fan XT, Zhu D, An XL, Su JQ, Cui L. Effect

of biochar amendment on the alleviation of antibiotic resistance in soil and phyllosphere of Brassica chinensis L. *Soil Biol Biochem*. 2018;119:74-82.

doi: 10.1016/j.soilbio.2018.01.015

87. Zhang X, Gong Z, Allinson G, Li X, Jia C. Joint effects of bacterium and biochar in remediation of antibiotic-heavy metal contaminated soil and responses of resistance gene and microbial community. *Chemosphere*. 2022;299:134333.

doi: 10.1016/j.chemosphere.2022.134333

88. Sharma A, Ahmad J, Flora SJS. Application of advanced oxidation processes and toxicity assessment of transformation products. *Environ Res*. 2018;167:223-233.

doi: 10.1016/j.envres.2018.07.010

89. Park JA, Pineda M, Peyot ML, Yargeau V. Degradation of oxytetracycline and doxycycline by ozonation: Degradation pathways and toxicity assessment. *Sci Total Environ*. 2023;856:159076.

doi: 10.1016/j.scitotenv.2022.159076

90. Wang F, Wang Z, Zhao Y, Zhang J. Performance of Traditional and Emerging Water-Treatment Technologies in the Removal of Tetracycline Antibiotics. *Catalysts*. 2024;14(4):269.

doi: 10.3390/catal14040269

91. Michael-Kordatou I, Iacovou M, Frontistis Z, Hapeshi E, Dionysiou DD, Fatta-Kassinos D. Erythromycin oxidation and ERY-resistant Escherichia coli inactivation in urban wastewater by sulfate radical-based oxidation process under UV-C irradiation. *Water Res*. 2015;85:346-358.

doi: 10.1016/j.watres.2015.08.050

92. Ågerstrand M, Berg C, Björlenius B, *et al*. Improving Environmental Risk Assessment of Human Pharmaceuticals. *Environ Sci Technol*. 2015;49(9):5336-5345.

doi: 10.1021/acs.est.5b00302

93. Backhaus T. Medicines, shaken and stirred: a critical review on the ecotoxicology of pharmaceutical mixtures. *Philos Trans R Soc Lond B Biol Sci*. 2014;369(1656):20130585.

doi: 10.1098/rstb.2013.0585

94. Marx C, Mühlbauer V, Krebs P, Kuehn V. Environmental risk assessment of antibiotics including synergistic and antagonistic combination effects. *Sci Total Environ*. 2015;524-525:269-279.

doi: 10.1016/j.scitotenv.2015.04.051