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Contaminants in agricultural soils

Challenges and solutions

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Antibiotic residues as contaminants in agricultural soils

Nikola Rakonjac, Wageningen University, The Netherlands[1]; Joost Lahr, National Institute for Public Health and the Environment (RIVM), The Netherlands; Sören Thiele-Bruhn, Trier University, Germany; Bjorn J. A. Berendsen, Wageningen Food Safety Research, The Netherlands; Carlos A. Faúndez Urbina, Pontificia Universidad Católica de Chile, Chile; and Coen J. Ritsema, Wageningen University, The Netherlands

1 Introduction

Soil contamination research has increased over the past decades, reflecting a growing awareness of this issue globally (Guo et al., 2014). The diversity of man-made contaminants in the soil is constantly evolving due to advancements in agrochemicals and industrial processes. Moreover, the escalated focus on ensuring high-quality food, particularly with the rising global population, has led to increased attention to the health of the soil. Notably, over the last three decades in particular, the intensified practices in livestock farming and activities like wastewater reuse in agriculture have underscored the existence

1 *Corresponding author:* nikola.rakonjac@wur.nl.

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of antibiotics in soils, categorizing them as an emerging concern. Figure 1 summarizes the number of scientific journal articles published each year from 1935 to 2023, sourced from the Scopus database using the search terms "antibiotics" and "soil".

Antibiotics are substances used, among other purposes, for the treatment or prevention of microbial diseases in both animals and humans. Their usage is global and is regulated, necessitating market authorization prior to application (EMEA, 2008; European Commission, 2021). The selection and quantity of these compounds used vary, influenced by factors such as the specific disease being addressed, geographical location (availability of certain antibiotics), and individual characteristics of the human or animal recipient (e.g. weight). Hutchings et al. (2019) provide a historical perspective on antibiotics, detailing their discovery, clinical introduction, and targeting principles.

A primary route through which veterinary antibiotics, specifically those administered to livestock animals, are introduced into the environment involves the excretion of urine and feces by treated animals, followed by the application of contaminated manure to agricultural soils (Boxall et al., 2004). In contrast, human antibiotics, typically reach agricultural soils through irrigation with treated wastewater (Negreanu et al., 2012). These pathways, along with other less prominent ones, are further discussed later in this chapter.

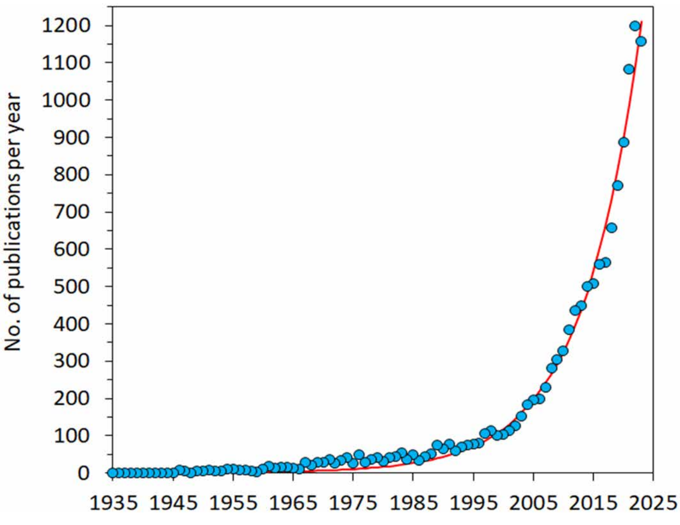

Figure 1 Number of scientific journal publications released annually from 1935 to 2023: results of a literature search conducted in the Scopus database, retrieved on February 8, 2024, using the search terms "antibiotics" and "'soil".

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The two extensive reviews (Thiele-Bruhn, 2003b; Cycoń et al., 2019), published nearly two decades apart, and focusing on antibiotic residues in soils, indicate that the prevalent antibiotic groups detected in soil have remained consistent. These include Tetracyclines, Sulfonamides, β-lactams, Macrolides, Fluoroquinolones, and Aminoglycosides. Some antibiotics within the mentioned groups potentially have adverse effects on soil processes, organisms, or, eventually, human health through the food chain (Katsivelou et al., 2023; Nguyen et al., 2023). However, a comprehensive understanding of the potential extent of these effects remains challenging, partly attributed to the widely varying behavior of antibiotics within the soil, as documented in the global literature. This variability is influenced by the physico-chemical properties of the substances, soil structure and characteristics, environmental conditions, or a combination of these factors. Hence, there is a need for a shift towards standardization in studying the behavior of antibiotics in soil. Straightforward laboratory experiments and fit-for-purpose analytical methodologies would allow us to study all these aspects in an efficient, yet effective way. This would facilitate a more profound grasp of the antibiotics' fate in soil, offering valuable insights for accurate risk and effect assessments.

The fate of antibiotics in soil can also be explored through modeling approaches. Modeling offers certain advantages over experimental efforts by being applicable to a broad range of conditions, scenarios, and various types of compounds. Additionally, modeling can assist authorities and policymakers in identifying antibiotics potentially present in the soil compartment and assessing conditions that may increase the exposure to them. However, the accuracy and reliability of modeling results heavily rely on the availability and (un)certainty of input parameters. Here, monitoring and experimental data are essential. Therefore, a combination of all aspects is crucial in tiered methodologies that comprehensively address the environmental fate of antibiotics.

Given that the two mentioned reviews (Thiele-Bruhn, 2003b; Cycoń et al., 2019) already furnish a summary of concentrations found in soil worldwide over the past 40 years and their effects on soil microbial communities, these aspects are not extensively examined in this chapter but are rather briefly addressed. Therefore, the aim of this chapter is to:

- provide an overview of the potential environmental routes by which human and veterinary antibiotics may reach the agricultural soil;
- address processes affecting antibiotic fate in the soil;
- discuss modeling perspectives for predicting the residues and fate of antibiotics within soils;
- offer the latest insights into both the detection techniques for antibiotics in soil and the experimental efforts aimed at standardizing investigations into antibiotic behavior in soil;

- highlight key findings from the Dutch case study, where antibiotics were introduced into the soil through slurry manure injection;
- explore the issue of mixed antibiotic contaminations; and
- discuss potential future trends in this research area.

2 Antibiotic pathways to agricultural soils

There are various pathways through which antibiotic residues can reach the soil. These routes may involve variations in the types of antibiotics present in the soil, variances in the concentrations introduced, and differences in the duration of exposure over time periods.

2.1 Via manure (indirect application – veterinary antibiotics)

After administration to livestock animals, a significant proportion of veterinary antibiotics may be excreted unchanged through urine and feces. The amount excreted is influenced by various factors, such as the livestock sector, animal characteristics, antibiotic properties and type, and the method of administration (Kim et al., 2011). Depending on the combinations of these factors, the excreted portions of parent compounds may range from 3% to 90% (Rakonjac et al., 2022). However, certain antibiotics may undergo extensive metabolism post-administration. The degree to which an antibiotic is metabolized is an important

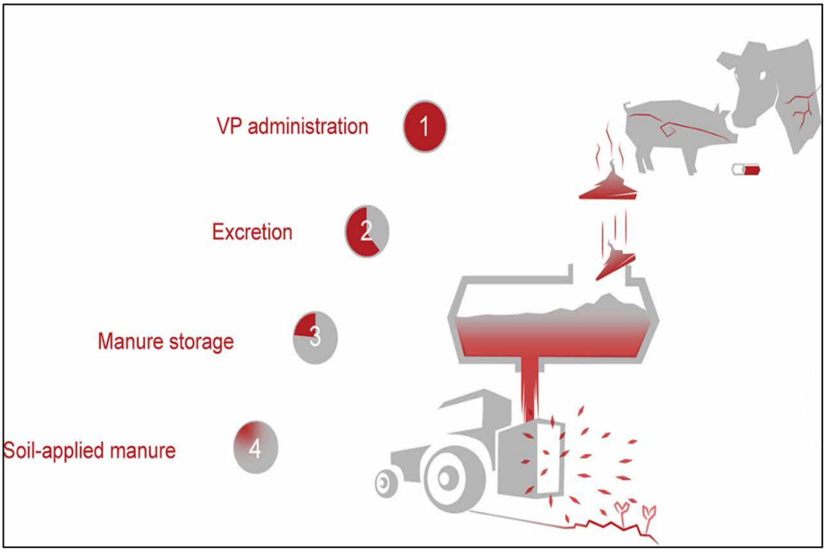

Figure 2 Visualization of the entry pathway of veterinary antibiotics into the environment through the application of post-storage manure to soil. Source: Reprinted from Rakonjac (2023).

factor in environmental exposure, as the metabolites of some compounds exhibit reduced or no antibacterial activity. Also, some metabolites may be more environmentally relevant than the parent compound, especially when the metabolite is more toxic.

Following excretion, antibiotic residues usually end up in manure storage, where they might undergo further dissipation. The degree of dissipation is influenced by factors like storage conditions, duration, temperature, physico-chemical properties of the antibiotics, and practices related to manure handling and management. After the storage period, which for instance lasts around 6 months in the Netherlands (Lagerwerf et al., 2019), any remaining antibiotic fractions are introduced into the soil through the application of manure. This pathway is depicted in Fig. 2.

2.2 Via grazing manure (direct application – veterinary antibiotics)

In addition to residues from stored manure, veterinary antibiotics can also directly reach the soil through manure or urine deposited by animals during pasture seasons (e.g. dairy cows). This pathway is noteworthy because it bypasses the storage period and associated dissipation processes, potentially leading to higher concentrations of antibiotics compared to those in stored manure. However, on a larger scale, this route is generally less significant due to lower quantities of produced manure and a limited number of agricultural fields used for this purpose (Rakonjac et al., 2023).

2.3 Products from manure processing facilities (veterinary antibiotics)

The processing of animal manure is considered, alongside feeding measures and manure export, as a potential solution to alleviate pressure on the fertilizer market and mitigate associated pollution. One approach involves the separation of manure into solid and liquid fractions. The solid fraction can be utilized as an energy source, while the liquid (thin) fraction serves as a substitute for fertilizers. Similar to raw manure, this liquid fraction may contain elevated concentrations of antibiotics, as observed in storage tanks of processing installations by Lahr et al. (2014). Therefore, these residues could potentially be deposited onto the soil when using this substitute fertilizer.

2.4 Via wastewater irrigation and sludge disposal (human antibiotics)

Worldwide, continuous efforts are underway to ensure a reliable and sustainable freshwater supply, with a critical emphasis on optimizing the utilization of current water resources. One notable initiative in this regard involves the reuse of (treated) wastewater for irrigation. This practice has been implemented for numerous years in arid and semi-arid regions, but it has also garnered increased attention in temperate climates (Bixio et al., 2006). While wastewater irrigation serves as a beneficial water and nutrient source, it also presents risks. The abundance of contaminants, antibiotics included, present in wastewater, can pose threats to public health and the environment.

Human antibiotics are usually released into the sewer, either after excretion from treated patients or through improper disposal via toilets (Kusturica et al., 2017). Consequently, in urban settings, hospitals are often considered hotspots for antibiotic emissions (Herrmann et al., 2015). Once these residues enter the sewer system, they are typically conveyed to wastewater treatment plants, where incomplete elimination may occur, leading to their potential transfer into surface water (Kümmerer, 2003). Subsequently, they may further infiltrate soils when this water is used for irrigation.

The diversity of antibiotics in wastewater used for agricultural soil irrigation depends on its source and the treatment technologies applied before reuse. Existing literature consistently notes significant variations in antibiotic concentrations in such water sources (Michael et al., 2013; Kulkarni et al., 2017), with the possibility of soil contamination by complex mixtures (Kostich et al., 2014). It is important to highlight that the treatment of wastewater before irrigation is not always feasible in low- and middle-income countries due to economic constraints, potentially leading to more severe pollution in such cases.

Agricultural soil pollution can also occur through waste disposal practices. The sludge generated in wastewater treatment plants is sometimes utilized as a soil fertilizer in agricultural fields. While this practice positively influences soil fertility and remains essential in many countries globally (Schmidt et al., 2006; Coors et al., 2016), it might introduce unwanted contaminants (e.g. human antibiotics) to agricultural soils. It is worth noting that this practice is not prevalent in some countries, such as the Netherlands, where the sludge is predominantly incinerated, processed, and exported rather than being applied locally.

2.5 Combination of veterinary and human antibiotics

While the individual pathways of these two groups are well-understood, a knowledge gap exists when considering the combination of these scenarios. For instance, when the same agricultural soils are affected by both manure application and treated wastewater irrigation, they are exposed to both human and veterinary antibiotics (Pan and Chu, 2017). This implies potential exposure to entirely different antibiotics during various time intervals, as well as to the same antibiotics over extended periods, encompassing both the manuring season and irrigation cycles. Therefore, it is crucial to consider this aspect when developing strategies for environmental risk assessment of these compounds.

3 Fate of (veterinary) antibiotics in the soil

Once mixtures of antibiotics and excreta penetrate into the soil, the antibiotics tend to remain in the soil solution, where they are exposed to various processes. The relevant processes, as well as biotic effects caused by antibiotics, are schematically depicted in Fig. 3. While points 1 (medication) and 2 (input) from this figure have been previously discussed and are primarily associated with veterinary antibiotics, this section will concentrate on points 3 and beyond.

In the case of application onto the soil surface, antibiotics may undergo photodegradation by sunlight (4.1, Fig. 3), a process that can be quantitatively significant depending on the substance and substrate properties (Boreen et al., 2005; Wolters and Steffens, 2005; Thiele-Bruhn and Peters, 2007). Yet, photodegradation is fully inhibited as soon as the antibiotics are translocated into soil depths deeper than 0.5 mm (Miller and Donaldson, 1994). Once in the soil, degradation rates of antibiotics can vary depending on the compound, soil type (Berendsen et al., 2021), and depth (Rakonjac et al., 2023). In contrast, volatilization from the soil into the atmosphere (4.2, Fig. 3) is negligible because all these polar chemicals are characterized by very low vapor pressure and Henry coefficients (Thiele-Bruhn, 2003a). Transport of antibiotics by erosion and surface run-off (4.3, Fig. 3) occurs on sloping surfaces, especially grassland,

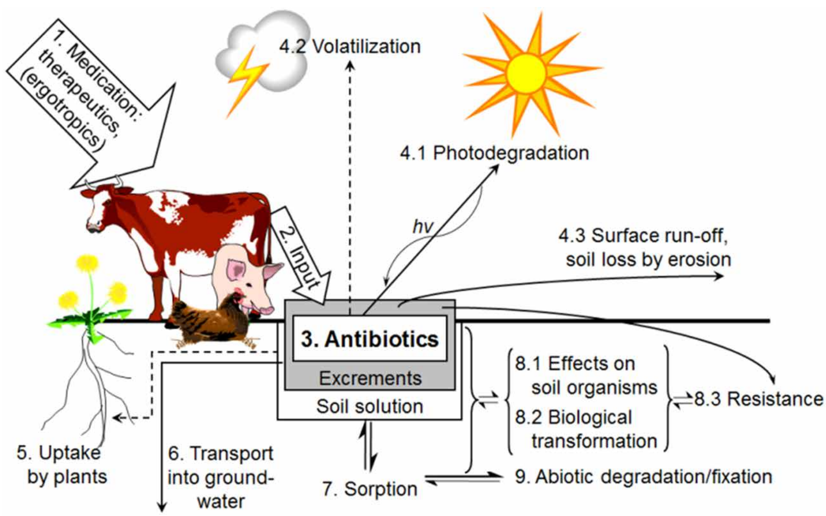

Figure 3 Input, distribution, degradation, and effects of veterinary antibiotics in the soil. Source: Reprinted from (Thiele-Bruhn, 2019).

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and is further accelerated by manure (Müller et al., 2003; Kreuzig et al., 2005). Thereby, run-off from grassland is increased due to the temporary surface sealing and hydrophobic effect of manure crusts (Stamm et al., 2003; Burkhardt et al., 2005). These transport processes are relevant especially in areas with dynamic relief. Yet, they are not specific for antibiotics but affect all chemicals and pollutants that have been introduced into soil by manure or other excreta.

Uptake by plants (5, Fig. 3) was confirmed for several plant types and antibiotics from different structural classes (Boxall et al., 2006; Dolliver et al., 2007; Liu et al., 2009). On one hand, the portions taken up rarely exceed a few percent of the total amount of antibiotics in soil (Langhammer et al., 1990; Grote et al., 2007; Freitag et al., 2008), on the other hand, adverse effects on plant growth and yield (Michelini, 2012; Michelini et al., 2012; Michelini et al., 2013), as well as on endophytes (Michelini et al., 2015), have been determined.

Transport and leaching into larger soil depths and groundwater (6, Fig. 3), respectively, occur (Kay et al., 2004; Kay et al., 2005; Höper et al., 2007; Weiß et al., 2007; Rakonjac et al., 2023). Yet, only smaller portions of the antibiotic contamination are dislocated (Aust et al., 2008b; Aust et al., 2010), while large fractions will remain in the soil (Förster et al., 2009; Rosendahl et al., 2011). The transport is largely bound to cotransport with particles and colloids or fast preferential flow (Hamscher, 2007; Höper et al., 2007; Xing et al., 2015; Zhou et al., 2016). Concentrations in groundwater of antibiotics that have been leached from manure treatment of soil are typically below 0.1 μg/L for each single compound (Hamscher, 2007; Hannappel et al., 2017), although also higher concentrations have been determined. Yet, analyses of groundwater samples showed that mixtures of several antibiotics frequently occur so that the summed concentration might even exceed 0.1 μg/L, a concentration that has been proposed as threshold value by the German UBA (Hannappel et al., 2017; Aust et al., 2010).

Sorption (7, Fig. 3) to the soil exchange sites, i.e. not only soil organic matter but also clay minerals and pedogenic oxides (Figueroa et al., 2004; Figueroa and Mackay, 2005; Gao and Pedersen, 2005; Schwarz et al., 2012), governs the mobility and bioavailability of antibiotics. Amphoteric and polar antibiotics are sorbed to soil components by specific and unspecific mechanisms (Tolls, 2001; Thiele-Bruhn et al., 2004). Sorption varies depending on the physico-chemical properties of the antibiotics, content and composition of soil organic matter as well as soil minerals, pH, and also soil moisture (Thiele, 2000; ter Laak et al., 2006a; ter Laak et al., 2006b). Furthermore, sorption and mobility, respectively, of antibiotics are significantly altered in the presence of organic waste material such as manure (Thiele-Bruhn and Aust, 2004; Kahle and Stamm, 2007; Bourdat-Deschamps et al., 2017). Comprehensive review articles on soil sorption of antibiotics were published by Wang and Wang (2015) and Wegst-Uhrich et al. (2014). Antibiotics are differently retarded in soils, which can be read from sorption coefficients. Differences largely depend on the structural class with smaller variation among individual compounds within an antibiotic class (Wegst-Uhrich et al., 2014; Wang and Wang, 2015). Antibiotics from some structural classes exhibit rather low sorption coefficients. Consequently, there is a higher risk that these antibiotics are leached from soil and may contaminate groundwater resources (Hamscher, 2007; Höper et al., 2007; Aust et al., 2010; Balzer et al., 2016). Sorption further varies with the pH-dependent speciation of the antibiotics. Compared to the sorption of the neutral species, it typically increases with the proportion of cationic species. On the other hand, sorption slightly declines with the increasing formation of anionic species (ter Laak et al., 2006b; Vasudevan et al., 2009; Figueroa-Diva et al., 2010). The formation of charged species depends on the pH of the soil and the acid dissociation constant (pK_a) of the specific antibiotic, resulting in the increased formation of cationic species at lower pH and of anionic species at higher pH. Among the different mechanisms responsible for the sorption of organic chemicals in soil, especially specific sorption mechanisms contribute to the sorption of antibiotics (Thiele-Bruhn et al., 2004; Figueroa-Diva et al., 2010; Schwarz et al., 2012; Wang and Wang, 2015). Consequently, it must be assumed that different individual antibiotic compounds compete for such specific sorption sites.

Antibiotics exert specific effects on biota even at very low dosage, hence, adverse effects on soil organisms (8.1, Fig. 3) and especially on microorganisms must be expected. In response, microorganisms react to contamination with the biological transformation (8.2, Fig. 3) of organic compounds and in this case of antibiotics (Schwarz et al., 2010; Girardi et al., 2011; Grossberger et al., 2014). However, this, as well as immobilization, in soil does not necessarily has a direct and/or immediate feedback on the – once initiated – negative effects of antibiotics on soil biota. It was shown that even soil-bound antibiotics can exert adverse effects and that effects can proceed even beyond the dissipation of the antibiotics (Chander et al., 2002; Chander et al., 2005; Hammesfahr, 2011; Peng et al., 2015). Effects on and reactions of microorganisms lead to an altered and increased resistance (8.3, Fig. 3) as a natural reaction (Schmitt et al., 2006; Heuer and Smalla, 2007; Heuer et al., 2011; Marti et al., 2013), which is triggered by the specific action of the antibiotics or through the input of resistant microorganisms with contaminated excrements. A reduction in the spread of resistance and in the resistance level subsequently occurs after the addition of an antibiotic to soil ended (Sengeløv et al., 2003; Heuer and Smalla, 2007). However, it is supposed that the restoration of a total population to its former status, including antibiotic sensitivity, is unlikely. Both types of pollution, the addition of antibiotics and of antibiotic-resistant microorganisms to soil, can affect the structure and function of environmental microbial populations. Regarding resistance genes, the situation is more complex, since genes are not "degradable pollutants" but auto-replicating elements (Grenni et al., 2018). Comprehensive information on the effect of antibiotics on resistance levels in the environment can be found in a literature study by Schmitt et al. (2017).

Furthermore, abiotic degradation and fixation/sequestration processes (9, Fig. 3) contribute to the reduction of antibiotic contents in soil (Schwarz et al., 2015; Hurtado et al., 2017). Thereby, it is assumed that the subsequent release of previously sequestered antibiotics is low (Rosendahl et al., 2011).

4 Modeling approaches

Sources, pathways, fate, and behavior of antibiotics in the soil environment can be evaluated through dedicated experiments and monitoring studies or by using modeling approaches. The advantage of a modeling approach is its applicability to a broad range of environmental conditions and different types of antibiotics, whereas experimental efforts provide results for specific situations only. Conducting scenario analyses is also a significant benefit of modeling. However, to ensure accuracy and reliability of modeling result, monitoring and experimental data are essential.

The European Medicines Agency provides a guideline on environmental impact assessment for veterinary medicinal products (EMEA, 2008). This guideline addresses different modeling perspectives per environmental compartment. In principle, it is recommended that when estimating medicine residues in the environment a stepwise (tiered) approach should be followed, using simple equations to provide an initial standard assessment and moving on to more complex modeling approach when a more refined estimate of exposure is required. Regarding the latter, a series of mechanistic environmental models and accompanying scenarios has been created by working groups in Europe known as FOCUS (Forum for the Co-ordination of Pesticide Fate Models and Their Use) to simulate the fate and transport of agrochemicals in the environment. Since FOCUS models are primarily designed for the exposure assessment of pesticides, before they can be used for modeling the exposure of antibiotics they have to be tailored likewise.

FOCUS soil calculations are relatively straightforward, focusing on the degradation rate of chemicals applied to a fixed soil depth while ignoring potential losses from volatilization, runoff, and leaching. This guidance recommends using “best fit” degradation kinetics instead of relying solely on conventional first-order fits to experimental data. In addition to determining the appropriate degradation kinetics of the compound in soil, these calculations require the associated application rates to soil (i.e. mass of chemical per land area). The EMEA guidelines (EMEA, 2008) offer further recommendations on incorporating antibiotics instead of pesticides in the calculations mentioned earlier and interpreting the modeling results.

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One of the major uncertainties in modeling approaches is how to address compound sorption. The default assumption is that sorption behavior is primarily influenced by sorption to organic carbon. However, since veterinary antibiotics could be large molecules with several functional groups and a tendency to speciate into ionic species at certain environmental pH values, other soil components with polar and/or charged surfaces might also act as sorbents. Sorption behavior is likely to be highly pH dependent. These factors may necessitate updates to existing models. For more information on models addressing sorption specifically related to veterinary antibiotics in soil-manure systems, we refer to Wang et al. (2015). The primary shortcoming of current modeling approaches is their focus on individual environmental compartments rather than the entire chain of processes. This compartmentalization overlooks how the outcomes in one compartment might significantly impact the subsequent phases. For instance, neglecting soil leaching and the role of macropore flow could be critical, depending on soil type and compound chemical characteristics, as these processes can contribute to groundwater contamination. Models that accurately conceptualize and simulate macropore flow are possibly essential for antibiotic risk assessments at regional, national, or global scales. However, these models often require numerous physical parameters, many of which are challenging to estimate independently under field conditions. Improving parameter estimation would enhance model accuracy and our understanding of antibiotic transport. The SWAP-PEARL model (Berg et al., 2016; Kroes et al., 2017) used for pesticide leaching (Faúndez Urbina et al., 2020) could serve as a valuable reference for similar studies on antibiotics. In general, to model the fate of antibiotics in soil, an interdisciplinary approach is essential. In addition to studying soil–compound interactions, there is a need for a better understanding of agricultural practices like manure spreading, which can vary widely across countries and regions. These practices, coupled with spatially variable environmental conditions such as hydrology, can greatly influence the interactions mentioned earlier and hinder accurate risk assessment of these compounds.

5 Controlled laboratory studies

As indicated earlier, the fate of chemical substances in soil depends on two important parameters: persistence and mobility. The persistence, usually expressed as DT50, refers to the kinetic degradation of the native antibiotic. The mobility, usually expressed as K_d, is related to the transport of the antibiotic from soil to other reservoirs, e.g. surface or groundwater.

The persistence of a chemical compound is contingent upon both its inherent physico-chemical characteristics and on the properties of the soil in which it resides, encompassing biotic and abiotic elements such as the fraction of organic matter, pH and the active microbiome. Some studies reported the fate (and environmental risks) of selected antibiotics in manure (Berendsen et al., 2018), or soil after applying manure containing antibiotics (Hamscher et al., 2002; Jacobsen et al., 2004; Brambilla et al., 2007; Martínez-Carballo et al., 2007; Stoob et al., 2007; Uslu et al., 2008; Domínguez et al., 2014; Spielmeyer et al., 2017; Rietra et al., 2024). The methodologies applied in these studies are partially derived from the standardized approach method 307 of the Organization for Economic Co-operation and Development (OECD, 2002). The OECD guidelines describe procedures for aerobic and anaerobic degradation experiments and also mention experiments with sterilized soils to determine the contribution of abiotic transformation. The guidelines suggest to study at least four different soils to determine degradation rates and rely on relatively large portions of soil (50–200 g) introducing potential errors related to inhomogeneity. Furthermore, many studies focus on the persistence of the native substance only. However, it is recognized that degradation products can still exert a negative impact on the environment. Therefore, degradation studies followed by a study to annotate potential degradation products should be included in strategies to assess the fate of chemicals in the agricultural environment.

The mobility of a chemical compound is related to the soil–water partitioning coefficient (K_d), representing the likelihood of the chemical being adsorbed to soil particles. As adsorption primarily takes place through the partition into soil organic matter, a K_d normalized to the organic carbon content of the soil is usually reported, which is expressed as an organic carbon–water partition coefficient (K_{OC}) (Wegst-Uhrich et al., 2014). Mechanisms other than hydrophobic partitioning, e.g. ionic interactions, complex formation, and hydrogen bonding, are not accurately accounted for using this approach which results in underestimation of K_{OC} (Tolls, 2001). Several methods have been used for the estimation of K_{OC}, of which most are derived from OECD method 106 (batch equilibrium method) (OECD, 2000) or method 312 (soil column leaching method) (OECD, 2004). The column leaching test is described for native and aged residues, the latter referring to the test substance and its transformation products present in soil after prolonged exposure to the soil. The test relies on a 30 cm soil column and requires careful uniform packing of this column to obtain reproducible results. Artificial rain is applied to the soil column at a rate of 200 mm over a period of 48 hours, which is equal to a 251 mL volume for a soil column with a 4 cm inner diameter. Both the soil and the leachate volume are relatively high. The soil has to be adequately homogenized before analysis to reduce additional uncertainty and the large leachate volume leads to a highly diluted aquatic fraction which demands high sensitivity of the analytical method for determination of the chemical's concentration.

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As the persistence and mobility of antibiotics clearly depend on soil composition, there is a need for good modeling approaches to allow estimation of the DT50 and K_d for a large range of different soils. Recently, a study was performed to predict these parameters for eight antibiotics based on selected soil properties (Rietra et al., 2024). It was concluded that both the range in DT50 and K_d can be predicted reasonably well using regression models that account for the variation in soil type and sampling depth. Organic matter, iron oxides, pH, and clay content appear to be the most important soil parameters impacting these parameters. Furthermore, simple yet effective laboratory procedures to determine DT50 and K_d in a large number of different soils and for a large number of different chemicals are required to obtain data to feed the models. Such procedures allow the determination of the persistence and mobility of specific substance–soil(-substrate) combinations and to understand the mechanistic principles of the interactions of the antibiotic in their environment.

5.1 Analytical detection techniques

Fit-for-purpose analytical techniques are crucial to study the fate of any chemical compound in the soil–water nexus. Several analytical challenges need to be overcome. First of all, the methods need to include a wide range of antibiotics with highly diverse structures and physico-chemical properties (Thiele-Bruhn, 2003b). Second, the matrix itself is a challenge due to its high variation: soil can be a rather simple sandy soil with relatively low organic matter, but it can also be a loamy soil or a soil with high clay content which makes effective extraction of the chemicals in it extremely challenging. Furthermore, the addition of highly diverse substrates to soil can alter the soil composition and structure and complicate the analysis. Preferably, the analytical methods are applicable to all types of soils. Third, methods should be able to achieve detection of antibiotic residues at extremely low concentrations, preferably in the low microgram per kilograom range. Only then the soil interactions can be studied at environmentally relevant concentrations. Last, the methods have to be accurate, precise, and highly selective to prevent false positives from occurring and to prevent natural soil constituents from interfering in the detection of the relevant chemicals and their transformation products.

To achieve such a high standard, the detection method of choice is tandem mass spectrometry. Currently, triple-quadrupole mass spectrometry hyphenated to liquid chromatography is the method of choice for antibiotic analysis. A review of methodologies applied for environmental and food matrices was presented by Thiele-Bruhn (2003b) in 2003. In the early 2010s methods for soil analysis or related complex matrices (e.g. sludge and manure) were scarce and included 1–5 different antibiotic groups. More recently multi-methods were developed (Blackwell et al., 2004; Janusch et al., 2014; Wallace and Aga, 2016) and in 2019 a study was published especially focusing on the extraction of also the bound fraction of antibiotics in manure and soil (Jansen et al., 2019). An extraction solvent of 0.125% trifluoroacetic acid in acetonitrile with the addition of McIlvain-EDTA buffer proved to be the most effective. In the latter study, it was also demonstrated that by changing the extraction solvent either the total residual concentration can be determined or only the biologically available fraction; the fraction of the antibiotic that is freely present in the soil environment and has the potential to move among reservoirs or be transported into a cell.

To achieve the low detection limits and the high selectivity required, methods usually rely on solid phase extraction (SPE) as a clean-up and concentration technique. The extract is transferred to an activated SPE cartridge which is designed to retain the antibiotics of interest. After washing the cartridge, the antibiotics are eluted by an organic solvent that is evaporated to minimize the final extract volume. The concentrated extract is then injected onto the LC-MS/MS system.

Even though not often reported in the literature, these methods are not applicable to all types of soils and substrates. Thorough validation studies are usually lacking or are only focused on relatively simple sandy soils. In general, absolute recoveries tend to be extremely low, especially for soils with high clay or peat content hampering the accurate and precise analysis of antibiotic content in such soils.

5.2 Standardized fate studies

The OECD procedures aim for standardization of the determination of persistence and mobility of chemicals in soil. They rely on the availability of relatively large portions of soil and for both fate parameters the difficulty in homogenizing these soils after the addition of the chemicals tested, adds to the uncertainty of the determined parameters. Aiming for an effective and high-throughput approach, a strategy to determine the fate of chemicals in soil was reported in 2021 (Berendsen et al., 2021). This strategy includes an easy-to-apply persistence and mobility study and also considers the degradation of antibiotics to antimicrobial active transformation products (Fig. 4).

First, the strategy aims to categorize the antibiotics into one of three groups: (1) persistent, (2) degradation to inactive transformation products, and (3) degradation to active transformation product(s). Note that from an environmental point of view, the second category is preferred. To achieve this, a simple degradation study is proposed based on a miniaturization of OECD guidelines 307 (OECD, 2002). The advantage of this miniaturized setup is that the complete soil aliquot is extracted and thus no errors due to inhomogeneity are introduced. Also this allows to perform a study without the need for kilograms of soil material.

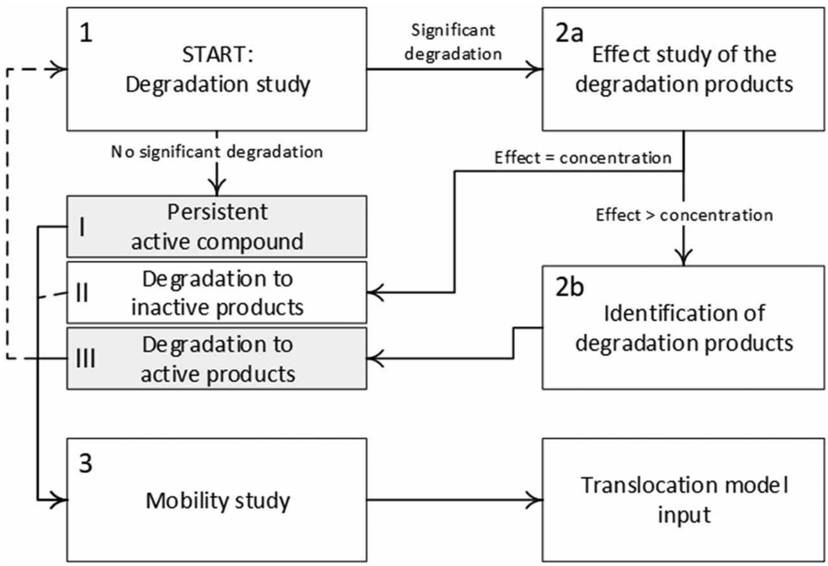

Figure 4 Schematic presentation of the reported strategy to determine the fate of chemicals in the soil water nexus. Source: Reprinted from Berendsen et al. (2021).

In case chemical degradation of the antibiotic was observed, the degradation was also examined using an effect-based test which recorded the antimicrobial activity instead of the concentration of the native antibiotic. In case chemical degradation was significantly larger than the decrease in antimicrobial activity over the same period of time, it was concluded that an antimicrobial active transformation product was formed during soil incubation. From the eight antibiotics included in that specific study, this was clearly the case for tylosin A. Further investigation using high-resolution mass spectrometry is required to (tentatively) identify the transformation product(s). For tylosin A, it was found that, during soil incubation, the native drug is transformed to tylosin A acid, a related substance that is still an antimicrobial (yet somewhat less potent) and more persistent.

Especially for the persistent chemicals, the persistence study should be followed by a mobility study. The use of a column leaching setup proved to be most suitable. Again, a miniaturized setup based on the OECD guidelines is most applicable, especially in case many different soils are included in the study. Soil columns, each containing 5 g of soil, were prepared using an SPE manifold. A 10 mL volume of artificial rain proved to yield K_{OC} values that were comparable to the values previously reported in the literature. The smaller volumes again prevent additional errors due to homogeneity, as the complete soil aliquot and leachate volume are analyzed to determine the antibiotic concentration in each of them. Furthermore, instead of 48 hours, this experiment lasted 1 hour to prevent any significant degradation of the native antibiotic inside the soil column. As a result, the throughput was increased allowing to study a larger number of different soils and soil-substrate combinations, and different types of chemicals.

6 Dutch case study

In the Netherlands, manure from intensive livestock farming is spread onto arable land and grassland in considerable amounts. Consequently, together with Belgium, the Netherlands represents one of the highest nitrogen input regions in Europe (de Vries et al., 2021). The majority of this manure, over 95%, is applied in a slurry form, with the common practice of injecting slurry into the soil to mitigate ammonia emissions into the air. Besides, applied manure may contain a broad range of veterinary antibiotics of different quantities, as detailed in Rakonjac et al. (2022), and could serve as a significant source for introducing these compounds into surface water (Rakonjac et al., 2024).

In order to study the environmental fate of various compounds injected with slurry, a field survey was conducted in the province of Gelderland in the Netherlands. Five pig-rearing and five veal calve-rearing farms situated on sandy soils were selected. In the spring of 2017, the slurry collected over the winter season at these facilities was injected into arable fields (9 locations) and grassland (1 location). During four months following injection, concentrations were monitored in soil (around 25 cm below the surface), in the shallow groundwater of the fields (max. 2.5 m) and in the surface water and sediment of adjacent ditches. The results were reported by (Lahr et al., 2018). Here, a summary of the findings for antibiotics is presented.

Most antibiotics reported to have been used at the farms were detected in slurry from the pits before land application. These included oxytetracycline, doxycycline, and sulfadiazine at both types of farms and flumequine and tilmicosin at veal farms only (Table 1). Concentrations were highest in slurry from veal farms. Other compounds were detected occasionally. Not all antibiotics used at the farms were represented in the analysis package (e.g. penicillin).

Several of the more hydrophobic antibiotics were found frequently in the soil of the fields after application and persisted to various degrees, especially

Table 1 Concentration ranges (μg/kg fresh weight) of the most frequently detected veterinary antibiotics in slurry from intensive pig and veal rearing facilities before application on land

Substance	Veal calve slurry (μg/kg f.w.)	Pig slurry (μg/kg f.w.)
Oxytetracycline	700–5000	<4–71
Doxycycline	350–2000	<5–700
Sulfadiazine	<1–200	<1–100
Flumequine	<3–1700	<7
Tilmicosin	24-300	<1

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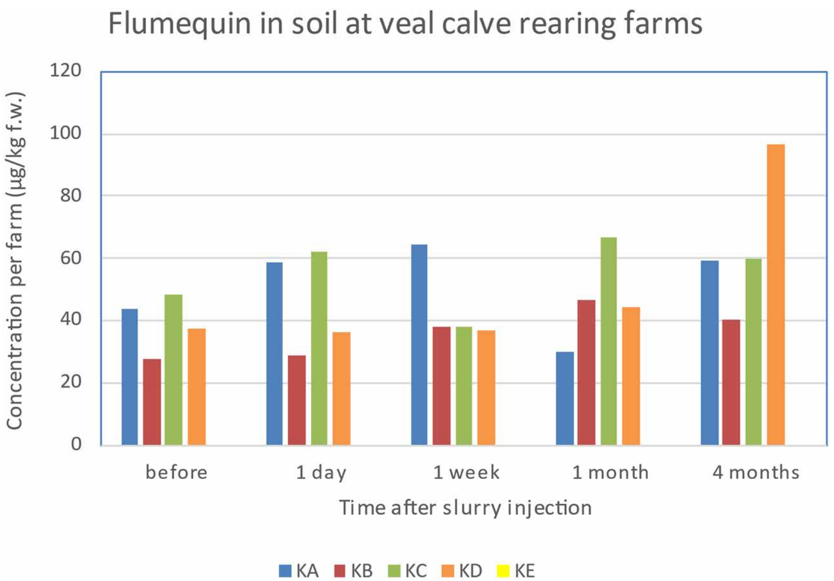

Figure 5 Concentrations (µg/kg fresh weight) of the quinolone antibiotic flumequine in the soil of five veal calve rearing farms (KA to KE) before and after slurry injection. Farm KE confirmed that flumequine was not used in their facility.

oxytetracycline, flumequine and tilmicosin. The latter two are typical of veal calve-rearing and may persist for over a year in soil because they were already found in the soils before the yearly slurry injection (see Fig. 5, for flumequine). Despite not being used at the farms, chlortetracycline was also detected in the soil at most farms. Its exact origin remains unclear.

As expected, instead of in soil, the more soluble antibiotics tended to be found more often in groundwater and/or surface water of adjacent ditches at the fields injected with slurry, most notably sulfadimidine (synonym of sulfamethazine) and sulfadiazine. The use of sulfadimidine as an antibiotic was not reported by the farms in the study. It is not sure where it stems from. Oxytetracycline, doxycycline, chlortetracycline, and flumequine were occasionally detected in the sediments of the ditches near the fields. In five regional streams in the same region as the farms, sampled once in July of the same year, only sulfadiazine was detected in the surface water and at only one occasion.

The results clearly demonstrate that slurry injection leads to contamination of the soil of agricultural fields by antibiotics and potentially also to some contamination of the groundwater and surface water around the fields of farms with intensive livestock rearing.

The findings of this limited study warrant a more in-depth assessment of the risks of substances injected with slurry to soil life, aquatic organisms, and the production of safe drinking water from groundwater.

7 Mixed contaminations with antibiotics in excreta and soil

Generally, it must be expected that contaminated soils not only contain one contaminant but mixtures of several pollutants (Posthuma et al., 2008; Backhaus, 2014; Jahnke et al., 2016). This also applies to antibiotics that have been recognized as widespread soil pollutants (Thiele-Bruhn, 2003b; Boxall et al., 2004; Sarmah et al., 2006). The fact that mixtures of veterinary antibiotics end up in soil is due to three major reasons. First, combinations of antibiotics are frequently administered to livestock (Fig. 6). Second, different antibiotics are given to different livestock animals and to different live stages of the animals because different pathogens and diseases typically occur and have to be treated. As a result, antibiotic mixtures are created when the excrements of different animals are collected in the same manure tank or lagoon. Third, excreta with mixed contamination are subsequently and often repeatedly spread onto agricultural fields when they are reused as fertilizer. The assumed accumulation of antibiotics after spreading to soil with organic fertilizer was previously confirmed in various studies (Hamscher et al., 2002; Höper et al., 2002; Hamscher, 2007; Aust et al., 2008a). This is also due to a

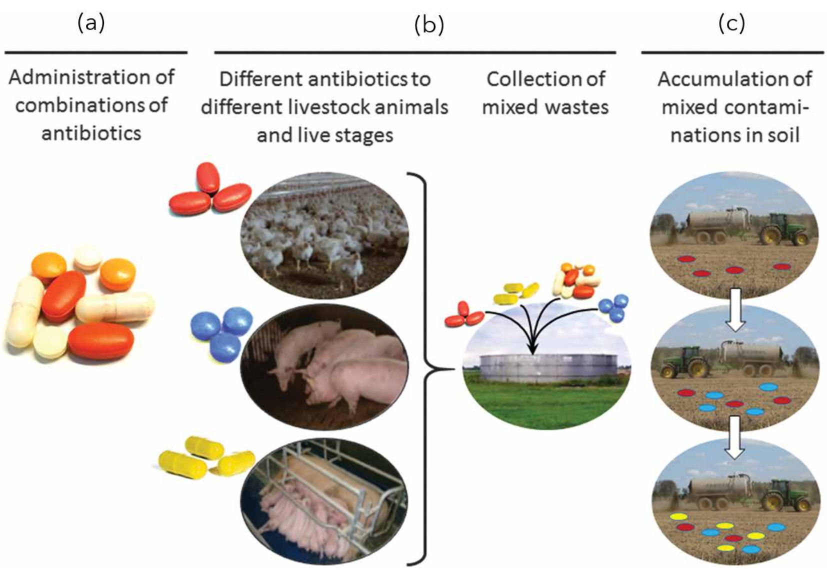

Figure 6 Formation of mixed contamination in the environment through (a) medication by using combinations of antibiotics, (b) on the farm by collecting wastes from different livestock animals and live stages, (c) by input and accumulation of residual contaminations from repeated manure application. Source: Own figure from S. Thiele-Bruhn (Univ. Trier). Single photographs with permission from M. Arenz-Leufen, I. Rönnefahrt, and UBA and from Christoph-Schulz (2018), doi: 10.1007/s00003-017-1144-7, open access.

retarded degradation in soil (Braschi et al., 2013), even of otherwise rather unstable antibiotics such as penicillins (Kotzerke et al., 2010). The repeated application of manure contaminated with antibiotics can lead to a seemingly continuous contamination level that is termed as apparent persistence or pseudo-persistence (Hamscher et al., 2005; Hamscher, 2007; Bottoni and Caroli, 2018). Not least, mixtures of antibiotic parent compounds and their metabolites, some of which exert an (altered) antibiotic activity as well, develop in soil upon biotransformation of the compounds (Pollard and Morra, 2018; Schwarz et al., 2010). A similar scenario for the accumulation of antibiotics exists for antibiotics for human use. There, also (i) combinations of antibiotics are often used for medication, (ii) mixtures accumulate over time in wastewater canals and wastewater treatment plants, and (iii) accumulate in soil with the repeated use of wastewater for irrigation and sewage sludge for fertilization, respectively (Monteiro and Boxall, 2010; Verlicchi et al., 2012; Verlicchi and Zambello, 2015).

Sorption competition between the components of a mixed contamination can occur in soil and increase the mobility and bioavailability of the contaminants. Sorption competition occurs in particular with mixtures of antibiotics of the same compound class, which compete for the same sorption sites in the soil. In addition, sorption competition increases with increasing concentrations. The resulting sorption coefficients are about 1/3 to 2/3 lower than for the individual compounds (Conkle et al., 2010; Ahmed et al., 2017). The sorption kinetics are considerably delayed, as has been shown for a single sulfonamide compared to a mixture of three sulfonamides (Thiele-Bruhn, 2019).

Regulatory limits of chemicals and ecological risk assessment are usually based on the effect of single compounds, but not taking into account mixture effects (Natal-da-Luz et al., 2011). Two characteristics in particular make the combined toxic effect of a mixture of antibiotics an important issue for hazard and risk assessment: (i) the ecotoxicity of a mixture of antibiotics is usually higher than the effect of the individual components, and consequently (ii) such a mixture may exhibit significant ecotoxicity even if all the individual contaminants are present in such low concentrations that they would not cause toxic effects on their own (Backhaus, 2014). Furthermore, it is expected that the environmental fate and effects of mixtures of antibiotics will differ from that of single compounds, because antibiotics from different structural classes have different modes of action leading to synergistically increased or antagonistically decreased effects, while in other cases additive effects are assumed. These different possibilities of a purely additive effect of mixed contaminations or antagonistic or synergistic interactions between the components are described by the two models of concentration additivity (CA) and independent action (IA) (Grimme et al., 2000). Although there are new developments, e.g. with thermodynamically based models (Gobas et al., 2018), these two models are widely used. This makes it important to note that the effects of antibiotic mixtures on the biological effects and chemical fate in the soil environment are still largely unknown (Boxall et al., 2012).

Using not single compounds but combinations of different antibiotics and/or further pharmaceuticals and components such as metals (e.g. silver Ag, copper Cu, zinc Zn) is common practice (Takahashi et al., 1987; Russell and Hugo, 1994; Haeili et al., 2014; Poole, 2017). Combinations of antibiotics and other pharmaceuticals are meant to synergistically increase the antibiotic effect and/or to reduce the formation of resistance (Riviere, 2011; Bollenbach, 2015). The use of antibiotic combinations and/or of antibiotics mixed with other synergistic compounds is actually increasing steadily and will probably continue to increase in the future (Keith et al., 2005; Caminero et al., 2010). Various studies showed that even old pharmaceuticals, sorted out in the past due to widespread resistance formation, can be revived in new combinations (Malik et al., 2014; Baym et al., 2016). The frequent use of mixtures of antibiotics in livestock production is exemplarily shown on veterinary prescriptions for medicated feedstuff during a one-year period on data from Mecklenburg-Western Pomerania, Germany (Table 2). It is shown that in 47% of all prescriptions mostly two but up to five antibiotics and other pharmaceuticals had been mixed (Thiele-Bruhn et al., 2003).

Since the use of different antibiotics in parallel and in close succession is common and significant amounts of antibiotics as well as other drugs and elements with antibiotic properties, such as copper and zinc, are excreted by the drug-treated organisms (Sarmah et al., 2006; Du and Liu, 2012), the mixed contamination of wastes is widespread. Published studies cover excreta and manure, wastewater and sewage sludge. They consistently report that manure, digestate, wastewater, sewage sludge, and compost are often contaminated

Table 2 Number of antibiotic compounds used and mixed in medicated feedstuff; data base: 2097 veterinary manufacturing orders for pharmaceuticals' admixture to feedstuff in the state of Mecklenburg-Western Pomerania, Germany (period October 2000 until September 2001; data from Thiele-Bruhn (2003a))

No. of components	5	4	3	2	2-5	1	0
			Including non-antibiotic compounds[a]				
No. of prescriptions	3	92	346	546	987	1110	
% of prescriptions	0.1	4.4	16.5	26.0	47.1	52.9	
			Only antibiotic pharmaceuticals				
No. of prescriptions	2	54	246	504	806	1290	1[b]
% of prescriptions	0.1	2.6	11.7	24.0	38.5	61.5	0.05

[a]Non-antibiotic compounds: Zn oxide, bromhexine hydrochloride, citric acid, vitamins A and D3, Mn carbonate.
[b]Feed contained only a vitamin mix.

with two or more (>20) individual antibiotic compounds (Sattelberger, 1999; Winckler et al., 2004; McClellan and Halden, 2010; Arenz-Leufen, 2012; Clara et al., 2013; Li et al., 2013; Ratsak et al., 2013; An et al., 2015; Hannappel et al., 2017). As the waste materials are usually used as organic fertilizer and applied to agricultural soils, the corresponding environmental samples are consequently often also contaminated with pharmaceuticals and antibiotics in particular (Monteiro and Boxall, 2010; Li et al., 2013; Łukaszewicz et al., 2017). The studies in Table 3 show that not only single antibiotic compounds but mixtures of several compounds frequently occur in soil, so that the combined concentrations clearly exceed natural background levels. Natural soil contents of antibiotics, which are formed by the secondary metabolism of autochthonous soil microorganisms, are in the range of a few µg/kg and are mainly found in the rhizosphere of soil (Soulides, 1965; Lumsden et al., 1992). The introduced anthropogenic antibiotics belong to different structural classes and exhibit different modes of action (Hu et al., 2008; Karci and Balcioğlu, 2009; Ok et al., 2011). Thereby, antibiotics from some structural classes are more abundant (e.g. tetracyclines) while others have been determined in much lower concentrations (e.g. sulfonamides) (Thiele-Bruhn, 2003b; Hembrock-Heger et al., 2011; Hamscher and Mohring, 2012). This is due to (i) a different usage and thus release into the environment, and (ii) a different persistence and mobility in soil. Based on these consistent findings, it can therefore be concluded that the occurrence of mixed contamination in the terrestrial environment is the rule rather than the exception.

In Table 3, data from eight studies were combined, presenting concentrations of two and up to 14 different antibiotics in soils. Often, however, the number of substances found in environmental samples seems to be limited less by the contamination status of the soil than by the number of substances investigated.

Interactions between antibiotics and also further pharmaceuticals are known from clinical and pharmacological research. Similar mixture effects are expected on non-target organisms in the (soil) environment. Interactions of antibiotic pharmaceutical were reviewed by Bollenbach (2015). Synergistic effects of pharmaceutical combinations can be due to

- effects on the uptake: the first pharmaceutical increases the permeability of the cell membrane for the second pharmaceutical,
- direct physical interaction: pharmaceuticals reciprocally stabilize the binding to the target site,
- targeting of sequential metabolic steps: e.g., sulfonamides and trimethoprim both affect the folic acid biosynthesis pathway with different mode of action (inhibit dihydrofolate reductase and dihydropteroate synthetase, respectively).

Table 3 Contamination level of soils with antibiotics. Numbers of samples (No. sample), numbers of antibiotics analyzed in the study (No. antibiotics), and highest numbers of antibiotics detected in one individual sample (max No. antib) are indicated

Study	Sattelberger (1999)			Hamscher et al. (2002)			Kues et al. (2002)			Clara et al. (2013)			An et al. (2015)			Gildemeister et al. (2011)			Hannappel et al. (2017)			Zhou et al. (2012)		
No. sample	61			14			42			8			70			2			104			5		
No. antibiotics	11			4			2			7			8			9			11			13		
max No. antib.	4			2			2			2			>4			7			2			14		
	#[a]	**av.**[b]	**max**[c]	**#**[a]	**av.**[b]	**max**[c]	**#**[a]	**av.**[b]	**max**[c]	**#**[a]	**av.**[b]	**max**[c]	**#**[a]	**av.**[b]	**max**[c]	**#**[a]	**av.**[b]	**max**[c]	**#**[a]	**av.**[b]	**max**[c]	**#**[a]	**av.**[b]	**max**[c]
		μg/kg	μg/kg		μg/kg	μg/kg		μg/kg	μg/kg		μg/kg	μg/kg		μg/kg	μg/kg		μg/kg	μg/kg		μg/kg	μg/kg		μg/kg	μg/kg
Tetracycline	0			14	31.7	191.3	30	21.5	85.0				27	241	976	2	168	332				3	350	1010
Oxytetracycline	0			0									34	609	1399	2	0.1	0.2				4	368	1410
Chlortetracycline	3	87	160	14	61.2	747.5	32	10.2	38.2				45	718	1590							4	3402	12900
Doxycycline													19	120	871							2	259	499
Trimethoprim	2	50	100							2	6.2	6.5				1	118	118	1	7.0	7.0	2	1.60	3.20
Sulfadimidine	2	50	100							0			4	3.5	11.5	1	589	589	0			2	1.85	3.69
Sulfadiazine	0									0			20	71.5	760				0			1	4.95	4.95
Sulfadoxine	0																		0					
Sulfathiazole	0																		0					
Sulfamethoxaz.	2	50	100							1	3.0	3.0	13	19.4	672				1	2.0	2.0			
Sulfamerazine													12	65.8	311				0					
Sulfamethoxypy.																			0					
Sulfachloropy.																			0			1	5.11	5.11
Sulfadimethox.																			0					
Sulfaethoxypy.																			0					

Sulfaguanidine													1	[d]<LOQ	[d]<LOQ
Sulfamonomethox.													2	2.69	5.37
Sulfaquinoxaline													1	2.02	2.02
Ciprofloxacin	5	200	370							2	2.6	3.4	3	8.10	14.0
Enrofloxacin	4	103	200										4	28.4	95.8
Norfloxacin													4	25.4	58.8
Ofloxacin													3	74.3	113
Pefloxacin													2	20.5	31.0
Difloxacin													1	4.01	4.01
Tylosin	0			0											
Erythromycin							5	23.6	35.0	1	0.3	0.3	1	[d]<LOQ	[d]<LOQ
Roxythromycin							0								
Clarythromycin							0								
Lincomycin													1	92.3	92.3
Polypeptide										1	33.1	33.1			

#[a] number of samples in which the specific antibiotic was detected.
av.[b] average concentration in all samples. Average was calculated only for data above the limit of detection.
max[c] highest concentration determined in one of the investigated samples.
[d]<LOQ detected below limit of quantification.

Altered effects of antibiotic mixtures vs. the single compounds have been reported for various combinations of antibiotics. These individual case studies showed deviating effects, e.g. on the soil bacterial community composition, microbial enzyme activities and abundance of functional genes in particular of those related to the nitrogen cycle, community level physiological profiles (Ma et al., 2014; Cleary et al., 2016; David et al., 2016). Additionally, deviating effects of mixtures of antibiotics with metals or other organic agents such as pesticides have been reported (Kong et al., 2006; Yan et al., 2011; Fang et al., 2016; Liu et al., 2016). Furthermore effects on faunal species such as earthworm *Eisenia fetida* or the soil nematode *Caenorhabditis elegans* have been reported (Jonker et al., 2004; Dong et al., 2011).

However, in the case of environmental contamination, it seems difficult to predict whether the effects of the mixture are additive or independent of each other. Typically, it is assumed that pharmaceuticals having the same mode of action cause additive toxic effects, when they are combined (Vasquez et al., 2014). However, experimental findings show that binary mixtures of antibiotics from the same chemical class exhibit not only additive (sulfonamides) but also synergistic effects (e.g. macrolides, tetracyclines, and fluoroquinolones) (Yang et al., 2008). Thiele-Bruhn (2015) reported synergistic effects also for a binary mixture of sulfonamides. On the other hand, interactions between bactericidal and bacteriostatic antibiotics are largely antagonistic (Ocampo et al., 2014) as was shown by a study on the effects of binary mixtures of 21 antibiotics on *E. coli* as test organism. However, the general conclusion by Ocampo et al. (2014) was somewhat disproved by the findings of Christensen et al. (2006). In the presence of sludge bacteria numerous synergistic effects and in less cases concentration additivity were found even for combinations of bacteriostatic and bactericidal antibiotics (Christensen et al., 2006). Results from Yang et al. (2008) point to the fact that combinations of bacteriostatic antibiotics (within one or between different compound classes) exert additive or synergistic effects, while combinations of bactericidal antibiotics are additive (within compound class) or are antagonistic (between different compound classes). Furthermore, effects of mixtures frequently change with the concentrations of the mixed compounds and combinatory effects may shift, e.g. from antagonism at a low concentration level to concentration additivity at a medium level and to a synergistic effect at high concentrations (González-Pleiter et al., 2013).

One particular aspect of the undesirable effects of antibiotics in the environment is the increase in antibiotic resistance. Synergistic combinations can lead to stronger and also faster resistance development compared to that in the presence of single antibiotics (Hegreness et al., 2008). A combination of tetracycline, sulfamonomethoxine and ciprofloxacin added to soil led within 7 days and 20 days to 1.7 to 11-fold higher abundance of sulfamonomethoxine

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and ciprofloxacin resistant bacteria but not to increased tetracycline-resistant bacteria compared to control soils contaminated with the single compounds (Ma et al., 2014). In contrast, antagonistic pharmaceutical combinations lead to slower resistance evolution than synergistic ones (Hegreness et al., 2008; Singh and Yeh, 2017). Also metals such as Cu, Zn, Cd, and Hg exert antimicrobial effects and can promote a co-selection for antibiotic resistance (Baker-Austin et al., 2006; Seiler and Berendonk, 2012). The release of such metals combined with antibiotics into the (soil) environment in the course of agricultural activities may result in a combined selection and co-selection of antimicrobial resistant bacteria, which even makes agricultural soils hot-spots of the formation of antibiotic resistance in the environment (Seiler and Berendonk, 2012). However, the state of knowledge about adverse effects of antibiotics and, in particular, antibiotic mixtures in the environment is still incomplete and contradictory, so that it is not actually possible to derive predicted no-effect concentrations (PNEC) that trigger an increased abundance of microbial resistance genes in the soil environment (Schönfeld et al., 2017).

8 Conclusion and future trends

The intensified practices in livestock farming and the adoption of waste (water) reuse in agriculture have highlighted concerns about the presence of antibiotics in soils. Various pathways contribute to the introduction of antibiotic residues into the soil compartment, leading to variations in the types of antibiotics, concentrations, and exposure durations. Once antibiotics enter the soil, they undergo diverse processes, with persistence and mobility being crucial parameters influencing their fate.

Soils are commonly exposed to a mixture of antibiotics rather than a single antibiotic, and this phenomenon can be attributed to three primary factors. First, livestock often receives combinations of antibiotics. Second, diverse antibiotics are administered to different livestock and at various life stages to address specific pathogens and diseases. Consequently, excrements from different animals are collected in the same manure tank or lagoon, resulting in the creation of antibiotic mixtures. Third, excreta with mixed contamination is subsequently and frequently spread onto agricultural fields during reuse as fertilizer. Consistent findings indicate that the occurrence of mixed antibiotic contamination in the terrestrial environment is the norm rather than the exception. This is a crucial consideration in the risk assessment of these compounds.

Furthermore, addressing the complex behavior of both individual antibiotics and mixtures in soil requires a shift toward standardization in studying their behavior. Implementing straightforward laboratory experiments and employing fit-for-purpose analytical methodologies would facilitate the investigation of these aspects efficiently and effectively. This approach would improve our understanding of antibiotics' fate in soil, offering valuable insights for accurate risk and effect assessments. This chapter offers some suggestions to move in that direction.

Here are a few challenges that remain to be addressed in the future:

- insufficient publicly disclosed data on the administration rates of veterinary antibiotics per animal sector. Ongoing initiative: Big Data strategy for veterinary medicines in the EU | European Medicines Agency (europa.eu);
- uncertainty about the excretion rates of antibiotics;
- clear definition of persistent antimicrobials and preference to use antibiotics that degrade within the system (animal/manure/soil);
- lack of established environmental threshold values for pharmaceuticals; and
- interaction between pharmaceuticals and other soil contaminants, particularly in relation to antibiotic resistance. For instance, recent research has explored soil plastispheres (Zhu et al., 2022) and Quaternary ammonium compounds (Mulder et al., 2018).

9 Acknowledgments

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10 Where to look for further information

A standard introduction to the topic of veterinary antibiotics in the environment can be found in Boxall et al. (2004) and Sarmah et al. (2006). If the focus is specifically on soil, a comprehensive overview is provided by Thiele-Bruhn (2003b). For more details on the uptake of veterinary medicines from soil into plants, Boxall et al. (2006) offers one of the foundational reviews. For further insight into experimental and testing procedures, the OECD guidelines, as mentioned earlier in this chapter, can be consulted.

In addition to the studies referenced in this chapter, valuable information on the (environmental) characteristics and behavior of specific antibiotic compounds can be found in the following sources:

- European Medicines Agency (https://www.ema.europa.eu/en/homepage);
- European Chemicals Agency (https://echa.europa.eu);
- Veterinary Substances DataBase (https://sitem.herts.ac.uk/aeru/vsdb/index.htm).

For a broader perspective on medicines in the environment and the interplay between scientific and regulatory issues, as well as how these have evolved over the years, one might refer to Boxall et al. (2012, 2024). For an even wider scope, Van den Brink et al. (2018) offers valuable insights.

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