TEXTE
32/2019

Environmental risks from mixtures of antibiotic pharmaceuticals in soils – a literature review

Final Report

For our Environment

Umwelt Bundesamt

TEXTE 32/2019

Project No. 92954
Report No. (UBA-FB) FB000071

Environmental risks from mixtures of antibiotic pharmaceuticals in soils – a literature review

by

Prof. Dr. Sören Thiele-Bruhn
Trier University, Soil Science Dept., Faculty VI, Trier

On behalf of the German Environment Agency

Imprint

Publisher:
Umweltbundesamt
Wörlitzer Platz 1
06844 Dessau-Roßlau
Tel: +49 340-2103-0
Fax: +49 340-2103-2285
buergerservice@uba.de
Internet: www.umweltbundesamt.de

/umweltbundesamt.de
/umweltbundesamt

Study performed by:
Trier University, Soil Science Department,
Faculty of Regional and Environmental Sciences
Behringstr. 21
54286 Trier

Study completed in:
November 2018

Edited by:
Section IV 2.2 Pharmaceuticals, Washing and Cleaning Agents and Nanomaterials
Dr. Sabine Konradi, Dr. Ines Vogel

Publication as pdf:
http://www.umweltbundesamt.de/publikationen

ISSN 1862-4804

Dessau-Roßlau, April 2019

Abstract

A literature research was done on the topic of 'environmental risks of mixed antibiotic contaminations in soils'. Overall aims were to identify antibiotics that are typically applied in veterinary and human medicine, to evaluate reports on mixtures and contamination levels occurring in soils and in organic waste materials such as manure that are applied to soil as fertilizer, to find information, which mixture effects result from this for soils and soil (micro) organisms, and to identify major knowledge gaps and to propose further steps for research and regulation.

Antibiotics are largely and increasingly consumed worldwide in human and veterinary medicine. It is well known that many agricultural soils are contaminated with antibiotics. Even more, it must be expected that contaminated soils not only contain one antibiotic but mixtures of several compounds. This has strong implications for the environmental relevance, since the ecotoxicity of a pharmaceutical mixture is typically higher than the effects of each individual component. Mixed contaminations occur for instance because the use of antibiotic combinations and/or of antibiotics mixed with other synergistic compounds is steadily increasing in human as well as veterinary medicine. Even old pharmaceuticals, sorted out in the past, are revived in new combinations. Comprehensive knowledge exists in pharmaceutical and medicinal literature, respectively, on the effects of various combinations of antibiotics. However, much less is known on antibiotics in the environment. Mixed antibiotics' contaminations in the environment may result from (i) the administration of combinations of antibiotics, (ii) the medication of different livestock animals and different live stages of the animals with different antibiotics and collection of all excreta in one manure tank and (iii) subsequent, repeated spreading of contaminated manure, sewage sludge or other organic waste materials onto agricultural fields. This results in contaminations of organic waste materials with mixtures of different antibiotics comprising up to 20 or more individual compounds. Consequently, soils fertilized with these substrates contain mixtures of different pharmaceutical antibiotics. This is supported by monitoring data of up to 13 antibiotics found in soil.

Existing knowledge on adverse effects of antibiotics on soil organisms is largely restricted to studies with individual compounds, which show clear dose-dependent adverse effects for example on microbial biomass, or soil community structure and functions. Additionally, numerous resistance genes are found in contaminated soils at a significantly and substantially increased abundance level. Synergistic antibiotic combinations can even lead to stronger and also faster resistance development. This applies not only for antibiotic mixtures but also for mixtures of antibiotics with other chemicals, which are in an agricultural context especially copper and zinc. Effects of antibiotic mixtures may be largely modulated by various influencing factors, i.e. the investigated endpoint and/or subject of analysis, the concentrations of the mixed compounds, the time-dependence of antibiotic effects, nutrients and nutrient substrates in soil. Furthermore, interactions exist with all other boundary conditions that affect the fitness of biota such as heat or frost. Also the fate of antibiotics may be altered in the presence of mixed contaminations, which is especially due to sorption competition in mixtures. Despite the outlined findings it must be stated that mostly incomplete knowledge exists on the various topics. More information from targeted, systematic research is needed. To this end, three successive research projects are proposed, aiming to increase the systematic knowledge on effects of mixtures in soil. The research would be ideally combined with scientific workshops. Not last, all these research efforts should lead to or even be flanked by regulatory measures for an improved use and management of antibiotics.

Kurzbeschreibung

Zu der Thematik „Umweltrisiken für Böden aufgrund der Belastung mit Antibiotikagemischen“ wurde eine Literaturstudie durchgeführt. Die generelle Zielsetzung war dabei Antibiotika zu identifizieren, die üblicherweise in der Veterinär- und Humanmedizin angewendet werden, Berichte über Gemische und Rückstandsgehalte von Antibiotika in Böden und organischen Abfallsubstraten, die wie z.B. Gülle als Dünger verwendet werden, auszuwerten, und Informationen zu bündeln, welche Effekte der Mischungstoxizität dies für Böden bzw. Boden(mikro)organismen bedeutet, und nicht zuletzt wesentliche Wissenslücken aufzudecken, um auf dieser Basis Schritte für weitere Forschung und Regulation vorzuschlagen.
Antibiotika werden weltweit in großem Umfang und weiter zunehmend in der Human und Tiermedizin angewendet. Es ist bekannt, dass viele landwirtschaftlich genutzte Böden mit Antibiotika kontaminiert sind. Darüber hinaus muss davon ausgegangen werden, dass Böden nicht nur durch eine antibiotische Substanz, sondern durch Gemische von Antibiotikawirkstoffen kontaminiert sind. Dies ist von wesentlicher Bedeutung für die Umweltrelevanz, da die Ökotoxizität von Pharmazeutikagemischen typischerweise größer ist als von Einzelsubstanzen. Belastungen durch Stoffgemische entstehen unter anderem durch die Anwendung von Kombinationen verschiedener Antibiotika bzw. von Gemischen von Antibiotika mit anderen, synergistisch wirkenden Substanzen. Die Verwendung von Antibiotikagemischen nimmt in der Human- wie auch Veterinärmedizin stetig zu, wodurch sogar alte, früher aussortierte Wirkstoffe wieder verwendet werden. Zu den Effekten verschiedener Antibiotikagemische liegt eine breite Wissensbasis in der medizinischen und pharmazeutischen Literatur vor. Dagegen ist der Wissenstand über Antibiotika in der Umwelt deutlich geringer. Belastungen durch Antibiotikagemische in der Umwelt ergeben sich außer (i) durch die Anwendung von Kombinationspräparaten auch durch (ii) die Medikation unterschiedlicher Tierarten und Altersgruppen mit unterschiedlichen Antibiotika und anschließender Sammlung aller Exkremente in einem Gülletank sowie (iii) durch die nachfolgende, wiederholte Applikation kontaminierter Exkremente wie Gülle oder Klärschlamm auf landwirtschaftliche Flächen. So ergeben sich Belastungen von organischen Abfallsubstraten mit Gemischen von bis zu 20 oder mehr antibiotischen Einzelsubstanzen. Böden, die mit diesen Substraten gedüngt werden, weisen Gemische verschiedener, pharmazeutischer Antibiotika auf. Dies bestätigen Monitoringergebnisse mit bis zu 13 verschiedenen Antibiotika, die in Böden nachgewiesen wurden.
Das vorliegende Wissen über Schadwirkungen von Antibiotika auf Bodenorganismen ist weitgehend auf Studien über Einzelsubstanzen beschränkt. Diese zeigen eindeutige, dosis-abhängige, schädliche Wirkungen von Antibiotika z.B. auf die mikrobielle Biomasse in Böden, die strukturelle Diversität mikrobieller Gemeinschaften und mikrobielle Funktionen in Böden. Zudem wurden in belasteten Böden zahlreiche Resistenzgene mit deutlich und signifikant erhöhter Abundanz nachgewiesen. Synergistische Kombinationen von Antibiotika können darüber hinaus zu einer noch verstärkten und schnelleren Resistenzbildung führen. Dies ist nicht nur für Antibiotikagemische, sondern auch für Gemische von Antibiotika mit anderen Substanzen festzustellen; im landwirtschaftlichen Kontext sind hier insbesondere Kupfer und Zink zu nennen. Die Effekte von Antibiotikagemischen können durch unterschiedliche Einflussfaktoren erheblich verändert werden. Dies sind unter anderem der untersuchte Endpunkt, die Konzentrationen der im Gemisch enthaltenen Substanzen, die Zeitabhängigkeit der antibiotischen Wirkung, wie auch Nährstoffe und Nährsubstrate im Boden. Außerdem bestehen Wechselwirkungen zu allen anderen äußeren Randbedingungen, die die Fitness der Mikroorganismen beeinflussen, wie z.B. Hitze oder Frost. Auch der Verbleib und das chemische Verhalten von Antibiotika werden in Böden in Gegenwart von Schadstoffgemischen verändert. Hier ist insbesondere die Sorptionskonkurrenz zwischen unterschiedlichen Antibiotika zu nennen. Die dargelegten Erkenntnisse sollten nicht darüber hinwegtäuschen, dass der Wissensstand über die verschiedenen Teilaspekte der Thematik meist noch sehr unvollständig ist. Daher ist zielgerichtete, systematische Forschung notwendig, um weitere Informationen zu erhalten. Dazu werden drei aufeinander aufbauende Forschungsprojekte vorgeschlagen, die darauf zielen, das Systemverständnis über Schadstoffgemische in Böden zu verbessern. Diese Projekte werden idealerweise mit wissenschaftlichen Workshops kombiniert. Nicht zuletzt sollten diese Forschungsaktivitäten dazu führen bzw. dadurch begleitet werden, dass regulatorische Maßnahmen getroffen werden, die darauf abzielen, den Einsatz und die planvolle Handhabung von Antibiotika zu verbessern.

Contents

List of figures

List of tables

List of abbreviations

Ag	Silver
AMX	Amoxicillin
Apr	Apramycin
Azithro	Azithromycin
BLs	ß-Lactams
BMELV	Federal Ministry of Food, Agriculture and Consumer Protection (Bundesministeriums für Ernährung, Landwirtschaft und Verbraucherschutz)
BVL	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit
CA	Concentration additivity – modelling concept in mixture toxicity
$CaCl_2$	Calciumchloride
Cd	Cadmium
CFU	Colony forming units
cidal	bactericidal
Cipro	Ciprofloxacin
Clary	Clarythromycin
Col	Colistin
CPs	Cephalosporins
CTC	Chlortetracycline
Cu	Copper
Dano	Danofloxacin
DDD	Defined daily dose
DGGE	Denaturating gradient gel electrophoresis
Diflox	Difloxacin
DNA	Desoxyribonucleic acid
DOM	Dissolved organic matter
DOX	Doxycycline
DT_x	Disappearance time of x% of the initial concentration
EC_x / ED_x	Effective concentration (EC) or effective dose (ED) causing an adverse effect of x%
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EFSA	European Food Safety Authority
EMEA	European medicines agency
Enro	Enrofloxacin
ERA	Environmental risk assessment
Ery	Erythromycin
ESAC-Net	European Surveillance of Antimicrobial Consumption Network (provided by European Centre for Disease Prevention and Control)
Fe(III)	Trivalent iron
Fen	Fenbendazole
FKZ	Project no. (German: Forschungskennzahl)
Flero	Fleroxacin
Flu	Flubendazole
FQs	Fluoroquinolones
Hg	Mercury
IA	Independent action
ISO	International Organization for Standardization
Iver	Ivermectin
Josa	Josamycin

K_d	Distribution coefficient representing the ratio between the dissolved and sorbed fraction of a chemical (L/kg)
K_{OC}	Distribution coefficient representing the ratio between the dissolved and sorbed fraction of a chemical, normalized to the organic carbon content of soil (L/kg)
LAVES	Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit
LC_x / LD_x	Effective concentration (EC) or effective dose (ED) causing a lethality of x%
Lin	Lincomycin
LOEC	Lowest observed effect concentration
Lome	Lomefloxacin
Marbo	Marbofloxacin
MEC	Measured environmental concentration
Mg	Megagram (tons)
MIC	Minimum inhibitory concentration
MICres	Minimum inhibitory concentration of resistant microorganisms
MICsusc	Minimum inhibitory concentration of susceptible microorganisms
Mino	Minocyclin
MLs	Macrolides
MRSA	Methicillin-resistant *Staphylococcus aureus*
n.d.	Not detected, concentration below detection limit
n.i.	No information available
Neo	Neomycin
NOEC	No observed effect concentration
Nor	Norfloxacin
OECD	Organization for Economic Co-operation and Development
Oflox	Ofloxacin
Orbi	Orbifloxacin
OTC	Oxytetracycline
PB	Lead
PEC	Predicted environmental concentration
Pen	Penicillin-G
PICT	Pollution induced community tolerance
pK_a	Acid dissociation constant
PLFA	Phospholipid fatty acids
PNEC	Predicted no effect concentration
PPCP	Pharmaceuticals and personal care products
Roxy	Roxythromycin
RQ	Risk quotient
SAA	Sulfanilamide
SAcA	Sulfacetamide
Sara	Sarafloxacin
SAs	Sulfonamides
SCP	Sulfachloropyridazine
SDM	Sulfadimidine
SDMX	Sulfadimethoxine
SDX	Sulfadoxine
SDZ	Sulfadiazine
SIA	Sulfaisoxazole
SIR	Substrate induced respiration
SMM	Sulfamonomethoxine
SMP	Sulfamethoxypyridazine

SMrZ	Sulfamerazine
SMT	Sulfameter
SMX	Sulfamethoxazole
SMZ	Sulfamerazine
Spec	Spectinomycin
Spira	Spiramycin
SPY	Sulfapyridine
SQO	Sulfaquinoxaline
static	bacteriostatic
Strep	Streptomycin
STZ	Sulfathiazole
TC	Tetracycline
TCs	Tetracyclines
TDT	Time dependence of toxic effect
Thiaben	Thiabendazole
TI	Toxicity index
Tia	Tiamulin
Tri	Trimethoprim
Triclo	Triclosan
Tyl	Tylosin
UBA	Umweltbundesamt (Environmental Protection Agency, Germany)
UV	Ultraviolet light
Zn	Zinc

Summary

Background

The terrestrial environment is exposed to the input of contaminants from anthropogenic origin. Due to the use and intended or unintended release of numerous pharmaceutical antibiotics onto soils, it must be expected that in most cases contaminated soils not only contain one antibiotic but mixtures of several compounds.

Research on the occurrence and effects of mixed contaminations considerably increased in aquatic sciences in the past two decades; anyhow, data on mixture toxicity effects on aquatic flora and fauna are still scarce. Even considerably less knowledge exists for soils regarding mixture toxicity of pollutants in general and of combinations of antibiotics in special. The set of bioassays available for the assessment and monitoring of contaminant mixtures is solely based on aquatic toxicity tests. Concerned about the unwanted input into soil and effects of pharmaceutical antibiotics, the board of experts for environment questions from the German Federal Government recommended measures in a position paper (Sachverständigenrat für Umweltfragen, 2007), for example:

- An environmental risk assessment of priority pharmaceuticals, including old products, and grouping of compounds for the integrated determination of possible consequences.
- Continuous documentation of the contamination of surface waters and soils.
- Assessment of the environmental risks of pharmaceuticals, taking into account all compounds that are present at the same site and have similar effects.

The latter aspect clearly addresses mixed contaminations through pharmaceuticals. Based on previous studies it must be expected that environmental contamination with mixtures of pollutants (pharmaceuticals and other chemicals) and not only with single compounds is rather the rule than the exemption. Analytical monitoring surveys routinely confirm that organisms in the environment are exposed to complex multi-component pharmaceutical mixtures. The dose dependent effects of mixtures cannot be explained by regarding single compounds.

The ecotoxicity of a pharmaceutical mixture is typically higher than the effects of each individual component, and, consequently, such mixtures supposedly have a considerable ecotoxicity, even if all individual contaminants are present only in low concentrations. Regulatory limits of chemicals and ecological risk assessment are usually based on the effect of single compounds. Instead, considerable few knowledge exists regarding the toxicity of pollutant mixtures in soils in general and of mixtures of antibiotics in special. Existing knowledge gaps include, in particular, the need for more and better empirical data on the effects of pharmaceutical mixtures on soil organisms, and the exploration of the quantitative consequences of toxicokinetic, toxicodynamic and ecological interactions. Increased focus should be put on investigating the ecotoxicology of pharmaceutical mixtures in environmentally realistic settings. The substantial lack of knowledge leads to strong deficits in assessment and decision-making.

Objective

A literature research was done on the topic of mixed antibiotic contaminations in soils. The aim was to collect existing knowledge as well as to identify gaps. More specifically, the objectives of this literature study were

- to identify antibiotics that are typically applied in veterinary and human medicine, and thus are likely to end up in the soil environment;
- to clarify, which antibiotics are regularly applied in combination with other active substances and which effects of these combinations are described in medical literature;
- to evaluate existing reports on mixtures and contamination levels occurring in soils and in organic waste materials such as manure that are applied to soil as fertilizer;

- to find information, which mixture effects result from this for soils and soil (micro)organisms, their structural diversity and functional ecosystem services as well as the level of antibiotic resistance in soil;
- to identify major knowledge gaps and general research strategies to assess mixture toxicity in soil;
- to propose specific research projects in order to gain better knowledge on mixed contaminations with antibiotics and effects of mixture toxicity in soil and how they can be assessed.

1. Introduction

Antibiotics are largely and worldwide consumed. More than 70 % of the substances used in veterinary medicine are antibiotic compounds, while antibiotics are the third-largest group among all pharmaceuticals used in human medicine. Penicillins are most often used in both veterinary and human medicine, and macrolides, tetracyclines and sulfonamides are also among the most often prescribed groups.

Why do mixtures of antibiotics end up in soil?

1. Combinations of antibiotics are frequently administered to livestock.
2. Different antibiotics are given to different livestock animals and to different live stages of the animals because different pathogens typically occur and have to be treated.
3. Mixtures of antibiotics result, when excreta from different livestock are collected in the same manure tank or lagoon.
4. The so collected mixtures of active agents are subsequently, and often repeatedly spread onto agricultural fields, when they are recycled as fertilizer.
5. 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.

In addition to antibiotic pharmaceuticals, also non-antibiotic pharmaceuticals reach soils. This especially applies to analgesics and anti-inflammatories. Consequently, it was estimated that 10 to 20 different pharmaceutical compounds will end up within one year on a typical agricultural soil in Germany with respective fertilization regime.

A similar scenario for the accumulation of antibiotics exists for antibiotics for human use:

1. Combinations of antibiotics are also often used for medication,
2. Mixtures accumulate over time in wastewater canals and wastewater treatment plants, and
3. Mixtures accumulate in soil with the use of wastewater for irrigation and sewage sludge for fertilization, respectively.

In addition to the above mentioned routes, i.e. using livestock or human excrements as organic fertilizer and wastewater for irrigation, antibiotics can reach the environment along the following pathways:

- Directly by grazing animals and animals held in confined animal feeding operations, respectively.
- Exhaust air from stables may contain contaminated dust and thus contaminate the surrounding area and soils, which is especially relevant, when antibiotics are used as an admixture to dry live-stock fodder (medicated feedstuffs).
- Dermal application as ointment, dipping and pouring agent can be washed off, e.g. by rain, or drip off directly after treatment and may lead to contamination especially of in-field treatment sites.
- Surface waters and soils near shores and banks are directly affected by the use of antibiotics in aquaculture, when these pharmaceuticals are poured into the water either directly or as admixture to fish food.
- Indirect contamination of surface waters results from eroded soil, surface run-off, drainage water, and lateral flow originating from contaminated field and farm sites.

It is expected that the environmental effects of mixtures of antibiotics will differ from that of single compounds, because antibiotics from different structural classes have different mode of action (independent action) leading to synergistically increased or antagonistically decreased effects, while in other cases additive effects (concentration additivity) are assumed.

2. Use of antibiotics in pharmaceutical mixtures for medical application

Antibiotics are highly and increasingly used in human and veterinary medicine. The three countries with largest consumption of antibiotics in 2010 were 1. India, 2. China and 3. the USA. Highest rates in the increase of antibiotic consumption are yet found in developing countries, especially in BRICS countries (Brazil, Russia, India, China, and South Africa) and French West Africa. The consumption of antibiotics for medicinal purposes in industrialized countries such as Germany is stagnating. Yet, it stagnates on a high level, so that in 2014 the total consumption of antibiotics in Germany was the third highest among 28 countries of the EU and European Economic Area (EEA) member states. Furthermore, there is a strong overlap in the classes of antibiotics used in human and veterinary medicine. Penicillins, macrolides, tetracyclines and sulfonamides are most often used in both veterinary and human medicine. This means that environmental contamination with antibiotics resulting from use in human medicine or veterinary medicine is – related to antibiotic classes – not largely different but will add up.

The use of antibiotic combinations and/or of antibiotics mixed with other synergistic compounds is steadily increasing in human as well as veterinary medicine and even old pharmaceuticals, sorted out in the past due to widespread resistance formation, are revived in new combinations. Concerning this, substantial knowledge exists in pharmaceutical and medicinal literature, respectively, on the effects of various binary combinations of antibiotics. Anyhow, the knowledge on the effects of mixtures containing antibiotics is incomplete. Studies on mixtures of three and more compounds are scarce. Even more, most studies deal with one or a few pathogens used as test organisms (e.g. *Escherichia coli* and *Staphylococcus aureus*) but antibiotic effects and even more so effects of antibiotic mixtures can largely vary between species, tested properties and endpoints.

Synergistic effects of binary mixtures are expected when (i) antibiotics with different target site are combined, (ii) combinations of antibiotics have a different mode of action, and (iii) antibiotics are combined with specific other bioactive chemicals. On the other hand, antibiotics with the same target site will have an antagonistic effect. Synergistic effects of pharmaceutical combinations can be due to

a) an altered uptake, whereby the first pharmaceutical increases the permeability of the cell membrane for the second pharmaceutical,
b) direct physical interaction, when pharmaceuticals reciprocally stabilize their binding to the target site, and
c) sequential metabolic steps that are targeted by the mixture.

It is further assumed that pharmaceuticals having the same mode of action cause additive toxic effects. On the other hand, it is reported that interactions between bactericidal and bacteriostatic antibiotics are largely antagonistic; 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). The few experimental findings on soil toxicity, however, show deviating results, e.g. binary mixtures of antibiotics from the same chemical class (sulfonamides) exhibit not only additive but also synergistic effects.

3. Mixed antibiotic contaminations in organic waste materials and soils

In order to identify the status of soil contamination with mixtures of pharmaceutical antibiotics, the literature was researched for publications on contamination levels of organic waste materials used as fertilizer and of soils. The number of studies, delivering detailed information on the mixed contamination of individual samples of (a) waste materials such as manure, used as soil fertilizer, and (b) of soils, is small, though. Irrespective of that, the number of published datasets evaluated in this study consistently shows that not only contaminations with single antibiotics but mixed contaminations occur. However, irrespective of that coherent state

of knowledge, the number of studies that can be used for further evaluation is still scarce. This is because one or several of the following drawbacks apply:

- Data are not fully published but aggregated data (e.g. average values over series of samples) are published (in some cases even with incomplete information which and how many samples have been averaged).
- Data are not available in tables but are only shown in figures.
- Data are restricted to few, targeted chemicals.
- Dissimilar and only partly matching (sets of) antibiotics, respectively, are researched in different studies.
- Identification of investigated soils is insufficient or lacking.
- In few cases data on residual contamination levels of both waste material and of soil samples have been published. However, in these publications it is seldom documented whether the analyzed soil was fertilized with the analyzed waste material. Consequently, it is hardly possible to exactly follow the contamination route and resulting contamination of soils based on information from the literature.

Unlike the controversial findings on antibiotic mixture toxicity in soil, existing studies on the supposed input scenario (outlined in section 1) are consistently confirmed by the few existing studies on the topic. Mixed contaminations with antibiotics in the environment result from the administration of combinations of antibiotics. On example of a specific study it was shown that almost 50% of all prescriptions to livestock comprise mixtures of mostly two but up to five antibiotics and other pharmaceuticals. Reports further confirmed the assumed input pathways. Antibiotic mixtures are formed when different antibiotics are given to different livestock animals and to different live stages of the animals and when excreta from that different livestock are subsequently collected in the same manure tank and/or are consecutively spread onto agricultural fields. Furthermore, mixed contaminations result from (bio)degradation, in case parent compounds together with antibiotic active metabolites are present in soil. Not last, repeated application of manure contaminated with antibiotics leads to a mixed contamination and a regular replenishment of the antibiotics' contents. Combined with in part slow degradation kinetics of antibiotics in soil, this results in an apparently persistent contamination level.

Contaminations with on average clearly more than two and up to seven different antibiotics are reported for manure, digestate and compost. Substantially more antibiotics (up to >20) were found in sewage sludge samples. The contamination levels with antibiotics in organic waste material are in the range of mg/kg, in soil they are in the range of µg/kg. Yet, it must be assumed that true soil contents of antibiotics in field soils are substantially higher than reported, which is due to the strong and fast formation of non-extractable residues. For example, extractable portions of different antibiotics decline within 1 h or less of soil contact to ≤90% (sulfonamides), ≤80% (fenbendazole) and ≤70% (tetracyclines) and the recovery further declines with prolonged contact time with soil. It is assumed that residual contents of sulfonamides might be higher by a factor of 2.5 than the extractable contents. This factor is supposedly even higher for much stronger adsorbing antibiotics such as tetracyclines, fluoroquinolones and ß-lactams.

Corresponding to the contamination level in organic waste materials, existing reports show that not only single antibiotic compounds but mixtures of several compounds frequently occur in soil. The combined concentrations clearly exceed natural background levels. Two and up to 13 different pharmaceutical antibiotics were discovered in individual samples from agricultural topsoils. For pairs of samples, i.e. organic waste material and the receiving soil, a significant correlation between the frequency of detection of antibiotics in waste material and in the receiving soils was found.

Antibiotics in soil are not only due to anthropogenic contamination but also occur naturally, being formed by the secondary metabolism of autochthonous soil microorganisms. For example, streptomycin and oxytetracycline are well-known soil-borne antibiotics and are produced by *Streptomyces* actinobacteria. Among numerous other soil microorganisms, 30 to 50 % of actinomycetes isolated from soil are able to synthesize

antibiotics. Typical resulting soil contents are reported to be in the range of µg/kg and especially occur in the soil rhizosphere. However, studies show that residual contents of single antibiotic pharmaceuticals may exceed the natural background level and in part even the trigger value of the European Medicines Agency of 100 µg/kg. Furthermore, not only single antibiotic compounds but mixtures of several compounds frequently occur in soil, so that the combined concentrations clearly exceed natural background levels. The determined antibiotics belong to different structural classes and exhibit different modes of action. Thereby, antibiotics from some structural classes are more abundant (e.g. tetracyclines) while others have been determined in much lower concentrations (e.g. sulfonamides). This is due to (i) a different usage and thus release into the environment, and (ii) a different persistence and mobility in soil.

4. Toxicity of single antibiotic compounds and of contaminant mixtures with antibiotics on soil organisms

Existing knowledge on adverse effects of antibiotics on soil microorganisms is largely restricted to studies with individual compounds but not with mixtures. Despite the largely increasing number of publications on the topic, the knowledge on adverse effects even of individual antibiotics on soil organisms is still fragmented, mostly related to different soil microbial properties and a few faunal indicator species, respectively. On top of that, the vast majority of studies has been conducted on tetracylines and sulfonamides, while the knowledge on other antibiotic classes is reduced to a few single studies or is completely missing (e.g. cephalosporins). Existing knowledge suggests that soil microbial properties and functions are affected, such as microbial biomass, structural diversity of microbial communities (biodiversity) and functional diversity. Especially the microbial N-cycling is impacted by antibiotics. Also tolerance related parameters are affected and significant alterations in the microbial community structural composition have been determined as another effect of antibiotics. This especially applies to decreases in the abundance of microbial groups related to the two functions potential nitrification and denitrification, again underlining the obvious specific susceptibility of organisms and functions related to the N cycle.

Effects of antibiotics and antibiotic mixtures vary, depending on influencing factors and boundary conditions. For example, they may be largely modulated by the concentrations present. Antagonistic effects occurred more often at low concentrations of the mixed chemicals while at higher concentrations even synergistic effects occured, depending on the combined chemicals. On the other hand, in several cases low-dose activations by antibiotics or mixtures thereof occur that must not be misinterpreted as a positive effect on (soil) organisms. In general, effects in the range from being only just significant to about 10 % inhibition can be expected from typical contents of the single compounds in the environment. Yet, effects of mixed contaminations in the soil environment may clearly exceed that level. Regarding the indispensable relevance of microorganisms for soil ecosystem services and functioning this may have significant effects on soil fertility. Additionally, effects and altered effects of antibiotic mixtures on soil faunal species and crop plants are reported in the literature. When the earthworm *Eisenia fetida* was exposed to the individual antibiotics tetracycline and chlortetracycline, a dose-dependent, significant DNA damage in earthworm coelomocytes was observed. The combination of both antibiotics, however, resulted in slight antagonism at the maximum dose of 300 mg/L.

A specific effect of antibiotics in the soil environment with particular relevance for human and animal health is the increased formation of antibiotic resistance. The selection of resistant bacteria occurs especially at low antibiotic concentrations, typically below the minimal inhibitory concentration (MIC). Such low concentrations are regularly found in contaminated soils. Consequently numerous resistance genes are found in contaminated soils at a significantly and substantially increased abundance level. Synergistic antibiotic combinations can even lead to stronger and also faster resistance development. On the other hand, antagonistic pharmaceutical combinations lead to slower resistance evolution than synergistic ones. However, the derivation of predicted no effect concentrations (PNEC) triggering increased abundance of microbial resistance genes in the soil environment is actually not feasible. Not last, stronger effects of antibiotic mixtures on the induction of an increased antibiotic resistance level in soil must be expected.

Not only mixtures of antibiotics but also of antibiotics with other chemicals can exert different mixture toxicity. Contamination of agricultural soil with mixtures of antibiotics and other agrochemicals such as pesticides, other non-antibiotic pharmaceuticals as well as heavy metals often occurs. Especially relevant appear to be mixtures with heavy metals that enhance the effects of antibiotics on soil microorganisms. Copper (Cu) and zinc (Zn) are often applied to livestock as feed additive and can also enter agricultural soils as contaminants in biosolids used as fertilizer and in untreated waste water used for irrigation. Also metals such as Cu, Zn, Cd and Hg exert antimicrobial effects and contribute to the increased formation of antibiotic resistance, making agricultural soils hot-spots of the formation of antibiotic resistance in the environment.

5. Properties and conditions influencing the effects of antibiotics and mixtures

Effects of antibiotic mixtures may be largely modulated by various influencing factors. First of all they depend on the investigated subject of analysis and endpoint. Furthermore, effects of mixtures frequently change with the concentrations of the mixed compounds and combinatory effects may shift from additive or even antagonistic to synergistic effects at higher concentrations. All toxic effects are time-dependent and adverse effects especially of bacteriostatic antibiotics increase over timescales of days and weeks. Additionally, synergistic combinations will have a prolonged effect duration while effects of antagonistic combinations will have a reduced duration.

Also antibiotics and nutrients in soil may interact, which interaction can be understood as an antagonistic mixed effect. This includes effects of organic waste materials with which pharmaceuticals are typically introduced to soil. The nutrient rich, degradable substrates tend to increase adverse effects of biostatic acting antibiotics. For example, antibiotic effects disproportionally increased with incremental liquid manure addition; impacts of manure even varied depending on whether fresh or stored manure was used.

Furthermore, interactions exist with all other boundary conditions that affect the fitness of biota such as heat or frost, drought or excess of water combined with oxygen depletion, starvation or nutrient substrate supply, and pathogens or predators. Such environmental stress mostly leads to a synergistically increased adverse effect of chemicals. However, the effect of non-contaminant impacts such as nutrient status and environmental stressors on the mixture toxicity of combined contaminations has hardly been tested and no such publications related to mixed antibiotics have been found with this literature research. It is assumed that the total effect of a contaminant mixture increases or decreases, yet, no change in the type of the interacting effect (e.g. synergism, antagonism) of mixed chemicals is expected, unless the physicochemical properties and bioavailability (effective concentration) of one chemical changes substantially, e.g. altered solubility through a temperature increase.

6. Effects of mixtures of antibiotics and other pollutants on the fate in soil

Also the fate of antibiotics may be altered in the presence of mixed contaminations. Sorption and degradation can be significantly different, which will feed back on their effects. A strongly reduced retardation of antibiotics in soil can be observed, when sorption competition increases with increasing concentrations. Sorption competition will especially occur with mixtures of antibiotics from the same compound class, competing for the same sorption sites in soil. For compounds from different antibiotic classes, however, competitive sorption is largely restricted to the high concentration range. In addition, also sorption competition between antibiotics and non-antibiotic substances from different compound classes occurs. This also encompasses natural organic matter as it is added to soil with manure and similar waste materials. Not last, the dissipation and degradation, respectively, of antibiotics in soil can be altered in the presence of mixed contaminations.

7. Conclusions on consequences for soil functioning and for further research

Knowledge gaps

Especially in the past 20 years, the knowledge on the input, fate and effects of antibiotics in soil, excreta and organic fertilizers largely expanded. However, the work is still very much focused on specific structural classes of antibiotics, i.e. tetracyclines and sulfonamides, while the level of knowledge is still fragmentary for classes such as benzimidazoles, lincosamides and cephalosporins. For example, penicillins are most used in human and veterinary medicine, 3rd generation cephalosporins are of particular significance as reserve antibiotics for human medicine, yet research on their input, fate and effects in soil are largely missing. So again, it must be stated that still many knowledge gaps exist, which are summarized as follows.

- Reports on the occurrence of antibiotics in environmental substrates such as manure, sewage sludge and soils are largely limited to compounds from the structural classes of tetracyclines and sulfonamides and a minor number of reports on fluoroquinolones, macrolides and lincosamides.
- The knowledge on the environmental inputs, fate and effects of antibiotics of other structural classes is fragmented and incomplete or fully missing.
- Even less information is available on the occurrence, composition and concentrations of mixed antibiotic contaminations as well as their fate in soil.
- Soils are heterogeneous with different soil horizons as macrostructure and aggregates, rhizosphere etc. as microstructure. The distribution of antibiotics and antibiotic mixtures within soils is largely unknown.
- There are too few data on toxic effects of pharmaceuticals in soil and even less in particular on effects of mixtures.
- The underlying processes of mixture effects such as antagonism and synergism are only rudimentary understood. It should be aimed to overcome empirical description but identify (and further model) principles. This especially applies to the lacking understanding of the ecological roles of antibiotics in nature and possible adverse effects of environmental pollution arising from that. A long-term goal will be to develop a systematic approach for unraveling the underlying causes of any given pharmaceutical interaction.
- The existing knowledge on toxic effects of pharmaceuticals in soil (not to speak of mixtures) is based on a rather broad, non-systematic number of different test methods and endpoints that hinders the integration of the existing knowledge. It must be expected that effects and effect concentrations largely vary between different tested subjects and endpoints.
- In this regard it is further necessary to investigate the factors that influence mixture toxicity such as number of contaminants, their (relative) concentrations, and environmental conditions such as soil moisture and temperature, chemical and physical soil properties, status and composition of soil (microbial) community.
- A specific aspect is the time dependence of antibiotic effects; especially from mixtures, long-term exposure and chronic effects are expected.
- Hormesis, which is a seemingly positive increase of the investigated parameter at low doses of the tested chemical, can be part of a toxicity response. It occurs in the presence of various pharmaceuticals and their mixtures. It remains unclear how to valuate a hormetic increase and even more so in the presence of mixtures with single compounds having different effects in this regard.
- Lastly, viable concepts for cumulative exposure assessment strategies need to be developed.
- All this is flanked by the need for uniform and at best standardized methods to determine total contents and bioavailable fractions of antibiotics in soil and to reliably determine antibiotic effects on soil organisms. It is assumed that a bioavailable fraction will best represent effect concentrations.

Additionally, many of the findings reported in the previous sections are based on one or a few studies, dealing with a limited number of compounds or even other chemicals than antibiotics, and many are not dealing with soil but with other environmental compartments and medicine, respectively. Hence, more information from targeted, systematic research is needed, (i) to quantify and assess the hazard and risk that a given pharmaceutical mixture poses for the environment; (ii) to predict which pharmaceutical mixtures, in terms of composition and concentration, can be tolerated at a given site or in a given environmental compartment; (iii) to identify which compounds are the ecotoxicological drivers at a given site. Three successive research projects are proposed to further elucidate the complex situation and effects of mixed antibiotic contaminations.

Proposal of successive research projects their use in regulation

1. Contamination status and effects of mixtures of pharmaceutical antibiotics in the terrestrial environment – a meta-analysis

A meta-analysis of existing quantitative data on the dissemination of antibiotics as well as their occurrence in waste materials and soils shall be carried out. From this, predicted and measured environmental concentrations (PEC, MEC) of antibiotics should be derived and related to effect concentrations (EC) or no-effect concentrations in order to identify risks from mixture toxicity of antibiotics in soils and b) to refine and improve the state-of-knowledge about soil contamination with antibiotic mixtures and their ecotoxicological significance and consequences. First recommendations for action could be given to stakeholders and regulatory authorities based on this approach and further experimental work could be designed.

2. A general suite of methods for testing adverse effects of mixtures of pharmaceutical antibiotics in soil

In a second, subsequent (mid-term) project, experimental work based on the previous findings should be carried out. The proposed aim should be i) to identify suitable methods to indicate adverse effects of pharmaceutical antibiotics on soil microbial abundance and functioning. Testing of existing standardized methods and eventually of further methods aimed to determine relevant functions of soil microorganisms could be done in a dose-response approach with a suite of soils and a set of selected, representative antibiotics from the structural classes most often used in veterinary and human medicine, i.e. penicillins, tetracyclines, polypeptide antibiotics, sulfonamides, macrolides, aminoglycosides, lincosamides, pleuromutilines, fluoroquinolones, folic acid antagonists, fenicols, cephalosporines. ii) A test battery should be arranged with a subset of the identified, suitable methods. This set should at best comprise methods and endpoints representing parameters of microbial abundance and indicators of relevant functions.

3. Mixture toxicity of mixed contaminations with pharmaceutical antibiotics and other pollutants in agricultural soils

A third study (executed on a longer-term) should be aimed to investigate effects of mixtures of antibiotics by using the test battery. Typical mixtures should be tested, starting from binary mixtures and proceed with ternary and more complex mixtures in order to identify and categorize mixture toxicity of antibiotics from similar as well as different structural classes and modes of action, respectively. The overall aim would be to identify underlying principles of antibiotic action of mixtures in soil to enable further modelling and prognosis, which knowledge would better enable to define regulatory standards and thresholds.

This research would be ideally combined with scientific workshops in order to define the latest state-of-the-art on the topic. All this should lead to or even be flanked by regulatory measures for an improved use and management of antibiotics for medicinal purposes that include environmental issues.

Conclusions

The proposed research activities are aimed to scientifically support regulation. In general, the overall aim should be to reduce and minimize the dissemination of pharmaceutical antibiotics and related heavy metals (Cu, Zn) in the environment in order to prevent adverse effects on soil function and fertility and to impede the increase and spread of antibiotic resistance in the environment with its increasing health risks for animals (livestock) and humans. Regulatory measures could cover the analytical determination of antibiotic contamination of waste materials such as manure used as soil fertilizer and/or the determination of the resulting contamination level in soils. This could be especially relevant for manure exports and imports between farms and countries. Acceptable contamination levels (regulatory limit values) could be oriented to no-effect concentrations determined in soil toxicity studies and concentration levels inducing increased antibiotic resistance. Knowledge about and the consideration of environmental issues would enable to better plan and organize the use of pharmaceutical antibiotics on the farm level. With no doubt, the priority should still be the curing of infectious diseases of humans and animals. This, however, requires much more than before the consideration of environmental issues, especially to avoid the increasing ineffectiveness of antibiotics due to the formation of antibiotic resistance in the environment. There is strong conviction that this will also contribute to an improved sustainable soil use and protection of water resources.

Zusammenfassung

Hintergrund

Die terrestrische Umwelt ist dem Eintrag von Schadstoffen anthropogenen Ursprungs ausgesetzt. Aufgrund der Verwendung und dem gewollten oder ungewollten Eintrag in Böden von zahlreichen pharmazeutischen Antibiotika, muss davon ausgegangen werden, dass kontaminierte Böden in den meisten Fällen nicht nur ein Antibiotikum, sondern Mischungen mehrerer Substanzen enthalten.

Untersuchungen zum Auftreten und den Effekten gemischter Kontaminationen haben in den letzten zwei Jahrzehnten in der Gewässerforschung erheblich zugenommen. Dennoch liegen nur wenige Ergebnisse zu Effekten durch Mischungstoxizität auf die aquatische Flora und Fauna vor. Noch deutlich geringer ist der Wissensstand über die Mischungstoxizität von Schadstoffen in Böden sowohl im Generellen wie auch insbesondere über Gemische von Antibiotika im Speziellen. Die existierende Auswahl an Biotests zur Bewertung und Überwachung von Schadstoffgemischen basiert allein auf aquatischen Toxizitätstest. In Besorgnis um die unerwünschten Einträge von antibiotischen Pharmazeutika in Böden hat der Sachverständigenrat für Umweltfragen der Bundesregierung in einem Positionspapier im Jahr 2007 Maßnahmen vorgeschlagen wie zum Beispiel:

- Eine Umweltbewertung prioritärer Pharmazeutika, einschließlich Altwirkstoffen, und eine Gruppierung von Substanzen für die integrierte Ermittlung möglicher Konsequenzen.
- Eine fortlaufende Dokumentation der Kontamination von Oberflächengewässern und Böden.
- Die Bewertung der Umweltrisiken durch Pharmazeutika unter Berücksichtigung aller Substanzen, die am selben Ort vorliegen und ähnliche Wirkungen haben.

Gerade der letzte Punkt adressiert eindeutig gemischte Kontaminationen durch Pharmazeutika. Auf der Basis vorliegender Studien muss davon ausgegangen werden, dass Umweltkontaminationen mit Gemischen von Schadstoffen (Pharmazeutika und andere Chemikalien) eher die Regel sind, während Kontaminationen durch nur einen Stoff die Ausnahme darstellen. Analytische Monitoringstudien bestätigen immer wieder, dass die Umwelt komplexen Multi-Komponentengemischen von Pharmazeutika ausgesetzt ist. Dabei können die dosis-abhängigen Wirkungen von Gemischen nicht durch die Betrachtung der Einzelwirkstoffe und ihrer Effekte erklärt werden.

Die Ökotoxizität eines Pharmazeutikagemisches ist typischerweise größer als die Wirkung der Einzelsubstanzen. Folglich haben solche Gemische eine erhebliche ökotoxikologische Relevanz, selbst wenn die Einzelstoffe in nur geringer Konzentration vorliegen. Regulatorische Grenz- oder Maßnahmewerte für Chemikalien und die Umweltrisikobewertung basieren üblicherweise auf der Wirkung von einzelnen Stoffen. Dagegen gibt es deutlich weniger Wissen über die Toxizität von Schadstoff- bzw. Wirkstoffgemischen in Böden und insbesondere über Gemische von Antibiotika. Die vorliegenden Wissenslücken umfassen insbesondere den Bedarf nach mehr und besseren empirischen Daten über die Wirkungen von Pharmazeutikagemischen auf Bodenorganismen und die quantitative Bestimmung der Folgen toxikokinetischer, toxikodynamischer und ökologischer Wechselwirkungen. Ein verstärkter Fokus sollte auf die Untersuchung der Ökotoxikologie von Pharmazeutikagemischen in realistischen Umweltsituationen gerichtet werden. Die vorliegenden, erheblichen Wissenslücken bedeuten wesentliche Defiziten bei der Bewertung und Ableitung von Maßnahmen.

Zielsetzung

Mit dieser Studie wurde eine Literaturrecherche über Antibiotikagemische und deren Toxizität in Böden durchgeführt. Ziel war es, vorhandenes Wissen zu sammeln und Wissenslücken zu identifizieren. Dabei wurden folgende Fragestellungen näher betrachtet:

- Identifikation von Antibiotika , die typischerweise in der Human- und Veterinärmedizin angewendet werden und damit vermutlich auch in die Umwelt und Böden gelangen;
- Klärung, welche Antibiotika regelmäßig in Kombination mit anderen Wirkstoffen verabreicht werden und welche Effekte dieser Kombination in der medizinischen Literatur beschrieben werden;
- Berichte über das Vorkommen von Antibiotikagemischen in Böden und organischen Abfallstoffen wie Gülle, die als Bodendünger verwendet werden, auszuwerten, und Informationen über Rückstandsgehalte zu entnehmen;
- Informationen zu finden, welche Effekte von Gemischen daraus für Böden und Boden(mikro)organismen resultieren, insbesondere deren strukturelle Diversität und funktionelle Ökosystemdienstleistungen sowie das Ausmaß von Antibiotikaresistenz in Böden;
- wesentliche Wissenslücken und generelle Forschungsstrategien zur Bewertung von Mischungstoxizität in Böden zu identifizieren sowie
- spezifische Forschungsprojekte vorzuschlagen, um gezielt einen besseren Wissenstand über Belastungen durch Antibiotikagemische und Folgen der Mischungstoxizität in Böden und deren Bewertung zu erlangen.

1. Einleitung

Antibiotika werden weltweit und in großem Umfang verbraucht. Mehr als 70 % der in der Veterinärmedizin verwendeten Substanzen sind Antibiotika; in der Humanmedizin stellen sie die drittgrößte Gruppe der verwendeten Pharmazeutika. Dabei werden in der Human- wie auch Veterinärmedizin am häufigsten Antibiotika aus der Gruppe der Penicilline verabreicht, gefolgt von Makroliden, Tetrazyklinen und Sulfonamiden.

Wieso gelangen überhaupt Mischungen von Antibiotika in Böden?

1. Häufig werden Kombinationen verschiedener Antibiotika an Nutztiere verabreicht.
2. Unterschiedliche Nutztierarten und Tieraltersstufen werden mit unterschiedlichen Antibiotika behandelt, weil unterschiedliche Krankheitserreger auftreten und behandelt werden müssen.
3. Daraus entstehen Gemische von Antibiotika, wenn Exkremente unterschiedlicher Nutztierarten bzw. –gruppen in demselben Güllekeller, Gülletank oder derselben Güllelagune gesammelt werden.
4. Die auf diese Weise gesammelten Mischungen von Wirkstoffen werden als Wirtschaftsdünger,häufig zudem wiederholt auf landwirtschaftliche Böden ausgebracht.
5. Nachfolgend können durch biologischen Abbau im Boden Mischungen von antibiotischen Substanzen und ihrer Metabolite, von denen einige eine (veränderte) antibiotische Aktivität aufweisen, gebildet werden.

Zusätzlich zu antibiotischen Pharmazeutika gelangen auch nicht-antibiotische Pharmazeutika in Böden. Dies trifft insbesondere für Schmerzmittel (Analgetika) und Entzündungshemmer (Antiphlogistika) zu. Demzufolge wurde geschätzt, dass bei einem entsprechenden Düngungsregime innerhalb eines Jahres 10 bis 20 unterschiedliche pharmazeutische Substanzen auf typische landwirtschaftliche Böden in Deutschland gelangen.

Ein ähnliches Szenario für die Einträge und Anreicherung von Antibiotika gilt für Antibiotika, die in der Humanmedizin angewendet werden:

1. Häufig werden Kombinationen von Antibiotika bei der Medikation eingesetzt,
2. Gemische akkumulieren über die Zeit in Abwassersystemen und Kläranlagen, und

3. akkumulieren in Böden infolge der Verwendung von Abwasser für die Bewässerung bzw. von Klärschlamm für die Düngung.Zusätzlich zu den oben genannten Eintragspfaden, d.h. die Nutzung von menschlichen oder Nutztier-Exkrementen als Dünger und von Abwasser zur Bewässerung, können Antibiotika über die folgenden Pfade in die Umwelt gelangen:

- Direkter Eintrag erfolgt durch Weidevieh und Tiere, die im Freiland gehalten werden (sog. „confined animal feeding operations“).
- Stallabluft kann belasteten Staub enthalten und dadurch die Umgebung und Böden kontaminieren, was insbesondere relevant ist, wenn Antibiotika als Zusatz zu trockenem Tierfutter verabreicht werden (Fütterungsarzneimittel).
- Dermale Anwendung der Antibiotika als Salbe, Tauchbad oder Gießmittel, die z.B. durch Regen abgewaschen werden können oder zum Teil unmittelbar abtropfen und dadurch zu einer Kontamination, insbesondere der Behandlungsbereiche im Gelände führen.
- Oberflächengewässer und ufernahe Böden werden direkt durch den Einsatz von Antibiotika in der Aquakultur beeinflusst, wenn Antibiotika allein oder mit dem Fischfutter unmittelbar in das Gewässer gegeben werden.
- Indirekte Kontaminationen von Oberflächengewässern resultieren aus dem erosiven Abtrag von Böden sowie Oberflächenabfluss, Drainagewasser und lateralem Fluss aus kontaminierten landwirtschaftlichen Flächen in die Gewässer hinein.

Es ist zu erwarten, dass die Umwelteffekte von Antibiotikagemischen von denen der Einzelwirkstoffe abweichen. Dies liegt daran, dass Antibiotika unterschiedlicher Strukturklassen unterschiedliche Wirkmechanismen haben (unabhängige Wirkung), was zu synergistisch verstärkten oder antagonistisch verminderten Effekten führt, während für Wirkstoffe derselben Strukturklasse additive Effekte (Konzentrationsadditivität) zu vermuten sind.

2. Verbrauch von Antibiotika und ihre medizinische Verwendung als Antibiotikagemische

Antibiotika werden stark und weiter zunehmend sowohl in der Human- als auch in der Veterinärmedizin angewendet. Die drei Länder mit dem höchsten Antibiotikaverbrauch in 2010 waren 1. Indien, 2. China und 3. die USA. Die höchsten Zuwachsraten beim Verbrauch von Antibiotika sind hingegen in Schwellen- und Entwicklungsländern zu finden, insbesondere in den BRICS-Staaten (Brasilien, Russland, Indien, China und Südafrika) sowie Französisch-Westafrika. Der Verbrauch an Antibiotika für medizinische Zwecke stagniert in Industrieländern wie Deutschland. Dies allerdings auf einem hohen Niveau; so war der Gesamtverbrauch an Antibiotika in Deutschland in 2014 der dritthöchste unter den 28 Mitgliedsstaaten der EU und der Europäischen Wirtschaftszone. Zudem ist anzumerken, dass es eine große Schnittmenge der in der Human- und Veterinärmedizin am stärksten angewendeten Antibiotikaklassen gibt. Penicilline, Makrolide, Tetrazykline und Sulfonamide werden am häufigsten in der Human- und Veterinärmedizin verabreicht. Das bedeutet, dass Umweltkontaminationen durch Antibiotika, die aus der Anwendung in der Human- bzw. Veterinärmedizin resultieren – bezogen auf Antibiotikaklassen – nicht wesentlich unterschiedlich sind, sondern sich aufaddieren.

Die Verwendung von Antibiotikagemischen und /oder Gemischen von Antibiotika mit anderen synergistischen Stoffen nimmt in der Human- wie auch Veterinärmedizin stetig zu und sogar wegen zu starker Resistenzentwicklung früher aussortierte Substanzen werden in solchen Gemischen wieder eingesetzt. Diesbezüglich besteht ein umfängliches Wissen in der pharmazeutischen und medizinischen Literatur über die Wirkungen von verschiedenen, binären Antibiotikakombinationen. Demgegenüber ist das Wissen über die Wirkungen von komplexeren Gemischen unvollständig. Es liegen nur wenige Studien über Gemische mit drei oder mehr Antibiotika vor. Zudem befassen sich die meisten Studien nur mit einzelnen oder wenigen Pathogenen als Testorganismen (z.B. *Escherichia coli* und *Staphylococcus aureus*), wohingegen antibiotische Wirkungen und noch mehr die Effekte von Antibiotikagemischen erheblich zwischen Testspezies, getesteten Eigenschaften und Endpunkten variieren können.

Synergistische Wirkungen binärer Mischungen werden erwartet, wenn (i) Antibiotika mit unterschiedlichem Wirkort kombiniert werden, (ii) die kombinierten Antibiotika einen unterschiedlichen Wirkmechanismus haben, und (iii) wenn Antibiotika mit spezifischen anderen bioaktiven Chemikalien kombiniert werden. Andererseits ist bei Antibiotika mit dem gleichen Wirkort eine antagonistische Wirkung zu erwarten. Synergistische Effekte von Pharmazeutika-Gemischen können darauf beruhen, dass

a) die Aufnahme zum Wirkort verändert wird, wobei das eine Pharmazeutikum die Permeabilität der Zellmembran für die zweite Substanz erhöht,
b) direkte, physikalische Wechselwirkungen bestehen, indem Arzneistoffe gegenseitig die Bindung am Wirkort stabilisieren, und
c) aufeinanderfolgende, metabolische Schritte durch das Gemisch beeinflusst werden.

Dagegen üben Gemische von Antibiotika mit demselben Wirkmechanismus additive toxische Wirkungen aus. Berichte weisen darauf hin, dass Wechselwirkungen zwischen bakterizid und bakteriostatisch wirkenden Antibiotika hingegen zumeist antagonistisch sind. Kombinationen bakteriostatischer Antibiotika (innerhalb einer oder zwischen verschiedenen Verbindungsklassen) üben additive oder synergistische Wirkungen aus, wohingegen Gemische von bakterizid wirkenden Antibiotika additive (innerhalb einer Verbindungsklasse) oder antagonistische (zwischen verschiedenen Verbindungsklassen) Effekte aufweisen. Die wenigen experimentellen Befunde über toxische Effekte von Gemischen in Böden zeigen jedoch zum Teil von diesen Regeln abweichende Ergebnisse, z.B. weisen binäre Mischungen von Antibiotika der gleichen Verbindungsklasse (Sulfonamide) nicht nur additive, sondern auch synergistische Wirkungen auf.

3. Kontamination von organischen Abfällen und Böden mit Antibiotikagemischen

Um den Status der Kontamination von Böden durch Gemische pharmazeutischer Antibiotika zu identifizieren, wurde eine Literaturrecherche bezüglich Publikationen über Belastungen von Wirtschaftsdüngern (organische Abfallsubstrate) und Böden durchgeführt. Die Anzahl an Studien ist jedoch gering, die detaillierte Informationen über gemischte Kontaminationen (a) von organischen Abfallsubstraten wie Gülle, die als Bodendünger verwendet werden, und (b) von Böden liefern. Unabhängig davon zeigen die in dieser Literaturrecherche ausgewerteten Publikationen bzw. die darin enthaltenen Datensätze einvernehmlich, dass Kontaminationen durch Antibiotikagemische und nicht allein durch Einzelsubstanzen auftreten und auch in anderen Fällen üblicherweise erwartet werden müssen. Trotz dieses konsistenten Erkenntnisstandes ist dennoch zu betonen, dass die Zahl der Studien, die ausgewertet werden können, gering ist. Dies liegt daran, dass bei anderen Studien einer oder mehrere der im Folgenden aufgeführten Nachteile vorliegen:

- Daten wurden nicht vollständig publiziert, sondern aggregierte Daten (z.B. Mittelwerte über Probensätze) werden präsentiert (in einigen Fällen auch ohne oder mit nur unzureichender Information wie viele bzw. welche Daten gemittelt wurden).
- Quantitative Daten sind nicht in Tabellen verfügbar, sondern werden nur in Form von Abbildungen präsentiert.
- Daten sind beschränkt auf nur wenige, ausgewählte Chemikalien.
- Uneinheitliche und nur zum Teil übereinstimmende Auswahlen an antibiotischen Stoffen wurden in den Studien untersucht.
- Die Kennzeichnung und/oder Zuordnung der untersuchten Böden ist unvollständig oder fehlend.
- Nur in wenigen Fällen werden Daten von Rückstandsgehalten in beiden, Wirtschaftsdünger und Böden präsentiert. Liegen solche Publikationen vor, wird wiederum selten dokumentiert ob bzw. welche Böden mit den entsprechenden Wirtschaftsdüngern beaufschlagt wurden. Folglich ist es auf Basis von Informationen aus der Literatur kaum möglich, Kontaminationspfade eindeutig zu verfolgen und resultierende Bodenbelastungen nachzuvollziehen.

Im Gegensatz zu den widersprüchlichen Funden zur Toxizität von Antibiotikagemischen, zeigen die wenigen vorliegenden Studien zum vermuteten Eintragsszenario in Böden (kurz dargestellt in Abschnitt 1) ein konsistentes Bild. Die in der Umwelt auftretenden Kontaminationen durch Antibiotikagemische resultieren unter anderem aus der medizinischen Anwendung von Kombinationen von Antibiotika. Am Beispiel einer spezifischen Studie konnte gezeigt werden, dass nahezu 50% aller Antibiotika-Verschreibungen an Nutztiere Kombinationen aus zumeist zwei aber auch bis zu fünf unterschiedlichen Antibiotika wie auch weiterer Pharmazeutika enthielten. Zudem bestätigen vorliegende Publikationen die vermuteten Eintragspfade. Mischungen von Antibiotika entstehen dadurch, dass unterschiedliche Antibiotika an unterschiedliche Tierarten und Tieraltersstufen verabreicht werden. Werden die Exkremente dieser unterschiedlichen Tiergruppen an einem Ort gesammelt und anschließende auf Böden ausgebracht, ergibt sich die Kontamination durch Antibiotikagemische. Dies ergibt sich auch durch (biologische) Abbauprozesse, wobei zumindest teilweise antibiotisch aktive Metabolite entstehen können. Nicht zuletzt führt eine wiederholte Gülleapplikation auf landwirtschaftliche Flächen zur Entstehung von Gemischen und Auffüllung des Kontaminationsniveaus, so dass in Kombination mit der teilweise stark verlangsamten Abbaukinetik von Antibiotika in Böden eine quasi konstante Belastungshöhe erreicht wird.

Die Berichte zeigen Belastungen durch Gemische mit im Durchschnitt mehr als zwei und bis zu sieben verschiedenen Antibiotika in Gülle, Biogasschlamm und Kompost auf. Noch deutlich mehr verschiedene Antibiotika (bis zu >20) wurden in Klärschlammproben gefunden. Die Rückstandsgehalte von Antibiotika in diesen organischen Abfallsubstraten liegen in der Größenordnung von mg/kg; in Böden liegen diese hingegen im Bereich von µg/kg. Allerdings ist zu vermuten, dass die wahren Rückstandsgehalte in Böden deutlich höher liegen. Dies kommt dadurch, dass viele Antibiotika rasch und in erheblichem Ausmaß nicht-extrahierbare Rückstände in Böden bilden. Zum Beispiel nehmen die extrahierbaren Anteile verschiedener Antibiotika bereits durch kurzzeitigen Bodenkontakt von 1 Stunde und weniger auf ≤90% (Sulfonamide), ≤80% (Fenbendazol) und ≤70% (Tetrazykline) ab und die Wiederfindung nimmt mit weiterer Kontaktzeit weiter ab. Es wird vermutet, dass gealterte Rückstandsgehalte von Sulfonamiden in Böden um den Faktor 2,5 höher sind als die extrahierbaren Gehalte. Dieser Faktor ist für die stark sorbierenden Antibiotika wie Tetrazykline, Fluorchinolone und ß-Lactame vermutlich noch größer.

Übereinstimmend mit den Berichten über die Kontamination von organischen Abfallsubstraten, zeigen die vorliegenden Studien über Böden, dass nicht nur einzelne Antibiotika, sondern Gemische verschiedener Antibiotika und Strukturklassen üblicherweise in Böden auftreten. Die Gesamtkonzentrationen der Antibiotikagemische überschreiten eindeutig natürliche Hintergrundgehalte. Zwei und bis zu 13 verschiedene pharmazeutische Antibiotika wurden in verschiedenen Oberbodenproben von landwirtschaftlichen Flächen nachgewiesen. Anhand von Probenpaaren, d.h. organisches Abfallsubstrat und damit beaufschlagter Boden, konnte eine signifikante Korrelation zwischen der Häufigkeit mit der Antibiotika im Abfallsubstrat und im Boden auftreten gefunden wurden.

Antibiotika in Böden werden nicht nur durch anthropogene Kontamination verursacht, sondern treten auch natürlich auf; sie werden durch den Sekundärstoffwechsel natürlich vorkommender Bodenmikroorganismen gebildet. Zum Beispiel sind Streptomycin und Oxytetrazyklin weithin bekannte, im Boden gebildete Antibiotika und werden durch *Streptomyces* Actinobakterien gebildet. Unter vielen anderen Bodenmikroorganismen sind 30 bis 50 % der aus Böden isolierbaren Actinomyceten in der Lage Antibiotika zu synthetisieren. In Berichten werden typische, resultierende Bodengehalte in der Größenordnung von µg/kg angegeben, die insbesondere in der Rhizosphäre in Böden zu finden sind. Demgegenüber können die Rückstandsgehalte bereits einzelner pharmazeutischer Antibiotika die natürlichen Hintergrundgehalte deutlich überschreiten und liegen mitunter sogar oberhalb des von der Europäischen Arzneimittelagentur (EMA) festgelegten Auslöserwertes von 100 µg/kg. Zudem treten häufig nicht nur Einzelwirkstoffe, sondern Gemische mehrerer Antibiotika in Böden auf, so dass die kombinierten Konzentrationen klar oberhalb der natürlichen Hintergrundgehalte liegen. Die festgestellten Antibiotika gehören zu unterschiedlichen Wirkstoffklassen und weisen dementsprechend unterschiedliche Wirkmechanismen auf. Dabei treten Antibiotika einiger Strukturklassen deutlich häufiger auf (z.B. Tetrazykline), während andere mit geringeren Konzentrationen nachgewiesen wurden

(z.B. Sulfonamide). Dies liegt an (i) einer unterschiedlichen Anwendung und damit Freisetzung in die Umwelt und (ii) einer unterschiedlichen Persistenz und Mobilität in Böden.

4. Toxische Effekte von Antibiotika als Einzelwirkstoff und in Gemischen von Umweltkontaminanten auf Bodenorgansimen

Das vorliegende Wissen über negative Wirkungen von Antibiotika auf Bodenmikroorganismen bezieht sich fast ausnahmslos auf Studien über Einzelwirkstoffe und adressiert nicht Antibiotikagemische. Zudem ist festzuhalten, dass, obwohl die Anzahl an Publikationen über Wirkungen einzelner Antibiotika auf Bodenmikroorganismen in den vergangenen Jahren ganz erheblich zugenommen hat, das Wissen nach wie vor lückenhaft und sehr fragmentarisch ist. Zumeist finden sich Bezüge zu unterschiedlichen bodenmikrobiellen Parametern und auch einigen wenigen faunistischen Indikatorspezies. Zudem wurde die große Mehrzahl der Studien mit Vertretern der Tetrazykline und Sulfonamide durchgeführt, wohingegen das Wissen über andere Antibiotikaklassen auf wenige Einzelstudien begrenzt ist oder überhaupt keine Erkenntnisse vorliegen (z.B. Cephalo sporine). Bisherige Studien zeigen, dass mikrobielle Eigenschaften und Funktionen wie die mikrobielle Biomasse, strukturelle Diversität von mikrobiellen Gemeinschaften (Biodiversität) und funktionelle Diversität, gestört werden. Insbesondere die mikrobielle Steuerung des N-Kreislaufs wird durch Antibiotika beeinflusst. Auch Parameter der mikrobiellen Toleranz werden deutlich verändert und Veränderungen der strukturellen Diversität mikrobieller Gemeinschaften sind häufige Folge der Wirkung von Antibiotika in Böden. Letzteres scheint insbesondere mikrobielle Gruppen zu betreffen, die in die Prozesse der Nitrifikation und Denitrifikation eingebunden sind, was noch einmal die besondere Empfindlichkeit von Organismen unterstreicht, die in Funktionen im N-Kreislauf ausüben.

Effekte von Antibiotika und Antibiotikagemischen variieren in Abhängigkeit von Einflussfaktoren und Randbedingungen. Zum Beispiel können sie erheblich durch die vorliegenden Konzentrationen verändert werden. Antagonistische Wirkungen treten häufiger bei niedrigen Konzentrationen der Wirkstoffe im Gemisch auf, während gleiche Gemische bei hohen Konzentrationen sogar synergistische Effekte hervorrufen können, was von den jeweils kombinierten Antibiotika abhängt. Andererseits kommt es in verschiedenen Fällen zur Aktivierung mikrobieller Aktivitäten durch gering dosierte Antibiotika bzw. Gemische von Antibiotika. Diese Steigerungen dürfen aber keinesfalls als positive Wirkung auf Bodenorganismen fehlinterpretiert werden. Im Allgemeinen werden durch einzelne Antibiotika in Konzentrationen, wie sie auch typischerweise in der Umwelt vorzufinden sind, Wirkungen hervorgerufen, die über einen Bereich gehen, der von gerade signifikanten Effekten bis zu ungefähr 10 % Hemmung reichen. Demgegenüber werden Wirkungen von Antibiotikagemischen in Böden diesen Effektbereich deutlich überschreiten. Unter Berücksichtigung der erheblichen und unersetzlichen Bedeutung, die Mikroorganismen für die Funktionen und Ökosystemdienstleistungen von Böden haben, werden auch die genannten Effekte bereits signifikante Auswirkungen auf die Bodenfruchtbarkeit haben. Darüber hinaus werden Effekte von Antibiotika und veränderte Effekte durch Antibiotikagemische auf Spezies der Bodenfauna wie auch auf Nutzpflanzen in der Literatur beschrieben. Wenn Regenwürmer der Art *Eisenia fetida* den Einzelwirkstoffen Tetrazyklin bzw. Chlortetrazyklin ausgesetzt wurden, traten dosisabhängige, signifikante DNS-Schäden in den Coelomocyten der Regenwürmer auf. Dagegen verursachte die Kombination beider Antibiotika bei der maximalen getesteten Konzentration von 300 mg/L eine leicht antagonistische Wirkung.

Eine spezifische Wirkung von Antibiotika in Böden, die von besonderer Relevanz für die Gesundheit von Menschen und (Nutz)Tieren ist, ist eine Verstärkung des Resistenzniveaus gegen Antibiotika. Die Selektion resistenter Bakterien tritt insbesondere bei geringen Antibiotikakonzentrationen auf, typischerweise unterhalb der minimalen Hemmkonzentration (minimal inhibitory concentration; MIC). Solche geringen Konzentrationen sind üblicherweise in belasteten Böden zu finden. In der Folge sind in belasteten Böden zahlreiche Resistenzgene mit signifikant erhöhter Abundanz enthalten. Synergistisch wirkende Antibiotikagemische führen sogar zu einer stärkeren und beschleunigten Resistenzentwicklung. Andererseits weisen antagonistisch wirkende Kombinationen von Pharmazeutika eine gegenüber synergistischen Gemischen verlangsamte Resistenzentwicklung auf. Die Ableitung vorhergesagter Konzentrationen, die keinen Effekt auslösen (predicted

no-effect concentration, PNEC) und die die Schwelle markieren, oberhalb der eine erhöhte Abundanz von mikrobiellen Resistenzgenen in Böden auftritt, ist dagegen derzeit noch nicht möglich. Nicht zuletzt müssen stärkere Effekte durch Antibiotikagemische als durch Einzelsubstanzen auf die Auslösung eines erhöhten Resistenzniveaus in Böden erwartet werden.

Nicht nur Gemische von Antibiotika, sondern auch Mischungen von Antibiotika mit anderen Stoffen können Effekte der Mischungstoxizität auslösen. Belastungen landwirtschaftlicher Böden mit Gemischen von Antibiotika und anderen Agrochemikalien wie Pflanzenschutzmittel, andere nicht-antibiotische Pharmazeutika sowie Schwermetalle treten häufig auf. Besonders relevant sind dabei Mischungen mit Schwermetallen, die die Wirkung der Antibiotika auf Mikroorganismen verstärken. Kupfer (Cu) und Zink (Zn) werden häufig als Futterzusatzstoff an Nutztiere verabreicht und gelangen in landwirtschaftliche Böden als Kontaminanten in Wirtschaftsdüngern (z.B. Gülle und Klärschlamm) und in unbehandeltem Abwasser, dass zur Beregnung verwendet wird. Auch Metalle wie Cu, Zn, Cd und Hg haben antimikrobielle Wirkungen und können zur verstärkten Bildung von Antibiotikaresistenzen beitragen. Dadurch werden gerade landwirtschaftliche Böden zu „hotspots“ der Bildung von Antibiotikaresistenzen in der Umwelt.

5. Einfluss von Faktoren und Umweltbedingungen auf Effekte von Antibiotikamischungen

Effekte von Antibiotikagemischen können durch verschiedenen Einflussfaktoren ganz erheblich verändert werden. Zu allererst hängt das Ausmaß der Wirkung vom untersuchten biotischen Parameter und Endpunkt ab. Zudem verändern sich Wirkungen von Gemischen häufig mit der Konzentration der Einzelsubstanzen und können sich von additiver Wirkung oder sogar antagonistischer Wirkung zu einer synergistischen Wirkung bei höheren Konzentrationen verschieben. Alle toxischen Effekte sind zeitabhängig und schädliche Wirkungen, insbesondere von bakteriostatischen Antibiotika nehmen über Zeiträume von Tagen und Wochen zu.

Diesbezüglich ist zu erwarten, dass die Wirkungsdauer von Antibiotika in synergistischen Gemischen verlängert, in antagonistischen hingegen verkürzt ist.

Ebenso können Antibiotika und Nährstoffe bzw. Nährsubstrate in Böden interagieren, was als antagonistischer Mischungseffekt aufgefasst werden kann. Das schließt die Wirkung von organischen Abfallsubstraten ein, mit denen zusammen Pharmazeutika typischerweise in Böden gelangen. Die nährstoffreichen, abbaubaren Substrate erhöhen tendenziell schädliche Effekte von biostatisch wirkenden Antibiotika. Beispielsweise nehmen antibiotische Effekte mit gesteigerter Güllezugabe disproportional zu; die Einflüsse von Gülle variieren sogar in Abhängigkeit von der Verwendung frischer oder gelagerter Gülle.

Außerdem bestehen Wechselwirkungen mit allen anderen Randbedingungen, die die Leistungsfähigkeit von Organismen beeinflussen, wie Hitze oder Frost, Dürre oder Wasserüberschuss verbunden mit Sauerstoffmangel, Nährsubstratmangel oder Angebot durch Nährsubstratzugabe, sowie Pathogene oder Prädatoren. Derartiger Umweltstress führt meist zu einer synergistisch verstärkten, schädlichen Wirkung von Chemikalien. Allerdings liegen kaum Untersuchungen zum Einfluss von Größen, die keine Schadstoffe darstellen, wie der Nährstoffstatus und Umweltstressoren, auf die Toxizität von Gemischen vor; entsprechende Publikationen mit Bezug zu Antibiotikagemischen wurden in dieser Literaturrecherche nicht gefunden. Es wird vermutet, dass der Gesamteffekt von Schadstoffgemischen durch diese Nicht-Schadstoffeinflüsse zu- oder abnimmt (Synergismus oder Antagonismus), es jedoch nicht zu einer Veränderung des Wirkungstyps kommt, solange nicht physikochemische Eigenschaften oder die Bioverfügbarkeit (effektive Konzentration) einer Substanz entscheidend verändert werden, z.B. die Löslichkeit durch einen Temperaturanstieg.

6. Veränderung des Schicksals von Antibiotika in Böden durch Antibiotikagemische und andere Kontaminanten

Auch das Schicksal und der Verbleib von Antibiotika in Böden können bei Vorliegen von gemischten Belastungen verändert sein. Sorption und Abbau bzw. Abnahme nachweisbarer Gehalte können signifikant verändert sein, was Rückkopplungen auf die Wirkungen hat. Eine wesentlich verminderte Immobilisierung von

Antibiotika im Boden ist zu beobachten, wenn Sorptionskonkurrenz mit steigenden Konzentrationen zunimmt. Sorptionskonkurrenz tritt insbesondere bei Gemischen von Antibiotika derselben Strukturklasse auf; die Einzelverbindungen konkurrieren um dieselben Sorptionsplätze im Boden. Bei Antibiotika unterschiedlicher Strukturklassen ist die Sorptionskonkurrenz hingegen weitgehend auf höhere Konzentrationsbereiche beschränkt. Zusätzlich kann Sorptionskonkurrenz auch zwischen Antibiotika und nicht-antibiotischen Komponenten anderer Verbindungsklassen auftreten. Dies betrifft auch Verbindungen natürlicher, organischer Substanz, die z.B. mit Gülle in die Böden gelangen. Nicht zuletzt werden die Abnahme bzw. der Abbau von Antibiotika in Böden in Gegenwart von gemischten Kontaminationen verändert.

7. Schlussfolgerungen und Konsequenzen für die Bodenfunktionen und zukünftige Forschung

Wissenslücken

Insbesondere in den letzten 20 Jahren wurde das Wissen über die Einträge, das Verhalten und die Wirkungen von Antibiotika in Böden, Exkrementen und Wirtschaftsdüngern ganz erheblich erweitert. Allerdings sind die meisten Forschungsarbeiten, die die Umwelt betreffen, auf Antibiotika von nur wenigen Strukturklassen fokussiert, insbesondere Tetrazykline und Sulfonamide, während der Wissensstand über andere Strukturklassen wie die Benzimidazole, Lincosamide und Cephalosporine nach wie vor fragmentarisch ist. Zum Beispiel werden in der Human- und Veterinärmedizin am häufigsten Penicilline verwendet, Cephalosporine der dritten Generation sind als Reserveantibiotika von besonderer Bedeutung für die Humanmedizin; Studien über deren Einträge, Verbleib und Wirkung in Böden fehlen dagegen. Noch einmal ist daher zu betonen, dass weiterhin viele Wissenslücken bestehen, die wie folgt zusammengefasst werden können.

- Berichte über das Vorkommen von Antibiotika in Umweltsubstraten wie Gülle, Klärschlamm und Böden sind im Wesentlichen auf Antibiotika weniger Strukturklassen, wie die Tetrazykline und Sulfonamide beschränkt, eine geringere Anzahl von Studien befasst sich mit Fluorchinolonen, Makroliden und Lincosamiden.
- Erkenntnisse über Einträge in die Umwelt, das Verhalten und Effekte von Antibiotika anderer Strukturklassen sind fragmentarisch und unvollständig oder fehlen sogar völlig.
- Noch schlechter ist der Kenntnisstand über das Vorkommen, die Zusammensetzung und Konzentrationen von Antibiotikagemischen und deren Wirkungen in Böden.
- Böden sind äußerst heterogen infolge ihres Aufbaus aus unterschiedlichen Horizonten als Makrostrukturen und die Aggregierung und andere Bereiche wie die Rhizosphäre usw. als Mikrostrukturen. Die Verteilung von Antibiotika und Antibiotikagemischen in strukturierten Böden ist weitgehend unbekannt.
- Es liegen zu wenige Daten über toxische Effekte von Antibiotika und insbesondere von Antibiotika-Gemischen in Böden vor.
- Die zugrundeliegenden Mechanismen von Gemischeffekten wie Antagonismus und Synergismus sind nur unvollständig bekannt. Es ist darauf abzuzielen über die experimentelle Erfassung hinaus die grundlegenden Prinzipien zu identifizieren (und zu modellieren). Dies gilt insbesondere für das fehlende Verständnis der ökologischen Bedeutung von Antibiotika in der Natur und daraus folgende, mögliche unerwünschte Effekte einer Umweltbelastung. Es wird ein langfristiges Ziel sein, einen systematischen Ansatz zu erarbeiten, um die zugrundeliegenden Ursachen für eine auftretende Wechselwirkung zwischen Pharmazeutika zu ermitteln.
- Das vorliegende Wissen über toxische Wirkungen von Pharmazeutika in Böden (von Gemischen ganz zu schweigen) basiert auf einer vergleichsweise breiten, unsystematischen Vielfalt unterschiedlicher Testmethoden und Endpunkte. Dies behindert die Zusammenführung des vorliegenden Wissens. Es ist zu erwarten, dass Effekte und Effektkonzentrationen erheblich zwischen verschiedenen Testmethoden und Endpunkten variieren.

- In diesem Zusammenhang erscheint es außerdem notwendig, die Faktoren, die die Mischungstoxizität beeinflussen, wie z.B. die Anzahl der Schadstoffe im Gemisch, deren (relative) Konzentrationen und Umweltbedingungen wie die Bodenfeuchte und Temperatur, chemische und physikalische Bodeneigenschaften, Status und Zusammensetzung der (mikrobiellen) Organismengemeinschaft im Boden.
- Ein spezifischer Aspekt ist die Zeitabhängigkeit der Wirkungen von Antibiotika. Insbesondere von Gemischen, und bei Langzeit-Exposition werden chronische Effekte erwartet.
- Hormesis, die durch eine geringe Schadstoffdosis hervorgerufene, scheinbar positive Zunahme eines untersuchten Parameters, kann Teil eines toxischen Effektes sein. Sie tritt in Gegenwart verschiedener Pharmazeutika und deren Gemischen auf. Es ist nach wie vor unklar wie eine hormetische Zunahme zu bewerten ist, was insbesondere auf Gemische zutrifft, deren Einzelkomponenten in dieser Hinsicht unterschiedliche Effekte ausüben.
- Nicht zuletzt sind tragfähige Konzepte für Strategien zur Bewertung kumulativer Belastungen zu entwickeln.
- All dies wird flankiert durch den Bedarf nach einheitlichen, im besten Fall standardisierten Methoden zur Bestimmung von Gesamtgehalten und verfügbaren Fraktionen der Antibiotika in Böden, und zur zuverlässigen Bestimmung von Effekten durch Antibiotika auf Bodenorganismen. Es wird erwartet, dass Effektkonzentrationen am besten durch eine bioverfügbare Fraktion repräsentiert werden.

Zudem ist zu betonen, dass viele der in den vorangegangenen Abschnitten dargestellten Befunde auf einzelnen oder nur wenigen Studien basieren, die oft nur wenige, einzelne Antibiotika oder sogar andere Chemikalien als Antibiotika untersucht haben. Zudem behandeln viele der Studien nicht Böden, sondern andere Umweltkompartimente bzw. stammen aus der Medizinforschung. Deshalb ist weitere, zielgerichtete und systematische Forschung notwendig, (i) um die Schadwirkungen und Risiken die Antibiotikagemische für die Umwelt und insbesondere Böden bedeuten zu quantifizieren und zu bewerten; (ii) um vorherzusagen, welche Antibiotikagemische in Bezug auf Zusammensetzung und Konzentrationen an einem bestimmten Ort oder in einem bestimmten Umweltkompartiment toleriert werden können; (iii) um herauszufinden, welche Verbindungen die ökotoxikologischen Treiber an einem bestimmten Ort sind. Dazu werden drei aufeinander aufbauende Forschungsprojekte vorgeschlagen, um die komplexe Situation und Wirkungen von Antibiotikagemischen in Böden zu identifizieren.

Empfohlene Forschungsprojekte und ihr Nutzen in der Regulatorik

1. Belastungsstatus und Effekte von Gemischen pharmazeutischer Antibiotika in der terrestrischen Umwelt – eine Meta-Analyse

Eine Meta-Analyse vorliegender, quantitativer Daten über die Abgabe von Antibiotika wie auch über deren Vorliegen in organischen Abfallsubstraten und Böden. Davon ausgehend sind vorhergesagte und gemessene Umweltkonzentrationen (predicted and measured environmental concentrations, PEC, MEC) von Antibiotika abzuleiten und gegen Wirkungskonzentrationen (effect concentrations; EC) oder Konzentrationen ohne Wirkung (no-effect concentrations; NOEC) abzugleichen, um Risiken von Antibiotikagemischen in Böden zu erkennen und b) den Wissensstand bezüglich der Kontamination von Böden mit Antibiotika und Antibiotikagemischen und deren ökotoxikologischer Relevanz und Folgen weiter zu präzisieren und zu verbessern. Erste Empfehlungen für Maßnahmen sollen auf dieser Basis an regulatorische Behörden und Interessenvertreter gegeben werden sowie nachfolgende experimentelle Arbeiten geplant werden.

2. Ein allgemein verwendbarer Methodensatz zur Bestimmung von unerwünschten Wirkungen von Gemischen pharmazeutischer Antibiotika in Böden

In einem zweiten, nachfolgenden Projekt mittlerer Zeitdauer, soll experimentelle Forschung basierend auf den bisherigen Erkenntnissen erfolgen. Die Zielstellung sollte sein i) geeignete Methoden zu identifizieren, mit deren Hilfe unerwünschte Effekte von pharmazeutischen Antibiotika auf die Abundanz und Funktionen von Bodenmikroorganismen bestimmt werden können. Die Überprüfung vorhandener, standardisierter Methoden und eventuell weiterer Methoden, die relevante Funktionen von Bodenmikroorganismen erfassen, könnte mit einem Dosis-Wirkungs-Ansatz mit einer Auswahl von Böden erfolgen. Dazu sind repräsentative Antibiotika verschiedener Strukturklassen auszuwählen, die am häufigsten in der Veterinär- und Humanmedizin angewendet werden, z.B. Penicilline, Tetrazykline, Polypeptidantibiotika, Sulfonamide, Makrolide, Aminoglykoside, Lincosamide, Pleuromutiline, Fluorchinolone, Folsäureantagonisten, Fenicole und Cephalosporine. ii) Eine Testbatterie sollte entwickelt werden als Auswahl der als geeignet identifizierten Untersuchungsmethoden. Dieses Set sollte idealerweise Methoden und Endpunkte umfassen, die Parameter der mikrobiellen Abundanz repräsentieren und Indikatoren relevanter, mikrobieller Funktionen sind.

3. Mischungstoxizität von Schadstoffgemischen pharmazeutischer Antibiotika und anderen Kontaminanten in landwirtschaftlichen Böden

Ein drittes Projekt (mit langfristigerer Ausführungsperspektive) sollte zum Ziel haben, die Wirkungen von Antibiotikagemischen unter Verwendung der Testbatterie zu bestimmen. Typische Gemische sind dabei zu untersuchen, anfangend von binären Gemischen bis hin zu ausgewählten ternären und noch komplexeren Gemischen, um die Mischungstoxizität von Antibiotika gleicher wie auch unterschiedlicher Strukturklassen bzw. Wirkmechanismen zu bestimmen und zu kategorisieren. Das übergeordnete Ziel wird es sein, die zugrundeliegende Wirkprinzipien von Antibiotikagemischen in Böden zu identifizieren, um darauf aufbauend weitergehende Modellierungen und Prognosen zu ermöglichen. Mit diesem Wissen wäre es besser möglich, regulatorische Standards und Grenzwerte zu definieren.

Diese Forschungsarbeiten sollten idealerweise mit wissenschaftlichen Workshops kombiniert werden, um den aktuellen Stand des Wissens zum Thema zu definieren. All dies soll dazu führen oder bereits dadurch begleitet werden, dass durch regulatorische Maßnahmen eine Verbesserung der Nutzung und des Umgangs mit für medizinische Zwecke eingesetzten Antibiotika erreicht wird, zu welchem Zweck auch Umweltaspekte sachgerecht berücksichtigt werden.

Schlussfolgerungen

Die vorgeschlagenen Forschungsarbeiten zielen darauf Regulierungsinitiativen und Regulierungsinstitutionen wissenschaftliche Unterstützung zu liefern. Das übergeordnete Ziel sollte es sein, die Verbreitung von pharmazeutischen Antibiotika und entsprechenden Schwermetallen (Cu, Zn) in der Umwelt zu minimieren, um unerwünschte, nachteilige Effekte auf Bodenfunktionen und -fruchtbarkeit zu vermeiden und die Zunahme und Verbreitung antibiotischer Resistenzen in der Umwelt mit ihren Gesundheitsrisiken für Menschen und (Nutz-)Tiere zu verhindern. Regulatorische Maßnahmen könnten die analytische Bestimmung der Antibiotikabelastungen von Abfallsubstraten bzw. Wirtschaftsdüngern wie z.B. Gülle und die Bestimmung resultierender Rückstandsgehalte in Böden festlegen. Dies könnte besonders relevant sein für Wirtschaftsdüngerexporte und –importe zwischen Landwirtschaftsbetrieben und Staaten. Akzeptable Kontaminationsbereiche (gesetzliche Grenzwerte) könnten sich an Konzentrationen orientieren, unterhalb derer kein Effekt auf Bodenorganismen oder das Resistenzniveau erwartet wird (no-effect concentration) und die in bodentoxikologischen Untersuchungen ermittelt wurden. Ein fundiertes Wissen über diese Umweltbelange und deren Berücksichtigung würde es ermöglichen, die Nutzung von pharmazeutischen Antibiotika auf landwirtschaftlichen Betrieben besser zu planen und zu organisieren. Ohne Zweifel muss die Heilung von Infektionskrankheiten bei Mensch und Tier weiterhin erste Priorität haben. Dies schließt aber ein, viel mehr als zuvor Umweltbelange zu berücksichtigen, insbesondere die zunehmende Unwirksamkeit von Antibiotika aufgrund der anwachsenden Bildung von Antibiotikaresistenzen in der Umwelt. Es besteht die feste Überzeugung, dass dies auch zu einer verbesserten und nachhaltigen Bodennutzung und Schutz von Wasserressourcen beiträgt.

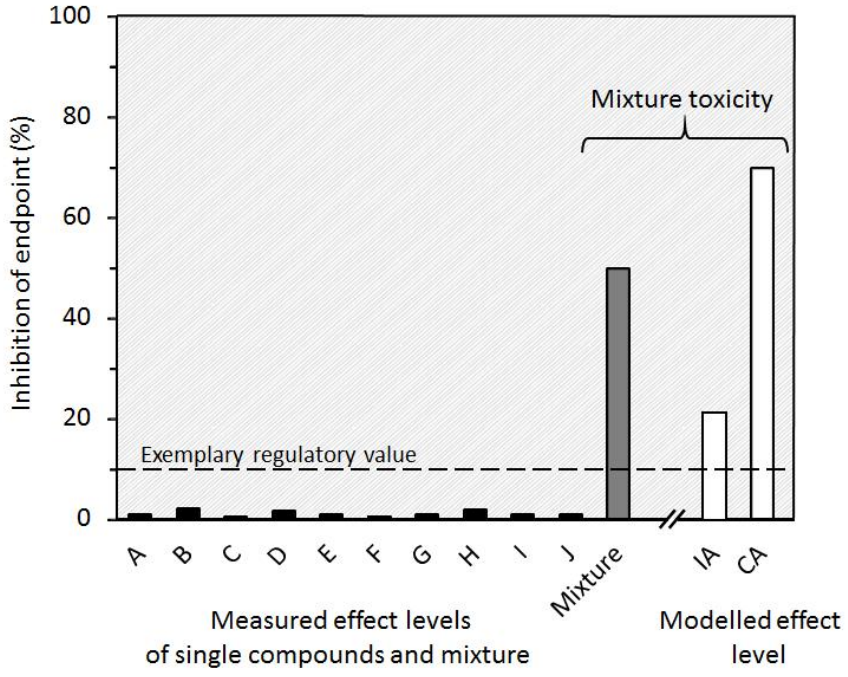
1 Introduction

The terrestrial environment is exposed to the input of contaminants from anthropogenic origin. Especially agricultural and urban soils are characterized by ubiquitous and ongoing input of pollutants. Due to the use and intended or unintended release of numerous chemicals onto soils, it must be expected that contaminated soils not only contain one contaminant but mixtures of several pollutants (Backhaus, 2014; Jahnke et al., 2016; Posthuma et al., 2008). It is expected that this also applies to pharmaceutical antibiotics that have been recognized as widespread soil pollutants (Boxall et al., 2004; Sarmah et al., 2006; Thiele-Bruhn, 2003b).

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 joint toxic effect of a pharmaceutical mixture a major issue for hazard and risk assessment: (i) the ecotoxicity of a pharmaceutical mixture is typically higher than the effects of each individual component, and, consequently, (ii) such a mixture can have a considerable ecotoxicity, even if all individual contaminants are present only in low concentrations that do not provoke significant toxic effects if acting singly on the exposed organisms (Backhaus, 2014).

This is shown on an example from Backhaus et al. (2000). In that study, a mixture of pharmaceuticals was used consisting of ten individual compounds at concentrations that cause low effects on a certain subjects of analysis below regulatory values when tested individually (Figure 1). However, the summed effect of the whole mixture is clearly larger, showing strong environmental impact and relevance.

Figure 1: Dose dependent effects of single compounds and of the mixture of these compounds as well as modelling of the mixture effect with the concepts of independent action (IA) and concentration additivity (CA).



Source: Figure modified from Backhaus et al. (2000)

Since the last two decades, effects of contaminant mixtures have been increasingly investigated in aquatic environments (e.g., Grimme et al., 2000; Vasquez et al., 2014); anyhow, data on mixture toxicity effects on marine flora and fauna are still scarce (Backhaus, 2014). Even considerably less knowledge exists for soils regarding mixture toxicity of pollutants in general and of combinations of antibiotics in special. The set of bioassays available for the assessment and monitoring of contaminant mixtures is solely based on aquatic

test methods (Jahnke et al., 2016). Related to the unwanted input into soil and effects of pharmaceutical antibiotics in soil, the board of experts for environment questions from the German Federal Government recommended measures in a position paper (Sachverständigenrat für Umweltfragen, 2007), for example:

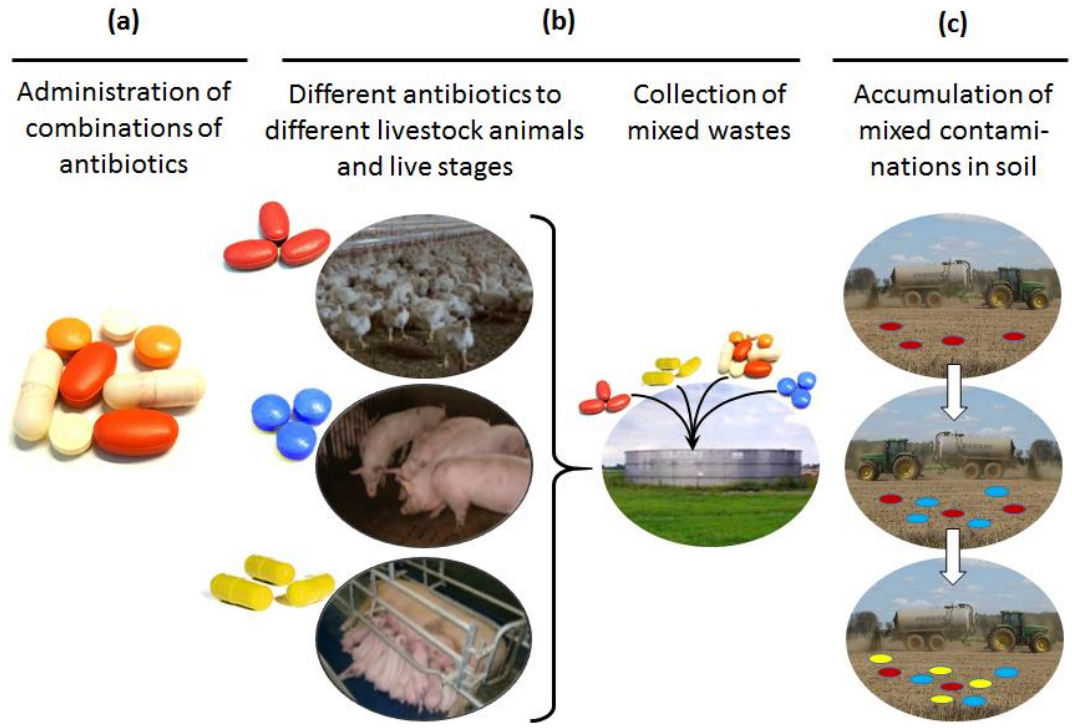
- An environmental risk assessment of priority pharmaceuticals, including old products, and grouping of compounds for the integrated determination of possible consequences.
- Continuous documentation of the contamination of surface waters and soils.
- Assessment of the environmental risk of pharmaceuticals, taking into account all compounds that are present at the same site and have similar effects.

The latter aspect clearly addresses mixed contaminations through pharmaceuticals. Previous studies showed that environmental contamination with mixtures of pollutants (pharmaceuticals and other chemicals) and not only with single compounds is rather the rule than the exemption. Analytical monitoring surveys routinely confirm that organisms in the environment are exposed to complex multi-component pharmaceutical mixtures (Backhaus, 2014). The dose dependent effects of mixtures cannot be explained by regarding the single compounds (see Figure 1) and the modelling of mixture toxicity, e.g. by using the classical concepts of concentration addition (CA) and independent action (IA; response addition). Existing knowledge gaps include, in particular, the need for more and better empirical data on the effects of pharmaceutical mixtures on soil organisms, and the exploration of the quantitative consequences of toxicokinetic, toxicodynamic and ecological interactions. Increased focus should be put on investigating the ecotoxicology of pharmaceutical mixtures in environmentally realistic settings (Backhaus, 2014).

Also for pharmaceutical antibiotics it is a realistic and very likely scenario that mixed contaminations occur in soil, yet there is a substantial lack of knowledge about possible adverse effects, and thus strong decision-making deficits. Why do mixtures of antibiotics end up in soil? This is shown for veterinary antibiotics in Figure 2. First, combinations of antibiotics are frequently administered to livestock. For example, existing studies show that mixtures of pharmaceuticals (two and more active compounds) are used in about 50 % of all veterinary medications. Second, different antibiotics are given to different livestock animals and to different live stages of the animals because different pathogens typically occur and have to be treated. Subsequently, mixtures of antibiotics will result, when excreta from different livestock are collected in the same manure tank or lagoon. The resulting mixtures of active agents are subsequently, and often repeatedly spread onto agricultural fields, when they are recycled as fertilizer. 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). In addition to antibiotic pharmaceuticals, also non-antibiotic pharmaceuticals reach soils. This especially applies to analgesics and anti-inflammatories (Gildemeister et al., 2011). Consequently, Gildemeister et al. (2011) estimated that 10 to 20 different pharmaceutical compounds will end up within one year on a typical agricultural soil with respective fertilization regime.

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 use of wastewater for irrigation and sewage sludge for fertilization, respectively (Monteiro and Boxall, 2010; Verlicchi et al., 2012; Verlicchi and Zambello, 2015).

Figure 2: **Formation of mixed contamination in the environment through a) medication by using combinations of pharmaceuticals, 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.**



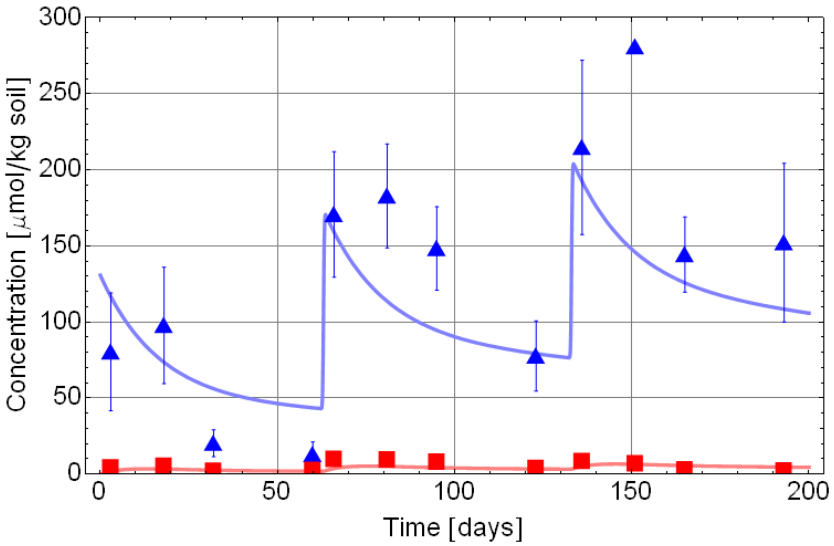
Source: Own figure from S. Thiele-Bruhn (Univ. Trier). Single photographs with permission from M. Arenz-Leufen, I. Rönnefahrt, and UBA and from Christoph-Schulz et al. (2018) doi: 10.1007/s00003-017-1144-7, open access.

The assumed accumulation of antibiotics after spreading to soil with organic fertilizer was previously confirmed in various studies (Aust et al., 2008a; Hamscher, 2007; Hamscher et al., 2002; Höper et al., 2002) and is exemplarily shown in Figure 3. Numerous studies showed that pharmaceutical antibiotics degrade rather slowly in soil, which even applies to penicillins whose lactam ring is very unstable and susceptible to degradation (Kotzerke et al., 2010). Consequently, repeated application of manure contaminated with antibiotics will lead to a replenishment and apparently continuous contamination level (Figure 3) that is termed as apparent persistence (Hamscher, 2007; Hamscher et al., 2005) or pseudo-persistence (Bottoni and Caroli, 2018). Furthermore, biodegradation of antibiotics in soil very much depends on soil moisture and pH and degradation half live times can increase under unfavorable conditions (acidic pH, dry soil) by factors up to 1000 (Braschi et al., 2013).

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 (independent action) leading to synergistically increased or antagonistically decreased effects, while in other cases additive effects (concentration additivity) are assumed. However, the impact of antibiotic mixtures on biological effects and chemical fate in the soil environment are largely unknown. Boxall et al. (2012) ranked the question "*How can effects from long-term exposure to low concentrations of PPCP* [pharmaceuticals and personal care products] *mixtures on non-target organisms be assessed?*" among the top 20 priority questions on pharmaceuticals in the environment.

Figure 3: Accumulation of sulfadiazine (SDZ) in arable field soil in the course of three times repeated soil fertilization using SDZ-contaminated manure.

Triangles: methanol extractable = potentially available SDZ; squares: $CaCl_2$ extractable = mobile SDZ



Source: Figure from A. Focks (DFG FOR566), unpublished.

In this study, a literature research was done using the Scopus literature database (Table 1). It confirmed that only very limited knowledge exists on the occurrence and effects of mixed soil contamination with antibiotics. In April 2018 a number of 7670 publications was found with the search terms "soil" and "antibiotic". Compared to this the number of hits largely declined, when combining the search term "soil" and search terms related to mixture toxicity (see Table 1). Only 17 publications were found with the additional search terms "antibiotic" and "pharmaceutical". Out of these 17 publications, one single publication truly dealt with soil and a binary mixture of antibiotics, i.e. tetracycline and chlortetracycline (Dong et al., 2011). In another study, the effect of a binary mixture, flubendazole and fenbendazole, on the water organism *Daphnia magna* was tested (Puckowski et al., 2017), while four studies investigated the fate and/or effects of binary mixtures of one antibiotic (chloramphenicol, oxytetracycline, sulfamonomethoxine, sulfadioxine) with a heavy metal (Hg, Pb, Cd, Cu) (Chen et al., 2011; Gao et al., 2014; Jin et al., 2010; Xu et al., 2017b). The other 12 studies – despite the search terms – dealt not with antibiotics.

Table 1: Numbers of references found by a literature research for the listed combinations of two, three and four search terms, respectively.

Use of the Scopus literature database on April 16, 2018.

Search term	AND soil	AND antibiotic	AND pharmaceutical
mixture toxicity	79	11	10
joint toxicity	49	7	6
joint effects	247	5	3
binary mixture	294	6	2
ternary mixture	44	3	3
toxic interactions	9	0	0
Sum w/o duplicates			17

The lack of studies and consequently of knowledge on mixture toxicity in soil is due to three facts. First, toxicity testing doing dose-response experiments is laborious work and especially time-consuming since the

number of samples to be tested exponentially increases for binary and even more for n-fold combinations. Second, antibiotics often exert time-delayed effects so that short acute test methods often are not applicable, but incubation times of days and even longer must be realized (Backhaus et al., 1997; Thiele-Bruhn and Beck, 2005). Third, studies with soil require, much more than with water, the testing of several soils with different composition and properties because the fate of the compounds may vary between soils and might be different in combinations compared to the single compounds (see chapter 7). Even more, the composition, fitness and resilience of the community of soil organisms will interact with both soil properties and pollutants.

Consequently, it must be the aim to fill knowledge gaps in so far that possible risks can at least be reliably estimated, so that decision guidance for policy consultation is available. Hence, the aims of this literature study are

- to clarify, which antibiotics are regularly applied in combination with other active substances and
- which mixture effects result from this for soils and soil (micro)organisms, their structural diversity and functional ecosystem services as well as the level of antibiotic resistance in soil;
- to identify combinations of antibiotics that are typically applied in agricultural practice, and thus are likely to end up as mixed contaminants in soil;
- to collect information on knowledge gaps and further research that is needed concerning the topic, which combinatory effects and mixture toxicity, respectively, may occur in contaminated soil with special emphasis on effects on soil microorganisms, their functions and antibiotic resistance level;
- to derive conclusions in which way mixed contaminations with antibiotics and effects of mixture toxicity may occur in soil and how they can be assessed.

1.1 Use of pharmaceutical antibiotics and properties of antibiotics

Pharmaceutical antibiotics reach the environment – strictly speaking – already with their application to an organism, since they will be inevitably transferred from the medicated organism to other compartments of the environment. Soils become particularly contaminated by the use of antibiotics in veterinary medicine for the treatment of companion animals (e.g., dogs, cats, horses) but even more for the use in livestock animals (e.g., pigs, poultry, cattle, sheep), when large herds are medicated.

Environmental contamination happens through

- intracorporal application of antibiotics (oral, intrauterine, intramuscular, intravenous), after which the substances are - mostly renal - excreted (Schadewinkel-Scherkl and Scherkl, 1995) and
- reach agricultural soils either directly by grazing animals and animals held in confined animal feeding operations, respectively, or indirectly through the use of excrements as organic fertilizer (Boxall et al., 2004; Halling-Sørensen et al., 1998; Kemper, 2008; Meyer et al., 2000).
- Additionally, exhaust air from stables may contain contaminated dust and thus contaminate the surrounding area and soils, which may especially be relevant when antibiotics are used as an admixture to dry livestock fodder (medicated feedstuffs) (Hamscher, 2008; Hamscher et al., 2003; Thiele-Bruhn et al., 2003).
- Dermal application as ointment, dipping and pouring agent can be washed off, e.g. by rain, or drip off directly after treatment and may lead to contamination especially of in-field treatment sites (Armstrong and Philips, 1998).
- Surface waters are directly affected by the use of antibiotics in aquaculture, when these pharmaceuticals are poured into the water either directly or as admixture to fish food (Hektoen et al., 1995; Kümmerer, 2009).

- Indirect contamination of surface waters results from eroded soil, surface run-off, drainage water, and lateral flow originating from contaminated field and farm sites (Burkhardt et al., 2005; Kay et al., 2004; Kay et al., 2005b; Kreuzig et al., 2005; Lapen et al., 2008; Topp et al., 2008).
- Pharmaceuticals from human medicine first of all reach the aquatic environment and may subsequently reach soils especially with the use of wastewater for irrigation and of sewage sludge for fertilization (Borgman and Chefetz, 2013; Chefetz et al., 2008; García-Galán et al., 2013; Rahube et al., 2014); see following paragraph.

Pharmaceuticals and antibiotics from human medicine are especially released to the environment via wastewater after they have been excreted mostly in its active form from the treated patient or are improperly disposed via the toilet (Daughton and Ternes, 1999; Paut Kusturica et al., 2017; Sattelberger, 1999). Because many pharmaceuticals are incompletely eliminated upon mechanical and biological cleaning stages of wastewater treatment plants, they are transferred into surface waters (Kümmerer, 2003). Additionally, leakages in sewer systems may contribute to the contamination of the subsurface (Ternes, 2000). The pollution of the environment through the production, transport and deposition of pharmaceuticals and pharmaceutical wastes is also possible (Holm et al., 1995; Reddersen et al., 2002) and may lead to a substantial contamination level (Velagaleti et al., 2002) at local sites, while with regard to the contaminated surface area this might be of less relevance. On the contrary, the use of antibiotic pesticides, e.g. aminoglycosides and nucleosides used as bactericides and fungicides in orcharding, is of high relevance in various countries (McManus et al., 2002). The restricted use of pesticides containing streptomycin is allowed in Germany in the course of the strategy to fight fire blight of fruit trees (*Erwinia amylovora*) and according to § 11(2) sentence 1 point 2 of the plant protection law (PflSchG) only when there is „danger ahead“ (BMELV, 2008). Interestingly, Misato et al. (1977) favored the use of antibiotics as pesticides, because “it is expected that the use of agricultural antibiotics will not generate environmental pollution”. Further possible discharge routes and paths of antibiotics into soils and groundwater and surface water have been summarized by Boxall et al. (2004).

In summary, especially the application of organic fertilizers, i.e. slurry, manure, sewage sludge (Sarmah et al., 2006), and in various countries also the increasing use of (treated) wastewater for irrigation of agricultural land (Siemens et al., 2010; Tamtam et al., 2011) are discharge routes of antibiotic contaminants into the terrestrial environment and especially onto agricultural soils. Being highly effective agents, produced to affect microorganisms, they cause adverse effects on soil microorganisms, their biodiversity and related soil ecosystem functions even at low environmental concentrations (Ding and He, 2010; Grenni et al., 2018). Even more, a continuing low environmental contamination level will particularly trigger antibiotic resistance that may lead to a long-term increased antibiotic resistance level in contaminated soil (Agga et al., 2015; Martínez-Carballo et al., 2007). The exchange of resistance genes among soil bacteria and human and animal pathogens has been confirmed (Forsberg et al., 2012). Furthermore, it was shown that food plants grown in fields that had been amended with contaminated organic fertilizer carried more and additional resistance genes compared to plants grown on soil without such amendment (Rahube et al., 2014). Although the risk level arising from an increased antibiotic resistance of soil microorganisms might be low, it is assumed that this may have a substantial impact on human health status that is even higher than the risk of hospital transmission because of the widespread distribution of pharmaceutical antibiotics and of the associated risk in the environment (Smith et al., 2005).

The pharmaceutical compound classes most often used in human and veterinary medicine, respectively, are listed in Table 2. More than 70 % of the substances used in veterinary medicine are antibiotic compounds (Halling-Sørensen *et al.* 1998), while antibiotics are the third-largest group among all pharmaceuticals used in human medicine making up a portion of 6 % (Schwabe und Paffrath 2001). These numbers were largely constant over the past years, although the consumption of antibiotics slightly declined in human medicine (Versorgungsatlas, 2016). Since the non-therapeutic use of antibiotics as ergotropics in livestock breeding

was more and more restricted in the EU and finally fully banned in 2006, the consumption of antibiotics for that purpose largely declined during that time (see also chapter 2). Especially farm animals, primarily pigs and poultry, are medicated with antibiotics, while only about 1 % of all veterinary prescriptions were for the remaining animal groups (Ungemach 2000). Due to the relevance and quantitatively substantial use of antibiotics, this literature study is focused on this group of pharmaceuticals.

Table 2: Most relevant indication groups of pharmaceuticals used in human and veterinary medicine.

Order according to the declining share of medical prescriptions.

Human medicine		Veterinary medicine	
Compound class	**Compound (examples)**	**Compound class**	**Compound (examples)**
Analgesics / Antirheumatics	acetylsalicylic acid, paracetamol / diclofenac	Antibiotics	amoxycillin, chlortetracycline, sulfadiazine, tylosin
Beta blockers / Ca-antagonists / ACE inhibitors	atenolol / verapamil / captopril	Ergotropics [a]	salinomycin, flavophospholipol
Antibiotics / Antiinfectives	phenoxymethylpenicillin, amoxicillin, tetracycline, sulfamethoxazole	Mineral nutrients, Trace elements	Zinc oxide, calcium phosphate
Antitussives / Expectorants	codeine / acetylcysteine, bromhexine	Coccidiostatics	avoparcin, lasalocid
Gastro-intestinal preparations	$Al(OH)_3$ $Mg(OH)_2$, ranitidine	Antiparasitics	ivermectin, fenbendazole
Psychotropic pharm.	bromazepam, diazepam	Analgesics	acetylsalicylic acid
Dermatics	prednicarbat, mometason	Vitamins	retinol, α-tocopherol
Bronchospasmolytics; Antiasthmatics	fenoterol, budenosid	Expectorants	bromhexinhydrochloride
Hormones	estradiol, tibolon	Hormones	estradiol, trenbolon

[a] Banned in the EU since 2006

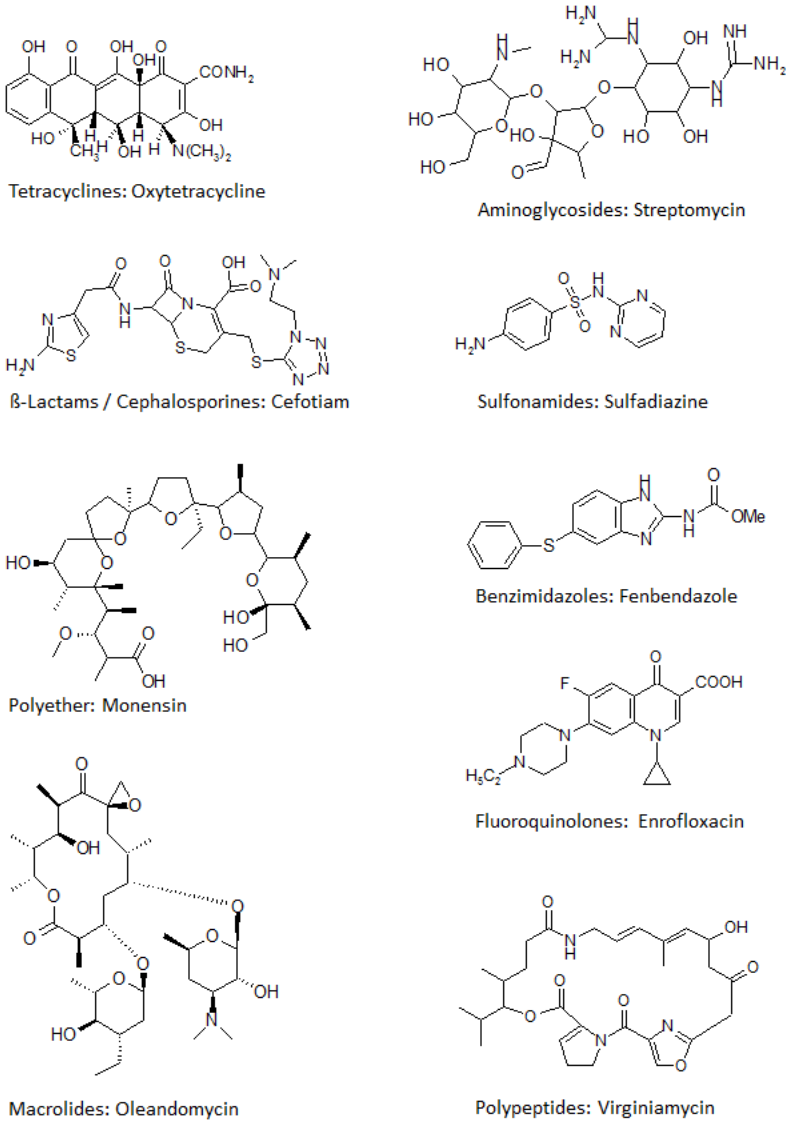
Data sources: Halling-Sørensen et al. (1998); Römbke et al. (1996); Schwabe and Paffrath (2001); Schwabe et al. (2017); Thiele-Bruhn (2003b).

In Germany alone there are more than 250 different antibiotic agents authorized (Kümmerer, 2001). In human medicine ß-lactams, tetracyclines and macrolides are among the most relevant structural classes (Schwabe et al., 2017), while in veterinary medicine especially antibiotics of the classes ß-lactams, tetracyclines, sulfonamides, aminoglycosides and macrolides are prescribed. Structural formulas of representative compounds from selected structural classes are shown in Figure 4. Already from the very different molecular structures it becomes obvious that „antibiotics“ is an umbrella term for a huge group of very heterogeneous compound classes, each with a multitude of different individual compounds.

Antibiotics are characterized by specific properties that distinguish them from numerous other compound groups. (i) Most antibiotics are amphoteric and polar substances. Hence, they are fundamentally distinct in their physicochemical properties from hydrophobic organic compounds (e.g., polycyclic aromatic hydrocarbons and polychlorinated biphenyls). Consequently, knowledge on the environmental fate of hydrophobic substances (many of which are well known and well investigated environmental pollutants) cannot be transferred to antibiotics. (ii) Antibiotics exert a specific biological effect against target and numerous non-target organisms, i.e. antibiosis, while the microorganisms in return can develop antibiotic resistance. (iii) Antibiotics reach the environment mostly together with organic waste material, and thus in complex heterogeneous organic substrates that substantially affect the composition, properties and biological status of soils (Halling-Sørensen et al., 1998; Thiele-Bruhn and Aust, 2004; Xing et al., 2015; Zhou et al., 2016).

Figure 4: Molecular structures of selected antibiotic compounds from often prescribed structural classes.

Triangles: methanol extractable = potentially available SDZ; squares: $CaCl_2$ extractable = mobile SDZ



Source: Figure according to Thiele-Bruhn (2003a), modified.

The following brief information on major compound classes of pharmaceutical antibiotics has been compiled from Gräfe (1992), Thiele-Bruhn (2003b) and Riviere (2011), if not indicated otherwise.

ß-Lactams

The ß-lactams comprise the penicillins and the cephalosporins.

Mode of action: Bactericidal. The bacterial peptidoglycan cell wall synthesis is inhibited through competitive inhibition of the bacterial transpeptidase. While penicillins and first generation cephalosporins act against gram-positive bacteria, second and third generation cephalosporins have also increasing activity against gram-negative bacteria.

Molecular structure: A ß-lactam ring, a thiazolidine ring (five-membered heterocycle; penicillins) or a dihydrothiazine ring (six-membered heterocycle; cephalosporins) and variable side chains are making up the structure. The antibiotic effect of penicillins is directly connected to the ß-lactam ring. Cephalosporins are

derivatives of 7-amino-cephalosporanic acid, condensed with a six-membered heterocycle in contrast to the five-membered heterocycle of penicillins.
Properties: The lactam ring is easily cleaved in acidic and basic media and by bacterial beta lactamases.
Examples: Penicillin G, amoxicillin, ampicillin (penicillins); cephapirin, cephalothin, cefotoxime (cephalosporins).

Tetracyclines
Mode of action: Bacteriostatic. Broad spectrum antibiotics against gram-positive and gram-negative bacteria as well as actinomycetes and protozoa. The bacterial protein synthesis is inhibited by binding to the 30S ribosomal subunit, blocking the binding of aminoacyl-transfer RNA to the ribosome-messenger RNA complex. Second generation tetracyclines doxycycline and minocycline also show greater activity against anaerobic bacteria.
Molecular structure: Tetracyclines are polyketides and comprise of a naphthacene ring structure.
Properties: The tetracyclines are amphoteric (zwitterionic) compounds, exhibiting three pK_a values. The strongest antibiotic effect occurs at pH around the isoelectric point. They are relatively stable in acids, but not in bases, and form salts in both media (Halling-Sørensen et al., 2002b; Riviere, 2011). The tetracyclines form chelate complexes with di- and trivalent metal ions and diketones and strongly bind to proteins and silanolic groups. Most tetracyclines are sparingly water soluble, while the solubility of the corresponding hydrochlorides is much higher. The tetracyclines strongly absorb light and thus, are susceptible to photodegradation (Mitscher, 1978).
Examples: Tetracycline, chlortetracycline, oxytetracycline, doxycycline.

Sulfonamides
Mode of action: Bacteriostatic. Sulfonamides interfere with the folic acid metabolism of bacteria. Competing with *p*-aminobenzoic acid, they hinder the formation of tetrahydrofolic acid. This inhibits bacterial DNA-synthesis. For more efficient antibiotic activity, they are typically combined with trimethoprim.
Molecular structure: All sulfonamides are derived from sulfanilamide. They have all a benzenesulfonamide basic structure with different substituents. The substituent at the N1-nitrogen atom is in most cases a heterocyclic five- or six membered ring.
Properties: Sulfonamides are relatively insoluble in water. They are characterized by two pK_a values indicating protonation of the amino group at a pH of 2 to 3 and deprotonation of the R1SO2NHR2 moiety at a pH of 5 to 11 (Ingerslev and Halling-Sørensen, 2000). In general, the amphoteric sulfonamides behave as weak acids and form salts in strongly acidic or basic solutions. Mostly, sulfonamides, substituted at the amino-N, have greatly reduced antibacterial activity.
Examples: Short acting – sulfathiazole, sulfaisoxazole; intermediate acting – sulfapyridine, sulfamethazine, sulfadiazine; long acting – sulfadimethoxine.

Aminoglycosides
Mode of action: Bactericidal. Bacterial protein synthesis is interrupted through aminoglycosides binding to the 30s ribosomal sub-unit, causing a misreading of the genetic code. Antibiotic activity is substantially reduced in environments with low oxygen partial pressure, acidic pH, and in the presence of divalent cations.
Molecular structure: The molecular structure is made up by two or more amino sugars that have glycosidic linkage to aminocyclitol.
Properties: All aminoglycosides are basic, strongly polar polycationic compounds. They are water soluble, mostly hydrophilic, and susceptible to photodegradation.
Examples: Streptomycin, kanamycin, gentamicin, amikacin, neomycin.

Macrolides

Mode of action: Bacteriostatic. Inhibition of translocation through binding to the ribosomal 50S subunit. Acting primarily against gram-positive bacteria including many penicillin-resistant microorganisms.
Molecular structure: Macrolides comprise a lactone ring with one or several deoxy sugars attached.
Properties: Many macrolides are weak bases and are unstable in acids. Their water solubility varies considerably between the different derivatives. Highest effectiveness in the neutral form at alkaline pH.
Examples: Tylosin, erythromycin.

Fluoroquinolones

Mode of action: Bactericidal. Interactions with both DNA gyrase and topoisomerase IV, a related type II topoisomerase. Typically DNA gyrase is more sensitive in gram-negative bacteria and topoisomerase IV more sensitive in gram-positive bacteria.
Molecular structure: Common are a 4-quinolone nucleus and various substituents such as carboxylate, fluorine, and alkanes (Wolfson and Hooper, 1985).
Properties: Most fluoroquinolones, also known as quinolones, exhibit large chemical stability. They are insensitive to hydrolysis and increased temperatures, but are degraded by UV-light. Their antibiotic potency depends mostly on the aromatic fluorene substituent at the C-6 position (Wetzstein, 2001).
Examples: Norfloxacin, ofloxacin, ciprofloxacin.

An overview on the antibiotic mode of action of different antibiotic classes is given in Table 3.

Table 3: Target sites of pharmaceutical antibiotics from different structural classes.

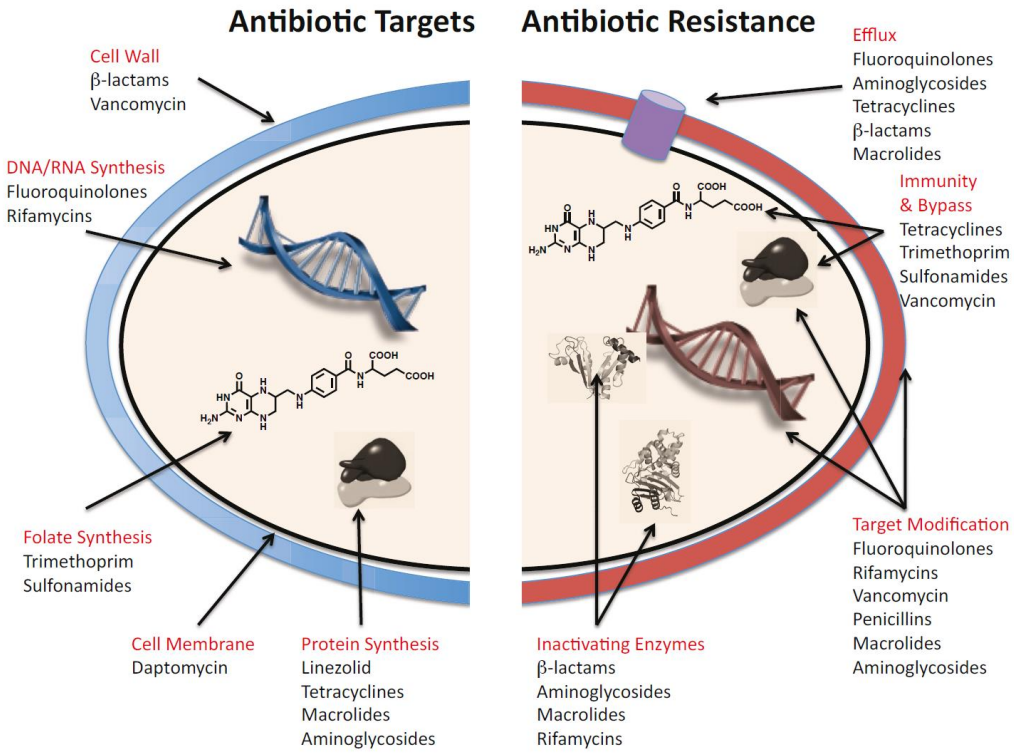
Single compounds named in brackets and specific mode of action in bold letters.

Antibiotic target site	Antibiotic class	
Cell wall synthesis	Penicillins Cephalosporins Vancomycin Carbapenems	Monobactams (Aztreonam) Oxazolidinones (Cycloserine) Bacitracin
Cytoplasmic membrane structure	Polymyxins	Daptomycin
Protein synthesis	Inhibition of 30s ribosomal subunit Aminoglycosides Tetracyclines Nitrofurans	Inhibition of 50s ribosomal subunit Macrolides (erythromycin, clarithromycin) Chloramphenicol Clindamycin Lincomycin Linezolid Oxazolidinones Streptogramins
DNA synthesis	Fluoroquinolones	Metronidazole
RNA synthesis	Rifamycines (Rifampin)	Streptovaricins
Mycolic acid synthesis	Isoniazid	
Folic acid synthesis	Inhibition of dihydropteroate synthase Sulfonamides	Inhibition of dihydrofolate reductase Trimethoprim

The antibiotic target sites at the bacterial cell are further depicted in Figure 5. As the other side of the coin, formation of antibiotic resistance is the natural reaction of targeted organisms. The mechanisms of resistance are the activation of membrane based protein efflux pumps, transporting the antibiotic compound out of the

bacterial cell (Blair et al., 2015; Gräfe, 1992). The antibiotic binding to the target is inhibited by the resistance mechanism of immunity, whereby antibiotics or their targets are bound by proteins. Similarly, molecular modifications of the target may occur through mutation of the targets themselves (Wright, 2010). All these mechanisms leave the antibiotic molecule unaltered, while inactivating enzymes as resistance mechanism catalyze the modification of molecular characteristics of the antibiotic relevant for the interaction with the targets.

Figure 5: Antibiotic targets and mechanisms of resistance at the bacterial cell.



Source: Figure and text from Wright (2010) doi: 10.1186/1741-7007-8-123, open access.

1.2 Fate of pharmaceutical antibiotics in soil

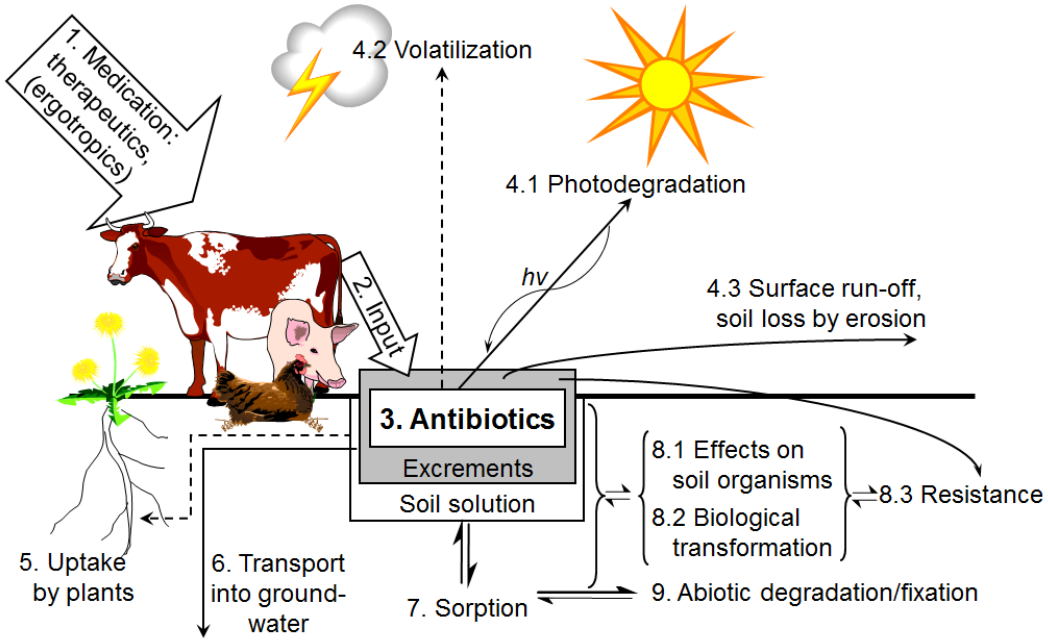
Pharmaceutical antibiotics are subject to various processes in soil. More in-depth information on the topic can be found in several reviews (Boxall, 2010; Chee-Sanford et al., 2009; Halling-Sørensen et al., 1998; Inyinbor et al., 2018; Pan and Chu, 2017; Pollard and Morra, 2018; Riaz et al., 2018). Relevant processes as well as biotic effects that are triggered by antibiotics are schematically depicted in Figure 6 and marked in bold print in the text outlined below. In Germany as well as in many other countries worldwide **soil contamination with pharmaceuticals** first of all results from their **use in farm animal treatment,** and thus especially antibiotics (**1.**) end up on agricultural soils. In semiarid and arid countries with a **higher reuse of wastewater for soil irrigation** this might be pharmaceuticals from human medicine instead (Borgman and Chefetz, 2013; Inyinbor et al., 2018). Anyhow, the input of antibiotics into soil happens first of all through contaminated **excrements** (**2.**). Consequently, mixtures of antibiotics and excreta and eventually mixtures of several antibiotics are introduced into soil. After penetration into the soil, the antibiotics **reach the soil solution** (**3.**) where they are exposed to various processes.

In case of application onto the soil surface it is possible that antibiotics are **photodegraded by sunlight** (**4.1**), which process can be, depending on the substance and substrate properties, quantitatively significant (Boreen et al., 2005; Thiele-Bruhn and Peters, 2007; Wolters and Steffens, 2005). Yet, photodegradation is

fully inhibited as soon as the antibiotics are translocated into soil depths deeper than 0.5 mm (Miller and Donaldson, 1994). In contrast, **volatilization from the soil into the atmosphere** (**4.2**) 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**) occur on sloping surfaces, especially of grassland, and are further accelerated by manure (Kreuzig et al., 2005; Müller et al., 2003). Thereby, run-off from grassland is increased due to the temporary surface sealing and hydrophobic effect of manure crusts (Burkhardt et al., 2005; Stamm et al., 2003). 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 into plants (**5.**) was confirmed for several plant types and antibiotics from different structural classes (Boxall et al., 2006; Dolliver et al., 2007; Liu et al., 2009a). On one hand, the portions taken up rarely exceed a few percent of the total amount of antibiotics in soil (Freitag et al., 2008; Grote et al., 2007; Langhammer et al., 1990), on the other hand, adverse effects on plant growth and yield (Michelini, 2012; Michelini et al., 2013; Michelini et al., 2012), as well as on endophytes (Michelini et al., 2015) have been determined. **Transport and leaching into larger soil depths and groundwater** (**6.**), respectively, occur (Höper et al., 2007; Kay et al., 2004; Kay et al., 2005a; Weiß et al., 2007). Yet, only smaller portions of the antibiotic contamination are dislocated (Aust et al., 2008b; Aust et al., 2010), while large fractions will remain in 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 (Aust et al., 2010; Hannappel et al., 2017).

Figure 6: Input, distribution, degradation and effects of pharmaceutical antibiotics in in soil following application of contaminated excrements as fertilizer.



Source: Figure according to Thiele-Bruhn (2003a), modified.

Sorption (**7.**) to the soil exchange sites, i.e. 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 (Thiele-Bruhn et al., 2004; Tolls, 2001). Sorption varies depending on the physicochemical properties of the antibiotics, content and composition of soil organic matter as well as soil minerals, pH, and also soil moisture (ter Laak et al., 2006a; ter Laak et al., 2006b; Thiele, 2000). Furthermore, sorption and mobility, respectively, of antibiotics is significantly altered in the presence of organic waste material such as manure (Bourdat-Deschamps et al., 2017; Kahle and Stamm, 2007; Thiele-Bruhn and Aust, 2004). Comprehensive review articles on soil sorption of antibiotics were published by Wang and Wang (2015) and Wegst-Uhrich et al. (2014).

Antibiotics exert specific effects on biota even at very low dosage, hence, **adverse effects on soil organisms** (**8.1**) and especially on microorganisms must be expected. In response, microorganisms react to contamination with the **biological transformation** (**8.2**) of organic compounds, and in this case of the antibiotics (Girardi et al., 2011; Grossberger et al., 2014; Schwarz et al., 2010). 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**) as a natural reaction (Heuer and Smalla, 2007; Heuer et al., 2011b; Marti et al., 2013; Schmitt et al., 2006), 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 (Heuer and Smalla, 2007; Sengeløv et al., 2003). 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.**) contribute to the reduction of antibiotic contents in soil (Hurtado et al., 2017; Schwarz et al., 2015). Thereby it is assumed that the subsequent release of previously sequestered antibiotics is low (Rosendahl et al., 2011).

The knowledge on the fate and effects of pharmaceutical antibiotics in soil increased very much in the past years. However, until now most studies on the exact fate and/or effects of antibiotics in soil are largely restricted to a few structural classes, i.e. tetracyclines, sulfonamides, macrolides, aminoglycosides and quinolones (see Table 17, pg. 89). The mentioned deficits contrast with the legal obligation to assess the environmental fate of pharmaceuticals in the course of the authorization procedure (EMEA, 1997; FDA, 1987). For example, the existing trigger value of 100 µg/kg of soil is the same for all antibiotic compounds and classes (EMEA, 1997; Straub, 2002), and not scientifically justified, while limit values are lacking.

2 Use of antibiotics in pharmaceutical mixtures for medical application

The worldwide consumption of pharmaceutical antibiotics in human and veterinary medicine is steadily increasing. Between 2000 and 2010, the usage of antibiotic pharmaceuticals for human medicine increased by 35% (Van Boeckel et al., 2014): Cephalosporins and broad-spectrum penicillins accounted for 55% of the total standard units consumed in 2010. The largest absolute increases in consumption between 2000 and 2010 were observed for cephalosporins, broad-spectrum penicillins, and fluoroquinolones. The most substantial relative increases from 2010 were observed for monobactams (2031%), glycopeptides (232%), cephalosporins (94%), and fluoroquinolones (65%). Also increases in consumption rates of two last-resort classes of antibiotics were significant, i.e. carbapenems (45%) and polymyxins (13%). The three countries with largest consumption of antibiotics in 2010 were 1. India, 2. China and 3. the USA. Antibiotic consumption was stable or had moderately decreased between 2000 and 2010 in Germany and other high-income countries. On the contrary, antibiotic consumption increased substantially in developing countries, with the highest rates found in BRICS countries (Brazil, Russia, India, China, and South Africa) and French West Africa; 76% of the overall increase in global antibiotic consumption between 2000 and 2010 was attributable to BRICS countries (Van Boeckel et al., 2014). Penicillins, macrolides and fluoroquinolones were the highest selling classes in human medicine, when expressed in mg/kg of estimated biomass.

Table 4: Consumption of antimicrobials' active substance in humans and food-producing animals.

Estimated biomass of the corresponding populations in 1,000 t and consumption expressed in mg/kg biomass[a] in 28 EU and European Economic Area (EEA) member states, 2014[b].

Country	Including 2014 consumption at hospitals	Consumption of active substance (t)			Estimated biomass (1,000 t)[c]			Consumption (mg/kg biomass)	
		Humans	Animals	Total	Humans[d]	Animals	Total	Humans	Animals
Austria	No	38	53	91	532	948	1,480	70.9	56.3
Belgium	Yes	107	266	373	700	1,678	2,378	153.4	158.3
Bulgaria	Yes	53	33	85	453	393	846	116.0	82.9
Croatia	Yes	34	31	65	265	273	539	128.4	114.8
Cyprus	Yes	7	42	48	54	107	160	124.7	391.5
Czech Republic	No	65	56	121	657	703	1,360	99.4	79.5
Denmark	Yes	50	107	157	352	2,415	2,767	143.5	44.2
Estonia	Yes	6	10	16	82	127	210	71.7	77.1
Finland	Yes	47	11	59	341	509	850	139.2	22.3
France	Yes	717	761	1,479	4,118	7,120	11,238	174.2	107.0
Germany	No	287	1,306	1,593	5,048	8,749	13,797	56.9	149.3
Hungary	Yes	53	150	203	617	779	1,396	86.6	193.1
Iceland	No	2	1	3	20	116	136	101.7	5.2
Ireland	Yes	45	90	134	288	1,866	2,154	155.6	48.0
Italy	Yes	634	1,432	2,064	3,799	3,977	7,776	166.9	359.9
Latvia	Yes	10	6	17	125	173	298	81.6	36.7
Lithuania	Yes	19	12	31	184	335	519	102.5	35.5
Luxembourg	Yes	4	2	7	34	52	86	130.2	40.9

Country	Including 2014 consumption at hospitals	Consumption of active substance (t)			Estimated biomass (1,000 t)[c]			Consumption (mg/kg biomass)	
		Humans	Animals	Total	Humans[d]	Animals	Total	Humans	Animals
Netherlands	Yes	52	214	264	1,052	3,135	4,187	49.9	68.4
Norway	Yes	45	6	50	319	1,866	2,185	140.1	3.1
Poland	Yes	263	578	829	2,376	4,109	6,485	110.7	140.8
Portugal	Yes	76	190	266	652	942	1,594	116.1	201.6
Romania	Yes	226	98	323	1,247	2,502	3,749	181.7	39.1
Slovakia	Yes	47	16	64	338	248	587	140.2	65.9
Slovenia	Yes	14	6	19	129	171	300	105.5	33.4
Spain	No	327	2,964	3,291	2,907	7,077	9,984	112.6	418.8
Sweden	Yes	72	9	82	603	811	1,414	119.8	11.5
United Kingdom	Yes	518	430	939	4,022	6,915	10,937	128.7	62.1
All [a]		3,821	8,927	12,720	31,314	58,914	90,228	123.7[e]	151.5

[a] Calculated from the exact figures (not rounded as shown). [b] The estimates presented are crude and must be interpreted with caution. Countries with less than 95% data coverage for community consumption in humans were Germany (85%) and the Netherlands (92%). In those countries, the consumption expressed in tons (t), without correction for population or biomass, will be an underestimate. For further limitations that may hamper the comparison of the consumptions of antimicrobials in humans and in animals, please see chapter 14 of ECDC et al. (2017). [c] Information on the estimation of human and animal biomass is given in chapter 4.3 of ECDC et al. (2017). [d] Population covered by data in ESAC-Net. [e] Population weighted mean.

Source: ECDC et al. (2017), modified.

It was reported by ECDC et al. (2017) that from a total of 12,748 t of antibiotic pharmaceuticals consumed within the EU and associated countries in 2014, 30% were used in human medicine and 70% were used in veterinary medicine (Table 4). It is further documented in Table 4 that the consumption of antibiotics in human as well as in veterinary medicine is even larger in some other EU countries (e.g. Spain, Ireland) compared to Germany.

2.1 Veterinary medicine

Antibiotics typically used in veterinary medicine

Reports from 2011 estimated an annual production of pharmaceutical antibiotics of 20,000 t (Li et al., 2011). It is expected that the amount produced will further increase with the increasing global livestock production, especially in emerging and developing countries (Łukaszewicz et al., 2017; Rehman et al., 2015; Steinfeld, 2004). The antibiotics that were dispensed most often in Germany in the years 2011- 2015 are listed in Table 5. For 2015 a total amount of 805 t antibiotics were used as veterinary medicine. These were penicillins (299 t), tetracyclines (221 t), polypeptide antibiotics (82 t), sulfonamides (73 t), macrolides (52 t), and fenicols (5 t) (BVL, 2016a). Even 3rd and 4th generation cephalosporins were administered to food-producing animals at a dosage of 0.2 mg/kg of estimated biomass, compared to 3.8 mg/kg of estimated biomass administered to humans (ECDC et al., 2017). The amounts of the 'Highest Priority Critically Important Antimicrobials' with highest relevance for the medication of humans, i.e. fluoroquinolones and cephalosporins (Cephalosporins of 3rd and 4th generation), slightly declined in 2015 to 10.6 t and 3.6 t after increases in the previous years. The usage of antibiotics in mixtures or combinations of antibiotics was not researched by ECDC et al. (2017),

though. Yet, it can be assumed that the most often consumed antibiotics will also contribute most to a mixed environmental contamination.

Table 5: Comparison of delivery quantities of antibiotic compound classes from 2011 to 2015 [a].

Compound class	2011 [t]	2012 [t]	2013 [t]	2014 [t]	2015 [t]	Difference 2011-2015[t]
Aminoglycosides	47	40	39	38	25	-22
Cephalosp., 1st gen.	2	2	2	2.1	1.9	-0.1
Cephalosp., 3rd gen.	2.1	2.5	2.3	2.3	2.3	0.2
Cephalosp., 4th gen.	1.5	1.5	1.5	1.4	1.3	-0.2
Fenicols	6.1	5.7	5.2	5.3	5.0	-1.1
Fluoroquinolones	8.2	10.4	12.1	12.3	10.6	2.8
Folic acid antagonists	30	26	24	19	10	-20
Fusidic acid [b]						
Ionophores [b]						
Lincosamides	17	15	17	15	11	-6
Macrolides	173	145	126	109	52	-121
Nitrofuranes [b]						
Nitroimidazoles [b]						
Penicillins	528	501	473	450	299	-229
Pleuromutilines	14	18	15	13	11	-3
Polypeptide antibiotics	127	124	125	107	82	-45
Sulfonamides	185	162	152	121	73	-112
Tetracyclines	564	566	454	342	221	-343
Sum	**1,706**	**1,619**	**1,452**	**1,238**	**805**	**-901**

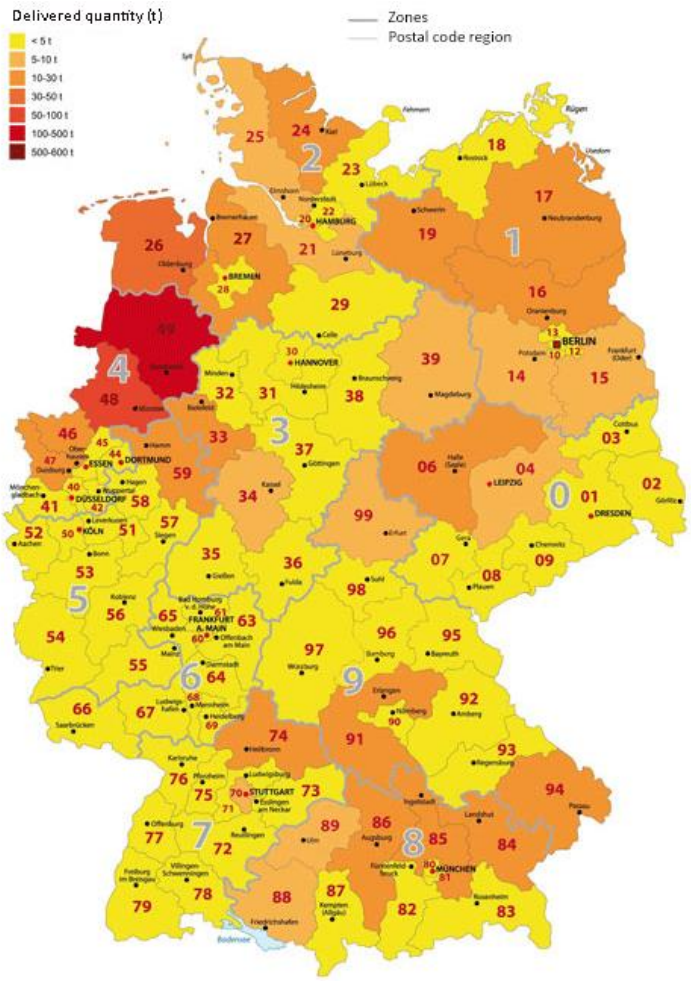
[a] Apparent inaccuracies or deviations in the quantities are due to round-off errors. [b] Data are not publicly available due to commercial and industrial confidentiality

Source: BVL (2016a).

The reported amounts of active ingredients cannot be assigned to individual animal species, because the majority of active ingredients is approved for the use with different animal species. Yet, the report of the LAVES (2012) documents exemplarily for the state of Lower Saxony, Germany, by average numbers for the years 2009 to 2010 the usage of antibiotics in livestock production, thus largely confirming previous EU wide studies (Ungemach, 2000). According to that report, 76% of broilers and between 84% and 100% of turkey (fattening and/or breeding) were treated with antibiotics in Lower Saxony. Furthermore, 68% of fattening pigs, 100 % of the fattening calves and 92% of animals in grazer breeding received antibiotics (Hannappel et al., 2017).

Figure 7: Regional allocation of delivered quantities of veterinary antibiotics in Germany in 2015.

Data in tons (t = Mg).



Source: Copyright Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL).

The use of veterinary antibiotics in Germany in the year 2015 was evaluated and geographically allocated by the BVL (2016a) using postal code regions (Figure 7). The highest delivered quantity was found in the postal code region 49. For this region as well as for the regions 16, 26, 27, 33, 47, 48, 49, 59 und 95 a decline in the delivered quantity was determined of 198 t (region 49) and of more than 10 t (the other above listed regions), respectively, from the year 2011 to 2015. On the other hand, the largest increase of about 1.2 t was found for the postal code region 70 (BVL, 2016a).

Antibiotic mixtures typically used in veterinary medicine

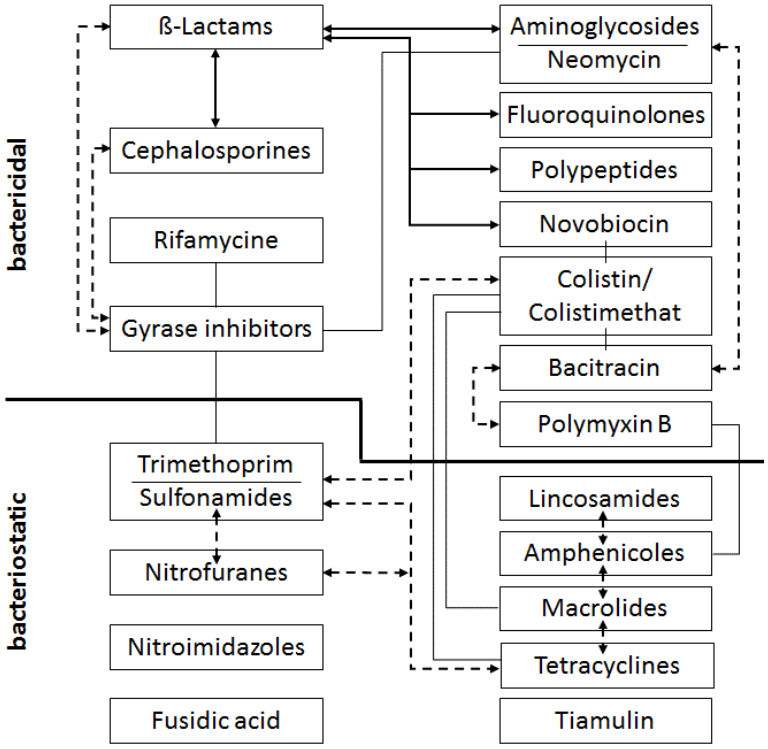
Often, not single compounds but combinations of different pharmaceutical antibiotics and/or further pharmaceuticals and components such as metals (e.g. silver Ag, copper Cu, zinc Zn) are administered in mixtures (Haeili et al., 2014; Poole, 2017; Russell and Hugo, 1994; Takahashi et al., 1987). Recommendations for antibiotic mixtures can be found in pertinent literature, (e.g. Bundestierärztekammer, 2015; Löscher et al., 2010; Schadewinkel-Scherkl and Scherkl, 1995). Combinations of antibiotics and other pharmaceuticals are meant to synergistically increase the antibiotic effect and/or to reduce the formation of resistance (Bollenbach, 2015). For example, well known and often applied is the combination of sulfonamides and trimethoprim.

While sulfonamides as structural analogues to *p*-aminobenzoic acid inhibit the formation of folic acid, trimethoprim blocks the dihydrofolate reductase enzyme so that both antibiotics synergistically interfere with the folic acid metabolism (Riviere, 2011). Remarkably, the mechanisms of pharmaceutical interactions are in most other cases unknown (Bollenbach, 2015).

The use of antibiotic combinations and/or of antibiotics mixed with other synergistic compounds is steadily increasing and will supposedly further increase in future (Caminero et al., 2010; Keith et al., 2005). Various studies showed that even old pharmaceuticals, sorted out in the past due to widespread resistance formation, can be revived in new combinations (Baym et al., 2016; Malik et al., 2014). However, not all combinations are meaningful or recommended because the joint effect can be synergistic on one hand but can also be antagonistic on the other (Bollenbach, 2015; Wood et al., 2012). Hence, specific combinations are recommended or should be avoided. Typical combinations of antibiotics and antibiotic classes, respectively, are shown in Figure 8 by the different lines.

Figure 8: Options for mixtures of veterinary antibiotic pharmaceuticals.

Typical combinations in one box, favorable combinations as thick arrows, useful combinations as solid lines and possible combinations as dotted lines.



Source: Modified from Schadewinkel-Scherkl and Scherkl (1995), Riviere (2011) and Bundestierärztekammer (2015).

Again, the knowledge on the effects of mixtures containing antibiotics is incomplete. Studies on binary combinations of two veterinary pharmaceuticals are quite numerous, while studies on mixtures of three and more compounds are scarce (Wood et al., 2012). Even more, most studies deal with one or a few pathogens used as test organisms (e.g. *Escherichia coli* and *Staphylococcus aureus*) but antibiotic effects and even more so effects of antibiotic mixtures can largely vary between species and endpoints (Bollenbach, 2015; González-Pleiter et al., 2013; Yan et al., 2011).

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 (Table 6). 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).

Table 6: 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.

Period October 2000 until September 2001.

Number of components	5	4	3	2	1	0
			including non-antibiotic compounds [a]			
Number of prescriptions	3	92	346	546	1110	
% of prescriptions	0.1	4.4	16.5	26.0	52.9	
			only antibiotic pharmaceuticals			
Number of prescriptions	2	54	246	504	1290	1 [b]
% of prescriptions	0.1	2.6	11.7	24.0	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.

Source: Thiele-Bruhn et al. (2003)

Table 7: Antibiotic compounds combined in 43 mixtures that were most often prescribed for use in medicated feedstuff; data base.

Data base: 2097 veterinary prescriptions of pharmaceuticals in the state of Mecklenburg-Western Pomerania; period October 2000 until September 2001

	Tetracyclines	Sulfonamides	Trimethoprim	Aminoglycosides	Benzimidazoles	Macrolides	Lincosamides	ß-Lactams	Polymyxines	Diterpenes
No. [a]	26	18	7	13	11	10	10	7	7	6
Mix of 5	CTC [b]	SDM			Flu			Pen	Col	
Mix of 4	CTC	SDM				Tyl		Pen		
	CTC			Spec	Flu		Lin			
	TC			Spec	Flu		Lin			
Mix of 3	CTC	SDZ	Tri							
	CTC	SDM			Flu					
	CTC	SDM						Pen		
	CTC	SDM				Tyl				
	OTC	SDZ	Tri							
	OTC	SDM+SMZ								
	TC	SDZ	Tri							
	CTC			Strep				Pen		
		SDZ	Tri		Fen					
		SDZ	Tri						Col	
		SDM				Tyl		AMX		
				Spec		Iver	Lin			
Mix of 2	CTC + TC									
	CTC	SDM								
	CTC			Apr						
	CTC				Flu					
	CTC					Iver				
	CTC						Lin			
	CTC								Col	

	Tetracyclines	Sulfonamides	Trimethoprim	Aminoglycosides	Benzimidazoles	Macrolides	Lincosamides	ß-Lactams	Polymyxines	Diterpenes
	CTC									Tia
	TC				Flu					
	TC								Col	
	TC									Tia
		SDZ	Tri							
		SDM				Tyl				
				Neo	Fen					
				Neo			Lin			
				Spec			Lin			
				Apr			Lin			
				Neo				AMX		
				Neo					Col	
				Neo						Tia
					Flu	Tyl				
					Flu		Lin			
Mix of 2					Fen					Tia
						Iver	Lin			
						Iver			Col	
						Iver				Tia
								AMX	Col	

[a] Number of mentions. [b] For abbreviations of antibiotics' names see list of abbreviations

Source: Thiele-Bruhn et al. (2003)

The antibiotic mixtures that were most often prescribed in the above mentioned study are listed in Table 7. It becomes clear that tetracyclines were the most dominant antibiotic class, followed by sulfonamides and aminoglycosides. Consequently, mixtures of antibiotics from these structural classes were most often prescribed. But also combinations with and of other antibiotics were used. Interestingly only in two mixtures antibiotics from the same structural class were combined, i.e. chlortetracycline combined with tetracycline and sulfadimidine with sulfamerazine (Table 7).

As was described in chapter 1 (Figure 2), mixtures of antibiotics might not only derive from the medical application of combination products but also from the consecutive use of different antibiotics and collection of all the compounds after excretion in one manure tank or lagoon. This is documented in Table 8 on example of the information of a veterinarian (Arenz-Leufen, 2012). The data listed in Table 8 show that different antibiotics from different antibiotic classes were used at the different life stages of the animals. At large farm units, animals of different life stages are typically reared in parallel. Consequently, the parallel use of different antibiotics will yield manure with mixed contamination.

Table 8: Administration of veterinary antibiotics in each pig production unit.

Written communication from anonymous veterinarian.

Pig unit	Antibiotic compound	Compound class	Therapeutic dose [a]	Application period
Sows + farrows	Enrofloxacin	FQs [b]	1.5 – 5	1 – 5 days
	Marbofloxacin	FQs	2 – 5	3 – 5 days
	Amoxicillin	BLs	20	24 hours
Weaners	Amoxicillin	BLs	20	24 hours
	Tulathromycin	MLs	2.5	1 injection
	Ceftiofur	CPs	3 – 5	3 – 5 days
	Tetracycline	TCs		1 injection
Fattening pigs (30 – 50 kg bodyweight)	Amoxicillin	BLs	20	24 hours
	Sulfadiazine/Trimethoprim	SAs	40/8	n.i.
	Tylosin	MLs	2 – 10	12 hours
Fattening pigs (>50 – 110 kg bodyweight)	Tylosin	MLs	2 – 10	12 hours
	Doxycycline	TCs	10 – 13	8 days
Sows – mating	Amoxicillin	BLs	20	24 hours
	Oxytetracycline	TCs	6 - 20	n.i.
Sows – finishing	Amoxicillin	BLs	20	24 hours
	Oxytetracycline	TCs	6 - 20	n.i.
Unspecified, application in all units	Sulfamethoxypyridazine + Trimethoprim (5:1)	SAs	30	3 – 5 days
	Tulathromycin	MLs	2.5	1 injection
	Amoxicillin	BLs	15	1-2 injections

[a] [mg/kg body weight]; [b] For abbreviations of the names of antibiotic classes see list of abbreviations; n.i. = no information

Source: Arenz-Leufen (2012)

2.2 Human medicine

Antibiotics typically used in human medicine

In many countries, more antibiotics are consumed in veterinary medicine compared to human medicine (ECDC et al., 2017) (see Table 4, pg. 45). In order to avoid overlaps, it is attempted to use different compounds in the two different fields of application. However, Table 9 shows that there is at least a strong overlap in the classes of antibiotics used for the two different purposes. Penicillins are most often used in both veterinary and human medicine, and macrolides, tetracyclines and sulfonamides are also among the most often prescribed groups. This means that environmental contamination with antibiotics resulting from use in human medicine or veterinary medicine is – related to antibiotic classes – not largely different but will add up.

The picture looks somewhat different when not only data on Germany but on 28 EU and European Economic Area (EEA) member states are combined (ECDC et al., 2017). However, it must be stated that only data on selected antibiotic classes where compiled in Table 10.

Table 9: Prescribed doses[a] of selected antibiotics and chemotherapeutics in Germany in recent years.

[a] In million defined daily doses – DDD.

	Antibiotic groups	2012	2014	2016
1	Penicillins and Aminopenicillins	114	116.4	121.9
2	Cephalosporins	72	75.6	78.3
3	Macrolides and Clindamycin	68	63.2	59.1
4	Tetracyclines	57	52.4	48.9
5	Fluoroquinolones	37	33.4	31.8
6	Sulfonamides and combinations with trimethoprim	13	12.5	11.6

Source: Data from Schwabe et al. (2017).

Table 10: Range, median and population-weighted average of the consumption of the antimicrobial classes selected for analysis in humans and food-producing animals in 28 EU/EEA member states in 2014.

Data expressed in mg/kg estimated body weight and results of Spearman's rank correlation analysis of consumption in animals and humans within country.

Antimicrobial class	Humans			Animals			ρ[b] (p-value)
	range	median	average[a]	range	Median	average	
Cephalosporins, 3rd and 4th generation	<0.01-12.1	2.0	3.8	<0.01-0.8	0.2	0.2	0.22 (0.251)
Fluoroquinolones and quinolones	3.1-17.4	6.2	8.1	0-11.6	1.7	3.5	0.56 (0.002)
Polymyxins	0-0.1	0.01	0.03	0-36.1	1.3	10.0	0.30 (0.122)
Macrolides	1.5-19.8	6.5	7.8	0-27.5	4.9	11.4	0.32 (0.100)
Tetracyclines	0.3-13.5	1.8	3.6	0.1-151.5	25.1	50.6	-0.035 (0.058)

[a] Population weighted mean.

[b] Spearman's rank correlation coefficient.

Source: ECDC et al. (2017).

Table 11: Share of the strongest consumed antibiotics of the total antibiotics consumption in German hospitals in the year 2013-2014.

	Antibiotic	Share of total consumption* (%)	Antibiotic class
1	Cefuroxime	15.3	Cephalosporin 2nd generation
2	Piperacillin-Tazobactam[a]	9.3	Penicillins
3	Ciprofloxacin	8.2	Fluoroquinolone
4	Ceftriaxon	7.8	Cephalosporin 3rd generation
5	Metronidazole	6.5	Nitroimidazoles

[a] Tazobactam is a ß-lactamase inhibitor

Data from BVL (2016b).

The individual antibiotic compounds that were most often prescribed in German hospitals in human medicine are listed in Table 11. It shows that especially ß-lactams and from this antibiotic class especially 2nd and 3rd generation cephalosporins are used that are reserved for human medicine only. So, on the level of individual compounds the overlap between antibiotics used for human and veterinary purposes is clearly less.

Antibiotic mixtures typically used in human medicine

The combination of pharmaceutical antibiotics for the treatment of infectious diseases is a usual measure in human medicine. Several positive effects can be reached (Füssle, 2011):

- Broadening of the spectrum of activity, e.g. by combining antibiotics against gram positive and gram negative pathogens,
- synergistic effect enhancement, e.g. by combining ß-lactams and aminoglycosides,
- retardation of resistance development, e.g. of pseudomonads,
- inhibition of degrading enzymes, e.g. ß-lactams and ß-lactamase inhibitor, by combinations of antibiotics and the respective enzyme inhibitors,
- inhibition of the synthesis of bacterial toxins (antibiotic effects on bacteria can trigger the production of bacterial toxins as reaction, which is called toxic-shock syndrome, leading to further deterioration of the human health status). For example, combinations of antibiotics with clindamycin inhibit toxic-shock with staphylococcus and streptococcus bacteria.

Combinations of antibiotics should follow some general rules (Füssle, 2011):

- Antibiotics with different target site should be combined, e.g. ß-lactams (cell wall) and aminoglycosides (ribosomes) or quinolones (DNA),
- combinations of antibiotics should have a different mode of action,
- even more, antibiotics with the same target site could hinder each other, so that antagonistic effects result, e.g. clindamycin and macrolides both targeting the 50S subunit of ribosomes,
- carbapenems induce ß-lactamase enzymes, degrading ß-lactams, and should thus not be combined with ß-lactams,
- the rule that bacteriostatic antibiotics should not be combined with antibiotics acting in the growth phase of bacteria (e.g. ß-lactams), however, is not always valid.

Transferring these rules to a soil contamination means, that a desirable effect enhancement in human therapy will lead to unwanted effect amplification on non-target soil organisms.
Some typical combinations of pharmaceutical antibiotics and other pharmaceuticals that are used in human medicine are listed in Table 12.

Not only combinations of antibiotic pharmaceuticals but also **combinations of antibiotics with other bioactive chemicals** can synergistically enhance the antibiotic effect. Ejim et al. (2011) screened bioactive compounds for possible combinations with antibiotics to yield mixtures with stronger antibiotic activity against human pathogens. Among the 156 compounds identified by a first data screen were numerous pharmaceuticals that were used for other purposes than antibiosis, and also vitamins and even pesticides such as DDT. Tests with the antibiotic minocycline showed that some of these combinations did not only synergistically enhance the antibiotic effect but in some cases antibiosis even against microorganisms with known resistance against minocycline was determined, including selected methicillin-resistant *Staphylococcus aureus* (MRSA) strains (Ejim et al., 2011). Antineoplastic agents such as 5-fluorouracil were shown to be bioactive as well, and some combinations with ß-lactams resulted in synergistic enhancement of the antibiotic effect, while other (methotrexate and cefotiam) resulted in an antagonistic mixture effect (Gieringer et al., 1986). Apitoxin, known as honey bee venom, is a protein-containing liquid with anti-inflammatory activity. Combinations of apitoxin with ampicillin, penicillin (both ß-lactams), gentamicin (aminoglycoside) or vancomycin (peptidoglycan) were tested for their effect on growth of MRSA strains (Han et al., 2016). Apitoxin exhibited

antibacterial activity and in combination with the four tested antibiotics at least partial, synergistic, antibiotic-enhancing effects against MRSA strains.

Table 12: Typical mixtures of antibiotic and other pharmaceuticals used or tested in human medicine to reach an effect amplification.

Color code: green = synergism; red = antagonism; yellow = none of both.

Compounds mixed	Effect	Test organisms	Reference
ß-Lactams with cytostatic 5-fluorouracil (amoxicillin, cefazolin, cefuroxim, piperacillin) + (5-fluorouracil)			(Alexy et al., 2006)
5FU : amoxicillin 5FU : cefazolin 5FU : cefuroxim 5FU : piperacillin	synergism	*Ps. Putida,* *E. faecalis*	
5FU : mix of amoxicillin + cefazolin + cefuroxim + piperacillin			
ß-Lactams with antineoplastic agents (cefoperazone, piperacillin, cefazolin, carbenicillin) + (mitomycin C, bleomycin, adriamycin, 5-fluorouracil, carboquone)			(Ueda et al., 1983)
Most combinations	neither synergistic nor antagonistic	*E. coli*	
Cefazolin : 5-fluorouracil Piperacillin : 5-fluorouracil Carbenicillin : 5-fluorouracil	slightly synergistic		
Most combinations	neither synergistic nor antagonistic	*K. pneumoniae*	
Piperacillin : bleomycin	synergistic		
Cefoperazone : carboquone Cefazolin : carboquone	antagonism		
Cefoperazone : adriamycin Cefazolin : adriamycin Piperacillin : adriamycin	approaching antagonism		
Most combinations	neither synergistic nor antagonistic	*P. vulgaris*	
Cefoperazone : mitomycin C Cefoperazone : bleomycin Cefoperazone : 5-fluorouracil Piperacillin : mitomycin C Piperacillin : bleomycin Carbenicillin : mitomycin C Carbenicillin : bleomycin Carbenicillin : adriamycin Carbenicillin : 5-fluorouracil	synergism		
Most combinations	neither synergistic nor antagonistic	*P. aeruginosa*	
Piperacillin : mitomycin C Piperacillin : 5-fluorouracil	synergism		

Compounds mixed	Effect	Test organisms	Reference
Cefoperazone : carboquone Piperacillin : carboquone Carbenicillin : carboquone Carbenicillin : adriamycin	antagonism		
4 Antineoplastic agents and 5 antimicrobial pharm. (ceftriaxone, ceftazidime, cefotiam, netilmicin, piperacillin) + (5-fluorouracil, mitoxantrone, methotrexate, vincristine)		*Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Serratia arcescens, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis*	*(Gieringer et al., 1986)*
5-Fluorouracil : ceftriaxone 5-Fluorouracil : ceftazidime 5-Fluorouracil : cefotiam 5-Fluorouracil : piperacillin	predominantly synergistic (> 55% of species strains)		
5-Fluorouracil : netilmicin Mitoxantrone : cefotiam Mitoxantrone : netilmicin Vincristine : ceftriaxone Vincristine : netilmicin	slightly synergistic (< 35% of species strains)		
Methotrexate : cefotiam	antagonistic (42 % of species strains)		
5-Fluorouracil : ceftriaxone 5-Fluorouracil : cefotiam 5-Fluorouracil : piperacillin Mitoxantrone : ceftriaxone Mitoxantrone : cefotiam Vincristine : ceftazidime Methotrexate : ceftriaxone Methotrexate : ceftazidime	slightly antagonistic (< 20% of species strains)		
5-Fluorouracil : netilmicin Mitoxantrone : ceftriaxone Mitoxantrone : ceftazidime Mitoxantrone : cefotiam Mitoxantrone : netilmicin Mitoxantrone : piperacillin Vincristine : ceftriaxone Vincristine : ceftazidime Vincristine : cefotiam Vincristine : netilmicin Vincristine : piperacillin Methotrexate : ceftriaxone Methotrexate : ceftazidime Methotrexate : cefotiam Methotrexate : netilmicin Methotrexate : piperacillin	predominantly indifferent (> 55% of species strains)		

3 Mixed antibiotic contaminations in organic waste materials and soils

The number of studies and publications, respectively, on the input to and residual contents of pharmaceutical antibiotics in the soil environment is steadily increasing. In all that studies several different antibiotics have been investigated and results show consistently that not only single antibiotics but mixed contaminations must be expected (e.g. Awad et al., 2014; Karci and Balcioğlu, 2009; Kuppusamy et al., 2018; Łukaszewicz et al., 2017; Ok et al., 2011; Sun et al., 2017; Watanabe et al., 2010). However, irrespective of that coherent state of knowledge, the number of studies that can be used for further evaluation is still scarce. This is because one or several of the following drawbacks apply:

- Data are not fully published but aggregated data (e.g. average values over series of samples) are published (in some cases even with incomplete information which and how many samples have been averaged). In case results from several individual samples, e.g. samples taken from different sites, are combined in one table or figure, it remains unclear, whether and which combinations of antibiotics existed in individual samples.
- Data are not available in tables but are only shown in figures.
- Data are restricted to few, targeted chemicals while studies analyzing larger numbers of antibiotics from various structural classes are scarce.
- Dissimilar and only partly matching (sets of) antibiotics, respectively, are researched in different studies.
- Methodical aspects of the trace level analysis of pharmaceutical antibiotics in waste materials and soils, respectively, are the real issue of the publication.
- Identification of investigated soils is insufficient or lacking.
- In few cases data on residual contamination levels of both waste material and of soil samples have been published. However, in these publications it is seldom documented whether the analyzed soil was fertilized with the analyzed waste material. Consequently, it is hardly possible to exactly follow the contamination route and resulting contamination of soils based on information from the literature.

In order to identify the status of soil contamination with mixtures of pharmaceutical antibiotics, the literature was researched for publications on contamination levels of organic waste materials used as fertilizer (chapter 3.1) and of soils (chapter 3.2).

3.1 Occurrence and residual contents of mixed contaminations with antibiotics and other pharmaceutical compounds and elements in organic waste materials

From pharmacokinetics it is well known that large amounts of antibiotics are released from the medicated body within time periods of hours and up to not more than several days (Riviere, 2011). The percentage of excreted parent compounds and antibiotic active metabolites, respectively, (Kümmerer, 2008) or of conjugates that can be reconverted to the parent compound in the environment (Lamshöft et al., 2007; Lamshöft et al., 2010; Langhammer, 1989) varies. It depends on the administration route, e.g. orally or by injection, the animal species and life stage of the animal as well as on boundary conditions affecting the physical condition of the medicated organism. Typically, between around 50% and up to >90% of the administered parent compound are quickly excreted (Kuppusamy et al., 2018; Riviere, 2011).

In any case, substantial amounts of antibiotics as well as of other pharmaceuticals and elements with antibiotic properties such as copper are excreted by medicated organisms and end up in waste material (Du and Liu, 2012; Sarmah et al., 2006). Manure is especially contaminated when antibiotics are (prophylactically) applied to complete herds so that large total quantities are dispensed (Cleary et al., 2016). Because the waste materials are often used as organic fertilizer and spread onto agricultural soils, environmental samples are frequently found to be contaminated with pharmaceuticals and antibiotics in special (Li et al., 2013;

Łukaszewicz et al., 2017; Monteiro and Boxall, 2010). Published studies cover excreta and manure, wastewater and sewage sludge. In all these publications mixed contaminations with numerous antibiotics and with other pharmaceuticals are reported. Hence, it must be concluded that the occurrence of mixed contaminations is the rule rather than the exemption.

A typical example for the use of various pharmaceutical antibiotics in livestock husbandry and their accumulation in manure is given in Table 13. From the antibiotic compounds analyzed in 24 manure samples, nine different antibiotics were detected in the samples alone or in mixtures with each other. None of the manure samples was free from antibiotics or contained only one compound, yet, at least two and up to six antibiotics were determined in concentrations above the detection limit.

Table 13: Antibiotic concentration in unprocessed pig manure from different stable sections housing pigs of different life stages.

Average of three replicates, standard deviation in brackets; for abbreviations of antibiotics' names see the list of abbreviations.

		Sulfonamides		Tetracyclines				Fluoroquinolones		
Sample		**SDZ** [a]	**SMP**	**CTC**	**DOX**	**OTC**	**TC**	**Cipro**	**Enro**	**Marbo**
		in µg/kg dry manure								
Sows + farrows										
1	*A*[b]	n.d.	n.d.	n.d.	138 (±19.9)	1820 (±395)	39.2 (±7.52)	121 (±18.8)	148 (±12.4)	1520 (±58.4)
2	A	n.d.	n.d.	n.d.	90.7 (±14.6)	n.d.	n.d.	567 (±17.5)	629 (±11.1)	2930 (±126)
3	*B*[b]	n.d.	n.d.	n.d.	n.d.	5055 (±635)	96.8 (±7.53)	n.d.	47.4 (±5.7)	256 (±33.8)
4	B	n.d.	n.d.	n.d.	n.d.	2180 (±470)	n.d.	n.d.	47.1 (±7.06)	138 (±13.3)
Weaners										
1	A	n.d.	n.d.	n.d.	202 (±15.9)	46.3 (±9.91)	n.d.	17.6 (±4.13)	62.7 (±4.90)	3.92 (±0.11)
2	A	6.58 (±1.85)	n.d.	n.d.	41.1 (±13.7)	n.d.	n.d.	31.3 (±10.7)	75.1 (±25.7)	8.36 (±2.29)
3	B	3.25 (±0.13)	n.d.	n.d.	n.d.	150 (±8.96)	n.d.	n.d.	n.d.	33.2 (±2.13)
4	B	14.0 (±1.72)	n.d.	n.d.	n.d.	541 (±38.4)	n.d.	n.d.	n.d.	26.6 (±4.84)
5	B	2.49 (±2.17)	n.d.	n.d.	n.d.	266 (±2.42)	n.d.	n.d.	n.d.	19.7 (±1.91)
Fattening pigs										
1	A	10.7 (±1.40)	n.d.	n.d.	86.3 (±8.91)	61.0 (±23.6)	47.6 (±5.41)	n.d.	2.75 (±0.47)	53.4 (±0.38)
2	A	4.76 (±0.77)	n.d.	n.d.	1890 (±290)	45.2 (±4.36)	7.34 (±0.74)	n.d.	3.31 (±0.56)	26.9 (±3.41)
3	A	n.d.	n.d.	n.d.	75.3 (±19.2)	n.d.	n.d.	n.d.	n.d.	75.8 (±27.4)
4	A	n.d.	n.d.	n.d.	41.6 (±7.19)	n.d.	n.d.	n.d.	n.d.	35.0 (±7.84)

Sample		Sulfonamides		Tetracyclines				Fluoroquinolones		
		SDZ [a]	SMP	CTC	DOX	OTC	TC	Cipro	Enro	Marbo
		in µg/kg dry manure								
Sows – mating										
1	A	25.5 (±6.45)	n.d.	n.d.	40.1 (±3.97	1445 (±262)	19.0 (±4.57)	n.d.	n.d.	37.2 (±4.94)
2	B	2.07 (±0.37)	11.4 (±1.05)	9.45 (±0.32)	n.d.	3295 (±407)	254 (±24.6)	n.d.	n.d.	3.39 (±0.27)
3	B	n.d.	5.11 (±0.82)	49.4 (±3.49)	n.d.	45 150 (±1775)	286 (±31.4)	n.d.	n.d.	2.48 (±0.49)
4	B	n.d.	n.d.	n.d.	n.d.	1475 (±250)	31.9 (±7.93)	n.d.	n.d.	n.d.
5	B	n.d.	n.d.	39.5 (±4.34)	n.d.	1990 (±177)	32.3 (±5.74)	n.d.	n.d.	n.d.
6	B	11.3 (±0.95)	8.44 (±0.95)	498 (±60.4)	n.d.	5330 (±503)	123 (±7.07)	n.d.	n.d.	10.0 (±1.58)
Young sows										
1	B	n.d.	n.d.	n.d.	n.d.	111 150 (±3285)	472 (±29.5)	n.d.	n.d.	n.d.
2	B	1.78 (±0.03)	n.d.	7.94 (±2.06)	n.d.	5995 (±319)	63.1 (±12.5)	n.d.	n.d.	n.d.
3	B	1.23 (±0.09)	n.d.	n.d.	n.d.	85.7 (±9.76)	n.d.	n.d.	n.d.	n.d.
Digested lagoon manure										
1	B	3.13 (±0.53)	n.d.	n.d.	n.d.	949 (±129)	27.1 (±1.79)	n.d.	n.d.	n.d.
2	B	24.4 (±1.99)	n.d.	n.d.	n.d.	6085 (±819)	74.7 (±3.52)	n.d.	n.d.	n.d.

[a] SDZ = sulfadiazine, SMPD = sulfamethoxypyridazine, CTC = chlortetracycline, DOX = doxycycline, OTC = oxytetracycline, TC =tetracycline, Cipro = ciprofloxacin, Enro = enrofloxacin, Marbo = marbofloxacin.
[b] The letters *A* and *B* represent the origin of the manure samples from two different farms.

Source: Table from Arenz-Leufen (2012), modified.

Out of the 20 individual antibiotics investigated in the study of Arenz-Leufen (2012) nine were identified in the different samples. In more detail that were two out of 12 sulfonamides plus trimethoprim, four out of four tetracyclines, three out of four fluoroquinolones. The antibiotics recovered in the lagoon samples even resisted anaerobic digestion for biogas production and further storage time that was estimated to range between 8 and 12 weeks (Arenz-Leufen, 2012). This study exemplarily shows that mixtures of antibiotic compounds emerge from the consecutive collection and storage of manure. This happens because (i) antibiotics are administered as a mixed medical preparation, (ii) excreta are collected over time periods covering different medications, and (iii) excreta are collected from different animal groups and life stages, receiving different medication (see Figure 2, page 34).

Data from nine studies on the occurrence and concentrations of four to 22 different pharmaceutical antibiotics in organic waste materials are compiled in Table 14. The studies cover in total 42 antibiotics from different structural classes and 649 samples from manure, digestate and mixtures of manure and digestate, sewage sludge and compost. The studies were selected because data were fully available (e.g. through appendices and supporting information, respectively) or at least clearly assignable. They show that the majority of the antibiotics that had been analyzed could be recovered in determinable concentrations in the investigated samples.

Table 14: Contamination level of organic waste materials used for soil fertilization with pharmaceutical antibiotics and metals (Cu, Zn).

Compounds that were analyzed in the evaluated studies are highlighted in blue. Numbers of samples (# sample), numbers of antibiotics analyzed in the study (# antib), average numbers of antibiotics detected in each individual sample (mean #) and highest numbers of antibiotics detected in one individual sample (max #) are indicated. (For abbreviations of antibiotics' names see the list of abbreviations).

Study	Sattelberger (1999)			Arenz-Leufen (2012)			Winckler et al. (2004)			An et al. (2015)			Ratsak et al. (2013)			Hannappel et al. (2017)			Ratsak et al. (2013)			Clara et al. (2013)			Li et al. (2013)			An et al. (2015)			McClellan and Halden (2010)			Clara et al. (2013)		
	Manure (especially pig slurry)															Man./Digest.[a]			Digestate			Sewage Sludge												Compost		
# sample	61			19			176			51			34			83		35			6	45			18			110			11					
# antib	11	13	Cu/ZnP[b]	6			4			11			20			11			20			7	9	Cu/Zn	22			8			20			7	9	Cu/Zn
mean #	2	4	Cu/Zn	2			1			>3			>2			2			>3			3	4.7	Cu/Zn	18			>3			>15			2	3.3	Cu/Zn
max #	5	7	Cu/Zn	4			4			>5			>3			7			>5			5	7	Cu/Zn	18			>5			>20			3	5	Cu/Zn
	#[c]	av[d] mg/kg	max[e] mg/kg	#	av mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av[f] mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av[f] mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av mg/kg	max mg/kg
TC	17	4.57	23	4	0.48	0.97	87	9.73	43.1	41	4.69	57.0	12	Pf	2.45				9	Pf	17.0							18	1.55	7.37	110	1914	2790			
OTC	21	6.95	29	15	45.9	451	9	21.5	136	48	18.5	47.3	7		3.60				0									18	1.01	2.17	110	87.5	114			
CTC	20	8.46	46	4	1.49	4.98	18	6.22	25.7	49	45.1	144	5		1.49				0									18	0.85	3.84	66	23.4	43.5			
DOX										32	1.74	6.50																10	0.75	2.10	110	966	1780			
Tri	11	3.37	17										1		0.05	15	0.19	0.59	0			2	0.03	0.03							66	26.0	60.5	5	0.03	0.03
SAcA													0						1		0.12															
SCP													0			13	0.06	0.10	1		0.03															
SDZ	10	16.1	91	9	0.09	0.26	86	4.89	35.3	28	0.80	4.98	5		0.65	41	0.56	13.0	14		6.25	0			48	0.01	0.02	14	0.02	0.06				0		
SDMX																34	0.25	2.10							0											
SDM	16	2.73	20							18	0.33	1.95	6		7.04	0			11		0.88	0			41	0	0.02	8	0.02	0.03				0		
SDX	0															0																				
SIA																									0											
SMrZ										14	1.17	4.59	0			12	0.03	0.06	1		0.07				0			9	0.01	0.04						
SMT													0						2		0.23															
SMX	0									21	1.14	18.0	0			1	0.03	0.03	3		0.16	6	0.01	0.01	45	0.01	0.02	10	0.03	0.67	22	0	3.3	5	0.003	0.01
SMP													4		0.02				1		0.05															
SMM																13	0.04	0.17							45	0.001	0.004									
SAA																															44	0	87.3			
SPY																1	0.03	0.03							47	0.01	0.02									
SQO													3		0.67				0																	

Study	Sattelberger (1999)			Arenz-Leufen (2012)			Winckler et al. (2004)			An et al. (2015)			Ratsak et al. (2013)			Hannappel et al. (2017)			Ratsak et al. (2013)			Clara et al. (2013)			Li et al. (2013)			An et al. (2015)			McClellan and Halden (2010)			Clara et al. (2013)		
	Manure (especially pig slurry)															Man./Digest.[a]			Digestate			Sewage Sludge												Compost		
	#c	av[d] mg/kg	max[e] mg/kg	#	av mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av[f] mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av[f] mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av mg/kg	max mg/kg	#	av mg/kg	max mg/kg
STZ	2	0.36	0.61													0									53	0	0.001									
Cipro	14	0.67	0.67	4	1.84	5.67							3		0.07				15		1.62				39	1.46	4.87				110	6858	10800			
Dano													1		0.05				7		0.97															
Diflox													0						6		3.40															
Enro	16	1.45	8.30	8	1.27	6.29							5		0.55				20		1.09				0						66	0	28.6			
Flero																									55	1.46	4.87									
Lome																									44	1.46	4.87				44	0	16.1			
Marbo													3		0.05				0						35	1.43	4.76									
Nor																									41	1.54	4.98				110	289	418			
Oflox																									46	1.50	4.98				110	5446	8140			
Orbi													1		0.02				0																	
Sara													1		0.06				1		0.02				43	0.10	0.33									
Azithro																															110	838	1220			
Clary																						4	0.15	0.38							110	66.2	94.6	5	0.02	0.04
Ery																						3	0.01	0.02	39	0.03	0.14				110	81.5	183	5	0.02	0.05
Josa																									32	0.003	0.02									
Mino																															88	1884	2630			
Roxy																						3	0.01	0.02	33	0.03	0.14							0		
Spira																									42	0.02	0.09									
Tylosin																									28	0.002	0.01									
Thiaben																															110	110	370			
Triclo																															110	12640	19700			
Cu	61	172	580																			5	234	270										8	144	180
Zn	61	524	1300																			5	876	1100										8	421	670

[a] Manure, digestate and mixtures thereof. [b] Cu/Zn = numbers including the detection of Cu and/or Zn in the samples. [c] # = number of samples in which the specific antibiotic was detected. [d] av = average concentration in all samples. Average was calculated only for data above the limit of detection, and thus without considering zero values. [e] max = highest concentration determined in one of the investigated samples. [f] no data on average concentrations were given in the study of Ratsak et al. (2013).

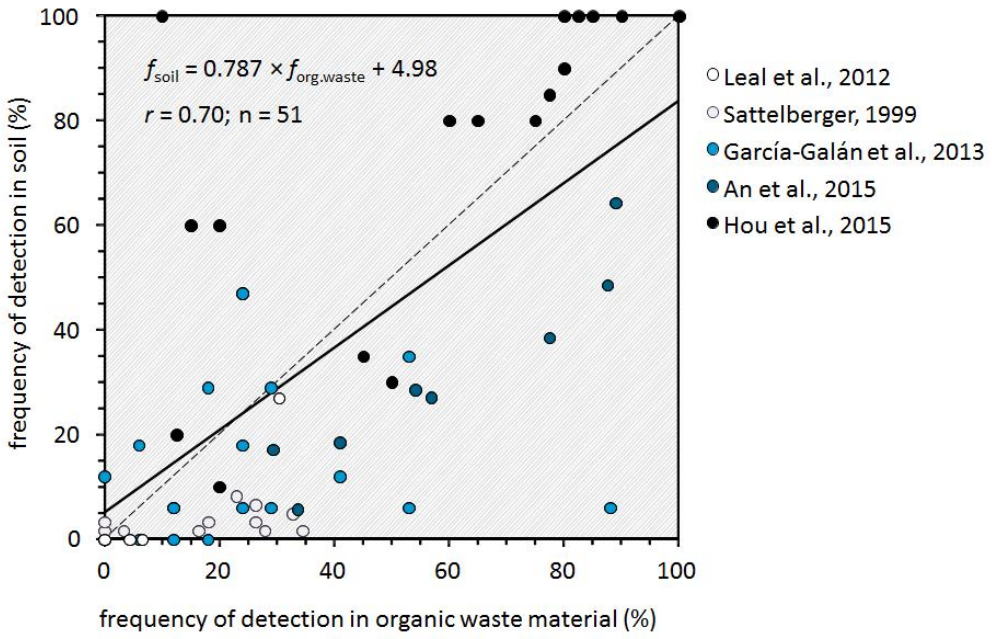
The studies consistently show that the waste materials are typically contaminated with more than one compound. In the study of Winckler et al. (2004), though, only one antibiotic was detected on average, yet out of a total of only four antibiotics investigated. In most studies contaminations with on average ≥2 and up to 7 different antibiotics are reported for manure (Table 14). Substantially more antibiotics (up to >20) were found in sewage sludge samples, while the reports on digestate and compost showed a similar picture as for manure. However, it must be noted that the intensity of investigation differed among studies and substrates. While in manure samples around 10 antibiotics were analyzed, it were around 15 antibiotics in sewage sludge samples. Additionally, the antibiotics investigated vary somewhat between studies. While tetracyclines and sulfonamides as typical pharmaceuticals in veterinary medicine were most of all investigated in manure samples, the spectrum of analytes was clearly broader for sewage sludge samples, including fluoroquinolones and macrolide antibiotics. It shows that the level of knowledge on the full bandwidth of contamination of organic waste material is still largely incomplete; data on other antibiotic classes such as ß-lactams were fully missing in the evaluated studies.

The contamination status of wastewater was not explicitly evaluated in this study. However, the existing literature clearly points out that wastewater and treated wastewater, after passing through a treatment plant, is typically contaminated with complex mixtures of pharmaceuticals, including pharmaceutical antibiotics (Kostich et al., 2014). Concentration levels of single compounds are in the range of ng/L up to the lower µg/L range (Clara et al., 2013; Hannappel et al., 2017).

Because organic waste materials are recycled as soil fertilizer, also the unwanted contaminants end up on agricultural soils. Hence, it can be expected that the contamination of soil is linked to that of organic waste material. This is shown in Figure 9 for which data of five studies were evaluated that had investigated both organic waste materials and soils.

Figure 9: Correlation between the frequency (f) of detection of different pharmaceutical antibiotics in organic waste materials (manure, sewage sludge) and in soils that had been fertilized with these materials.

The correlation line (solid) and the 1:1 line (dashed) are shown.



Source: Own figure from S. Thiele-Bruhn (Univ. Trier).

Although it is not fully clear from the studies whether the soils were in fact fertilized with the investigated waste materials and in which quantities and time periods before soil sampling this had happened, the relation between both is clear. The frequencies of detection of a specific antibiotic in a set of samples of organic waste materials and of soils are positively and significantly correlated (r = 0.7). This again emphasizes that pharmaceutical antibiotics that are contained in waste material used as soil fertilizer will end up in soil. Mixed contaminations in organic waste materials result in mixed contaminations in soil. Due to the dilution in soil (for example typical amounts of manure applied to soil are 30 t/ha and 1 ha of agricultural topsoil amounts to about 3000 t of soil) and the in part fast and strong immobilization in soil, it may not be possible to recover all these antibiotics in soil as is shown by the correlation line that deviates from the 1:1 line and by the typically lower contamination level determined in soil samples (see chapter 4). While contaminations with antibiotics in organic waste material are in the range of mg/kg, in soil they are in the range of µg/kg.

3.2 Occurrence and residual contents of mixed contaminations with antibiotics and other pharmaceutical compounds and elements in soil

Natural background level

Antibiotics naturally occur in soil and are formed by the secondary metabolism of autochthonous soil microorganisms (Raaijmakers and Mazzola, 2012; Scott Wells et al., 1982; Thomashow et al., 1997). For example, streptomycin and oxytetracycline are well-known soil-borne antibiotics and are produced by *Streptomyces* actinobacteria (Nkanga and Hagedorn, 1978; Schatz et al., 1944). Among numerous other soil microorganisms, 30 to 50 % of actinomycetes isolated from soil are able to synthesize antibiotics (Topp, 1981). Typical resulting soil contents are reported to be in the range of µg/kg and especially occur in the soil rhizosphere (Lumsden et al., 1992; Soulides, 1965). As an extreme, Shanahan et al. (1992) reported soil contents of up to 5 mg/kg for the lipopeptide iturin A. However, no such natural background exists for antibiotics that are exclusively produced by pharmaceutical industry and especially for fully synthetic antibiotics, i.e. chemotherapeutics such as the sulfonamides. In publications working with artificially spiked soils, background concentrations of antibiotics in the unamended control samples are consistently reported to be below the detection limit (e.g. Thiele-Bruhn, 2005).

Contamination status

Appropriate fertilization of soil using manure and other organic waste materials or even unwanted inputs, e.g. with stable dust (see chapter 1.1), can result in detectable residue contents of veterinary antibiotics in soils (Table 15). The significant correlation between the frequency of detection of antibiotics in waste material used as fertilizer and in the receiving soils is shown in Figure 9 (pg. 62). Gildemeister et al. (2011) showed that through these pathways also non-antibiotic pharmaceuticals are introduced into agricultural soils, which are especially analgesics and anti-inflammatory pharmaceuticals. Antibiotics from human medicine reach soils with sewage sludge, used as fertilizer, and by irrigation of soil using wastewater, which is especially relevant in semiarid countries (Christou et al., 2017; Khalid et al., 2018).

Typical soil contents of antibiotics are in the order of µg/kg (Table 15). Thus, residual contents of single antibiotic compounds may exceed the natural background level and in part even the trigger value of the European Medicines Agency of 100 µg/kg (EMEA, 1997). The studies in Table 15 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. The determined 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) (Hamscher and Mohring, 2012; Hembrock-Heger et al., 2011; Thiele-Bruhn, 2003b). This is due to (i) a different usage and thus release into the environment, (ii) a different persistence and mobility in soil.

Table 15: Contamination level of soils with pharmaceutical antibiotics, some other pharmaceuticals and metals (Cu, Zn).

Compounds that were analyzed in the evaluated studies are highlighted in blue. Numbers of samples (# sample), numbers of antibiotics analyzed in the study (# antib), average numbers of antibiotics detected in each individual sample (mean #) and highest numbers of antibiotics detected in one individual sample (max #) are indicated. (For abbreviations of antibiotics' names see the list of abbreviations).

Study	**Sattelberger (1999)**			**Hamscher et al. (2000), 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)**		
# sample	**61**			**14**			**42**			**8**			**70**			**2**			**104**			**5**		
# antibiotics	**11**			**4**			**2**			**7**			**8**			**9**			**11**	**14**	**+pha.**	**13**		
mean # antib	**0.2**			**1.9**			**1.5**			**1.0**	**3**	**Zn/Cu**	**>2**			**6.5**			**0.02**	**0.04**	**+pha.**	**8.4**		
max # antib	**4**			**2**			**2**			**2**	**4**	**Zn/Cu**	**>4**			**7**			**2**	**4**	**+pha.**	**14**		
	#[a]	**av.[b] µg/kg**	**max[c] µg/kg**	**#[a]**	**av.[b] µg/kg**	**max[c] µg/kg**	**#[a]**	**av.[b] µg/kg**	**max[c] µg/kg**	**#[a]**	**av.[b] µg/kg**	**max[c] µg/kg**	**#[a]**	**av.[b] µg/kg**	**max[c] µg/kg**	**#[a]**	**av.[b] µg/kg**	**max[c] µg/kg**	**#[a]**	**av.[b] µg/kg**	**max[c] µg/kg**	**#[a]**	**av.[b] µg/kg**	**max[c] 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	340	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	<LOQ	<LOQ
Sulfa-Sulfaquinoxaline																						2	2.69	5.37
																						1	2.02	2.02
Ciprofloxacin	5	200	370													2	2.6	3.4				3	8.10	14.0

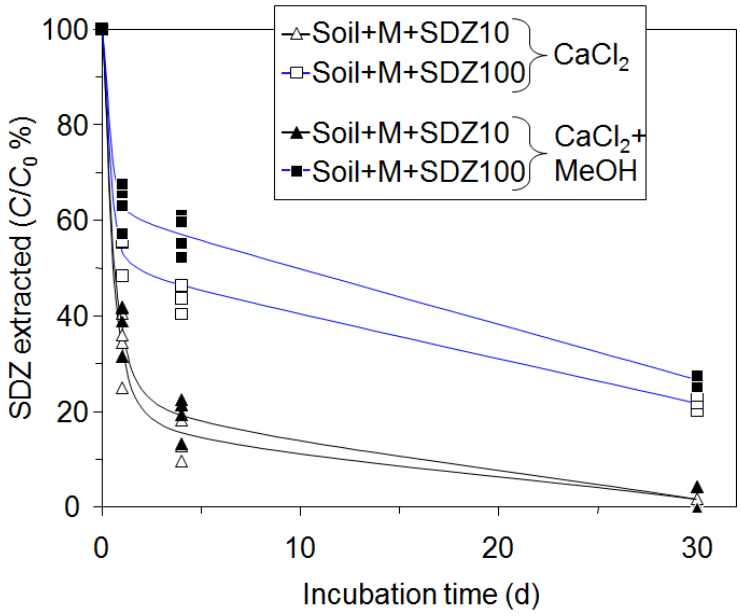
Study	Sattelberger (1999)			Hamscher et al. (2000), Hamscher et al. (2002)			Kues et al. (2002)			Clara et al. (2013)			An et al. (2015)			Gildemeister et al. (2011)			Hannappel et al. (2017)			Zhou et al. (2012)		
Enrofloxacin	4	103	200																			4	28.4	95.8
	#[a]	av.[b] µg/kg	max[c] µg/kg	#[a]	av.[b] µg/kg	max[c] µg/kg	#[a]	av.[b] µg/kg	max[c] µg/kg	#[a]	av.[b] µg/kg	max[c] µg/kg	#[a]	av.[b] µg/kg	max[c] µg/kg	#[a]	av.[b] µg/kg	max[c] µg/kg	#[a]	av.[b] µg/kg	max[c] µg/kg	#[a]	av.[b] µg/k	max[c] µg/kg
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	<LOQ	<LOQ
Roxythromycin										0														
Clarythromycin										0														
Lincomycin																						1	92.3	92.3
Polypeptide																1	33.1	33.1						
Carbamazepine																			1	0.5	0.5			
Caffeine																			1	160	160			
Acesulfam K																			0					
Cu [mg/kg]										8	45.3	52.0												
Zn [mg/kg]										8	139	150												

In Table 15, data from eight studies were combined, presenting concentrations of two and up to 13 different pharmaceutical antibiotics in soils. In these studies a total of 30 antibiotics from different structural classes as well as three other pharmaceuticals and two metals (Cu, Zn) were analyzed in 306 soil samples. The studies were selected because data were fully available (e.g. through appendices and supporting information, respectively) or at least clearly assignable to individual samples. They show that many of the antibiotics that had been analyzed could be recovered in determinable concentrations in the investigated samples. However, this was clearly less than the recovery of antibiotics in organic waste samples (Table 14, pg. 60).

It is important to consider that in the different studies field soil samples were taken at different and often unknown time periods after the last addition to soil of antibiotics together with contaminated waste material. Yet, the extractability and thus detectability of most antibiotics substantially declines within a short contact time of hours in a way that they are no more extractable from soil even by harsh extraction techniques such as microwave extraction and pressurized liquid extraction (Förster et al., 2008; Rosendahl et al., 2011; Stoob et al., 2006). For example, extractable portions of different antibiotics decline within 30 to 60 min of soil contact to ≤90% (sulfonamides), ≤80% (fenbendazole) and ≤70% (tetracyclines) (Müller et al., 2012; Thiele-Bruhn and Peters, 2007) and the recovery further declines with prolonged contact time with soil. Consequently, it must be assumed for many studies that the true soil contents of antibiotics in field soils are substantially higher than reported, especially when rather mild extraction methods have been used. This is shown in Figure 10 on example of the sulfonamide sulfadiazine that was spiked to soil together with manure. The extractable contents, using rather mild aqueous and organic extractants, strongly declined to <70% already within the first day after addition to soil (Hammesfahr, 2011). So it must be assumed that the contents of antibiotics are somewhat higher than indicated by the extractable contents. Förster et al. (2008) assumed that residual contents of sulfonamides might be higher by a factor of 2.5 than the extractable contents. It is supposed that this factor is even higher for much stronger adsorbing antibiotics such as tetracyclines, fluoroquinolones and ß-lactams. In the technical regulations, the DWA-AG GB-7.4 (2017) recommended to multiply by two the detectable concentrations of organic contaminants that persisted in soil for 1 year or more.

Figure 10: Time related decline of the extractable (detectable) concentration of sulfadiazine (SDZ) in Luvisol topsoil.

Determined after application with manure (M) to the soil sample at contents of 10 and 100 mg/kg, respectively Sequential extraction using 0.01 M $CaCl_2$ and methanol (MeOH).



Source: Own figure from S. Thiele-Bruhn (Univ. Trier) with data from Hammesfahr (2011).

4 Toxicity of single antibiotic compounds and of contaminant mixtures with antibiotics on soil organisms

4.1 Effects on functions and structural diversity of soil biota

Effects of single compounds

More and more studies on effects of single antibiotic compounds on soil organisms have been published in the past years so that the level of knowledge largely increased. It was lastly summarized in different reviews (Ding and He, 2010; Grenni et al., 2018; Nesme and Simonet, 2015; Qiao et al., 2018). An overview on tested compounds, species or groups of biota and endpoints is given in Table 16. The different studies show that adverse effects on many different test subjects must be expected. Many of the test concentrations used are rather high, though, and are not expected in the environment, where typical contents are in the order of µg/kg (see chapter 4). However, in general effects in the range of significant and about 10 % inhibition can be expected from typical, environmental contents of the single compounds (Thiele-Bruhn, 2005).
It is generally recognized that biostatic antibiotics such as tetracyclines and sulfonamides only act on growing microorganisms (Thiele-Bruhn and Beck, 2005). This on one hand requires in most cases the addition of a nutrient substrate (Gutiérrez et al., 2010) together with the antibiotics, in order to initiate growth of the soil microorganisms that are largely resting (Joergensen and Wichern, 2018). On the other hand it explains why tests such as soil basal respiration, working without a nutrient substrate, often show no effect of antibiotics (Table 16). Effects are often observed with tests including nutrient substrate addition to soil such as the substrate induced respiration (SIR) and the Fe(III) reduction or that directly use microbial growth as tested subject (e.g. leucine incorporation) (Demoling and Bååth, 2008; Thiele-Bruhn and Beck, 2005). In this context it should be noted that in a field situation antibiotics typically reach soils together with manure, sludge, wastewater etc., and thus together with a nutrient substrate. To continue the previous statement, activities within the N cycle, tolerance related parameters and properties of structural diversity are further sensitive indicators of antibiotic effects in soil (Table 16).
Despite the largely increasing number of publications on the topic, the knowledge on adverse effects of antibiotics on soil organisms is still fragmented, mostly related to diverse soil microbial properties and a few faunal indicator species, respectively. Additionally, existing research largely focused on only a few antibiotic structural classes and individual compounds from that classes. Although the list of studies compiled in Table 16 is not complete, it clearly shows that the vast majority of studies has been conducted on tetracylines and sulfonamides, while the knowledge on other antibiotic classes is reduced to a few single studies or is completely missing (e.g. cephalosporins). Hence, still numerous uncertainties and open questions remain. Consequently, it was stated that the number of data on toxic effects of pharmaceuticals in soil are still too few (Boxall et al., 2012; Brandt et al., 2015).

Table 16: Effects of single pharmaceutical antibiotics on soil microorganisms (soil microorg.), other microorganisms and faunal species.

For abbreviations used for effects and endpoints see the list of abbreviations.

Antibiotic	Substrate	Organism [a]	Effect	Endpoint	Concentration	Unit	Reference
Tetracyclines							
Chlortetracycline	Orthic Luvisol, sand	soil microorg.	soil basal respiration	no effect	1–50	mg/kg	(Zielezny et al., 2006)
	Paddy Soil, silt loam	soil microorg.	soil basal respiration	no effect	1–300	mg/kg	(Liu et al., 2009b)
	Typic Hapludalf, silt loam	soil microorg.	nitrification, Fe(III)reduction, soil basal respiration	no effect	0.0003–0.03	mg/kg	(Toth et al., 2011)
		green algae / cyanobacteria		EC_{50}	0.05 / 3.1	mg/L	(Halling-Sørensen, 2000)
	Sewage sludge	aerobic sludge bacteria		EC_{50}	0.03	mg/L	(Halling-Sørensen et al., 2002b)

Antibiotic	Substrate	Organism [a]	Effect	Endpoint	Concentration	Unit	Reference
Oxytetracycline	Silty sand / loamy sand	soil microorg.	SIR	ED_{10}	0.81 / 0.93	mg/kg	(Thiele-Bruhn and Beck, 2005)
	Silty sand / loamy sand	soil microorg.	SIR	ED_{50}	19.1 / 31.2	mg/kg	(Thiele-Bruhn and Beck, 2005)
	Sandy loam	soil microorg.	Fe(III) reduction	complete inhibition	>10	mg/kg	(Molaei et al., 2017)
	Alfisol, silt	soil microorg.	microbial biomass carbon	decrease	1–30	mg/kg	(Ma et al., 2016)
	Alfisol, silt	soil microorg.	nitrification	decrease	1–30	mg/kg	(Ma et al., 2016)
	Entic Cryumbrept, sand	fungi	length and activity of hyphae	decrease to 48%	10	mg/kg	(Colinas et al., 1994)
	Entic Cryumbrept, sand	soil microorg.	cultivable bacterial number	reduction to 71%	10	mg/kg	(Colinas et al., 1994)
	sand / sandy loam	springtail *F. fimetaria*	lethality	LC_{10}/EC_{10}	>5.000/ >5.000	mg/kg	(Baguer et al., 2000)
	sand / sandy loam	earthworm *A. caliginosa*	lethality	LC_{10}/EC_{10}	>5.000 / 1.954	mg/kg	(Baguer et al., 2000)
	sand / sandy loam	enchytraeid *E. crypticus*	lethality	LC_{10}/EC_{10}	>5.000 / 3.000	mg/kg	(Baguer et al., 2000)
		green algae / cyanobacteria		EC_{50}	0.207 / 4.5 / 1.6	mg/L	(Holten Lützhøft et al., 1999)
	sewage sludge	sludge bacteria		EC_{50}	0.4	mg/L	(Halling-Sørensen, 2001)
	sewage sludge	aerobic sludge bacteria		EC_{50}	0.08	mg/L	(Halling-Sørensen et al., 2002b)
Tetracycline	Paddy Soil, loam	soil microorg.	soil basal respiration	122 / 110 % of control (7/20d)	100	mg/kg	(Ma et al., 2014)
	Paddy Soil, silt loam	soil microorg.	soil basal respiration	no effect	1-300	mg/kg	(Liu et al., 2009a)
	Loam	soil microorg.	CLPP using Biolog	altered physiological profile	0-100	mg/kg	(Liu et al., 2014)
		13 soil microbial strains	minimum inhibitory concentration	MIC	< 1 – 1,000	µg/L	(Van Dijck and van de Voorde, 1976)
		8 soil microbial strains	minimum inhibitory concentration	MIC	10	µg/L	(van Gool, 1993)
		green algae / cyanobacteria		EC_{50}	0.09 / 2.2	mg/L	(Halling-Sørensen, 2000)
	sewage sludge	sludge bacteria		EC_{50}	2.2	mg/L	(Halling-Sørensen, 2001)
	sewage sludge	aerobic sludge bacteria		EC_{50}	0.08	mg/L	(Halling-Sørensen et al., 2002b)
Sulfonamides							
Sulfanilamide	diverse soils	soil microorg.	Fe(III)reduction	ED_{50} / EC_{50}	> 5,800	µmol/kg	(Thiele-Bruhn, 2005)
Sulfadiazine	diverse soils	soil microorg.	Fe(III)reduction	ED_{50} / EC_{50}	195/10.3	µmol/kg	(Thiele-Bruhn, 2005)
	sandy loam	soil microorg.	bacterial growth (leucin incorporation)	LOEC	0.001	mg/kg	(Brandt et al., 2009)
Sulfadiazine	Gleyic Cambisol, loamy sand / Orthic Luvisol, silt loam	soil microorg.	potential nitrification	Cambi/Luvi [b]: 9% / 25%	100 (32d) [c]	mg/kg	(Kotzerke et al., 2008)
	Gleyic Cambisol, loamy sand / Orthic Luvisol, silt loam	soil microorg.	potential denitrification	Cambi/Luvi [b]: 11% / 15%	100 (4d) [c]	mg/kg	(Kotzerke et al., 2008)
	Gleyic Cambisol, loamy sand / Orthic Luvisol, silt loam	soil microorg.	resistance: log(*sul1* / *rrn*) [d]	Cambi/Luvi [b]: +38%/+56%	100	mg/kg	(Heuer et al., 2011b)
	Gleyic Cambisol, loamy sand / Orthic Luvisol, silt loam	soil microorg.	resistance: log(*sul2* / *rrn*) [d]	Cambi/Luvi [b]: +42%/+102%	100	mg/kg	(Heuer et al., 2011b)
	Luvisol, loamy sand	soil microorg.	FDA hydrolysis	significant inhibition (28d)	10 / 100	mg/kg	(Xu et al., 2016)

Antibiotic	Substrate	Organism [a]	Effect	Endpoint	Concentration	Unit	Reference
Sulfadiazine	Luvisol, loamy sand	soil microorg.	dehydrogenase activity	significant inhibition: 10 14d, 100 28d	10 / 100	mg/kg	(Xu et al., 2016)
	Luvisol, loamy sand	soil microorg.	soil basal respiration	significant inhibition: 10 7d, 100 28d	10 / 100	mg/kg	(Xu et al., 2016)
	Luvisol, loamy sand	soil microorg.	total PLFA	significant reduction (28d)	10 / 100	mg/kg	(Xu et al., 2016)
	Gleyic Cambisol	soil microorg.	SIR	no effect	15.8 / 23.2	µmol/kg	(Hammesfahr et al., 2011b)
	Gleyic Cambisol	soil microorg.	nitrification / N mineralization / ammonification	decrease / decrease / increase	15.8 / 23.3	µmol/kg	(Hammesfahr et al., 2011b)
	sewage sludge	sludge bacteria		NOEC	60	mg/L	(Halling-Sørensen, 2001)
	sewage sludge	sludge bacteria		EC_{50} 0/10 h	15.9 / 16.8	mg/L	(Halling-Sørensen, 2001)
Sulfamonomethoxine	Paddy Soil, loam	soil microorg.	soil basal respiration	406 / 231 % of control (7 / 20d)	100	mg/kg	(Ma et al., 2014)
Sulfadimethoxine	diverse soils	soil microorg.	Fe(III)reduction	ED_{50} / EC_{50}	58.3 / 2.65	µmol/kg	(Thiele-Bruhn, 2005)
	Typic Hapludalf, silt loam	soil microorg.	Fe(III)reduction	1.5 %; 1 d	0.025	mg/kg	(Toth et al., 2011)
	Typic Hapludalf, silt loam	soil microorg.	Fe(III)reduction	11.5 %; 50 d	0.025	mg/kg	(Toth et al., 2011)
Sulfadimidine	Typic Hapludalf, silt loam	soil microorg.	soil basal respiration	no effect	0.025–0.200	mg/kg	(Toth et al., 2011)
	Typic Hapludalf, silt loam	soil microorg.	Fe(III)reduction	complete inhibition	>0.1	mg/kg	(Toth et al., 2011)
Sulfamethazine	diverse soils	soil microorg.	Fe(III)reduction	ED_{50} / EC_{50}	270/16.3	µmol/kg	(Thiele-Bruhn, 2005)
	silt loam	soil microorg.	soil basal respiration	Increase	20 / 100	mg/kg	(Awad et al., 2016)
	Paddy Soil, silt loam	soil microorg.	soil basal respiration.	EC_{10}, 2 d	20	mg/kg	(Liu et al., 2009a)
Sulfamethoxazole	Paddy Soil, silt loam	soil microorg.	soil basal respiration	EC_{10}, 2 d	7	mg/kg	(Liu et al., 2009a)
	loamy sand	soil microorg.	PICT (leucine incorporation)	increase by factor of 2	20 / 500	mg/kg	(Demoling et al., 2009)
	sandy loam	soil microorg.	Fe(III)reduction	complete inhibition	10	mg/kg	(Molaei et al., 2017)
Sulfapyridine	diverse soils	soil microorg.	Fe(III)reduction	ED_{50} / EC_{50}	432 / 37.4	µmol/kg	(Thiele-Bruhn, 2005)
	silty sand	soil microorg.	SIR 24h	ED_{50} / EC_{50}	6.2 / 0.89	mg/kg	(Thiele-Bruhn and Beck, 2005)
	loamy sand	soil microorg.	SIR 48h	ED_{50} / EC_{50}	11.5 / 0.55	mg/kg	(Thiele-Bruhn and Beck, 2005)
Sulfachloropyridazine	loamy sand	soil microorg.	pollution induced community tolerance - Biolog	tolerance +10%	7.3	mg/kg	(Schmitt et al., 2005)
Trimethoprim	Paddy Soil, silt loam	soil microorg.	soil basal respiration	Decrease	1-300	mg/kg	(Liu et al., 2009a)
	sewage sludge	sludge bacteria		EC_{50}	17.8	mg/L	(Halling-Sørensen, 2001)
Macrolides							
Tylosin	Paddy Soil, silt loam	soil microorg.	soil basal resp.	no effect	1-300	mg/kg	(Liu et al., 2009a)
	Humic Podzol, sand	soil microorg.	soil basal resp.	no effect	2,000	mg/kg	(Müller et al., 2002)
	sandy loam	soil microorg.	bacterial growth	EC50	960	mg/kg	(Demoling and Bååth, 2008)
	sandy loam	soil microorg.	PICT (bacterial growth)	increase by factor of 11	1500 preexposure	mg/kg	(Demoling and Bååth, 2008)
	sand / sandy loam	springtail *F. fimetaria*	lethality	LC_{10}/EC_{10}	>5,000 / 149	mg/kg	(Baguer et al., 2000)
	sand / sandy loam	earthworm *A. caliginosa*	lethality	LC_{10}/EC_{10}	>5,000 / 3,306	mg/kg	(Baguer et al., 2000)

Antibiotic	Substrate	Organism [a]	Effect	Endpoint	Concentration	Unit	Reference
Tylosin	sand / sandy loam	enchytraeid *E. crypticus*	lethality	LC_{10}/EC_{10}	2501 / 632	mg/kg	(Baguer et al., 2000)
	sewage sludge	sludge bacteria		EC_{50}	54.7	mg/L	(Halling-Sørensen, 2001)
Quinolones							
Ciprofloxacin	Paddy Soil, loam	soil microorg.	soil basal resp.	156 / 115 % of control (7 / 20d)	100	mg/kg	(Ma et al., 2014)
	Ustic Cambisol	soil microorg.	soil basal resp.	increase	1 / 5 / 50	mg/kg	(Cui et al., 2014)
	Ustic Cambisol	soil microorg.	nitrification	1: increase; 50: inhibited	1 / 5 / 50	mg/kg	(Cui et al., 2014)
	Haplic Chernozem	soil microorg.	soil basal resp.	decrease to 70% (2 d) and 35% (77 d)	0.2 / 2 / 20	mg/kg	(Girardi et al., 2011)
	sewage sludge	sludge bacteria		EC_{50}	0.61	mg/L	(Halling-Sørensen, 2001)
Oxolinic acid	sewage sludge	sludge bacteria		EC_{50}	0.1	mg/L	(Halling-Sørensen, 2001)
Difloxacin	Gleyic Cambisol, loamy sand	soil microorg.	soil basal resp.	increase	10 / 100	mg/kg	(Kotzerke et al., 2010)
Norfloxacin	acidic soil	soil microorg.	soil basal resp./nitrogen transformation	no effect / slight effect	5 / 10 / 30	mg/kg	(Yang et al., 2012)
Aminoglycosides							
Cycloheximid	Ustollic Haplargid	fungi/bacteria/protozoa	number of cultivable organisms	no effect	1	g/kg	(Ingham and Coleman, 1984)
Streptomycin	Ustollic Haplargid	fungi/bacteria/protozoa	number of cultivable organisms	no effect	1	g/kg	(Ingham and Coleman, 1984)
	sewage sludge	sludge bacteria		EC_{50}	0.47	mg/L	(Halling-Sørensen, 2001)
Pleuromutilins							
Tiamulin	sewage sludge	sludge bacteria		EC_{50}	14.3	mg/L	(Halling-Sørensen, 2001)
Quinoxalines							
Olaquindox	sewage sludge	sludge bacteria		EC_{50}	95.7	mg/L	(Halling-Sørensen, 2001)
ß-Lactams							
Amoxicillin	Orthic Luvisol, silt / Gleyic Cambisol, loamy sand	soil microorg.	microbial community composition (DGGE)	altered community composition	10-100	mg/kg	(Binh et al., 2007)
Penicillin G	sewage sludge	sludge bacteria		EC_{50}	84.6	mg/L	(Halling-Sørensen, 2001)
Imidazoles, Anthelmintics							
Fenbendazole	diverse soils	soil microorg.	Fe(III)reduction	no effect	3.3	µmol/kg	(Thiele-Bruhn, 2005)
Fenbendazole	Dystric Cambisol, sand	nematode *G. rostochiensis*	number of cycsts	decrease	10	mg/kg	(Thiele-Bruhn et al., 2006)
	Dystric Cambisol, sand	redworm *E. fetida*	lethality	NOEC	100	mg/kg	(Thiele-Bruhn et al., 2006)
	Dystric Cambisol, sand	redworm *E. fetida*	reproduction / biomass	LOEC	18 / 56	mg/kg	(Thiele-Bruhn et al., 2006)
	agar plate	soil nematode *P. maupasi*		ED_{50}	9	mg/kg	(Grønvold et al., 2004)
Ivermectin	Luvisol, silty loam		SIR	no effect	11	mg/kg	(Pfeiffer et al., 1998)
		springtails	lethality	LD_{50}	10	mg/kg	(Jensen and Scott-Fordsmand, 2012)
	topsoil, agricultural soil	soil invertebrates	feeding activity (Bait Lamina)	NOEC community/ EC10 individuals	0.25 / 0.05	mg/kg	(Jensen and Scott-Fordsmand, 2012)
	agar culture	soil nematode *P. maupasi*		ED_{50}	4.5	mg/kg	(Grønvold et al., 2004)
Metronidazol	sewage sludge	sludge bacteria		NOEC	100	mg/L	(Halling-Sørensen, 2001)

Antibiotic	Substrate	Organism [a]	Effect	Endpoint	Concentration	Unit	Reference
Polyether							
Monensin	Luvisol, silty loam	soil microorg.	soil basal resp.	EC_{50}	176	mg/kg	(Pfeiffer et al., 1998)
	Luvisol, silty loam	soil microorg.	SIR	increase	176	mg/kg	(Pfeiffer et al., 1998)
	Typic Hapludalf, silt loam	soil microorg.	soil basal resp.	no effect	0.01–0.100	mg/kg	(Toth et al., 2011)
	Typic Hapludalf, silt loam	soil microorg.	Fe(III) reduction	transient inhibition	0.01–0.100	mg/kg	(Toth et al., 2011)
	Typic Hapludalf, silt loam	soil microorg.	nitrogen transformation	EC_{50}	ca. 150	µg/kg	(Toth et al., 2011)
Lincosamides							
Lincomycin	Cambisol, silt loam / Podzol, sand	soil microorg.	16S rRNA gene, bact. Diversity	significant shift	0.05 - 500	mg/kg	(Čermák et al., 2008)
	Cambisol, silt loam / Podzol, sand	soil microorg.	CFU total bacteria and actinomycetes	significant, dose dependent decline	0.05 - 500	mg/kg	(Čermák et al., 2008)

[a] Soil microbial community, if not indicated otherwise.

[b] Cambisol (loamy sand) and Luvisol (silty loam), respectively.

[c] Determination after 32 d (pot. nitrification) and after 4 d (pot. denitrification) following addition of 100 mg/kg sulfadiazine with manure to soil.

[d] Increase in the abundance of *sul* resistance genes relative to the abundance of ribosomal *rrn* genes two months after addition of sulfadiazine with manure to soil (comparison to control with addition of manure only).

Effects of mixtures of antibiotics and/or other compounds on community activities and composition

Still a substantial lack of knowledge on the adverse effects of single antibiotics in the soil environment exists. In regard to this, there is an even stronger need for research on effects of pollutant mixtures and in particular on joint effects of mixtures of antibiotics in the environment, where large knowledge gaps still exist (Backhaus, 2014). Furthermore, most of the existing research on mixture toxicity has been done on aqueous environments and species (Vasquez et al., 2014), respectively, while studies on soils are scarce.

From clinical and pharmacological research, several interactions between antibiotics and further pharmaceuticals are known and 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

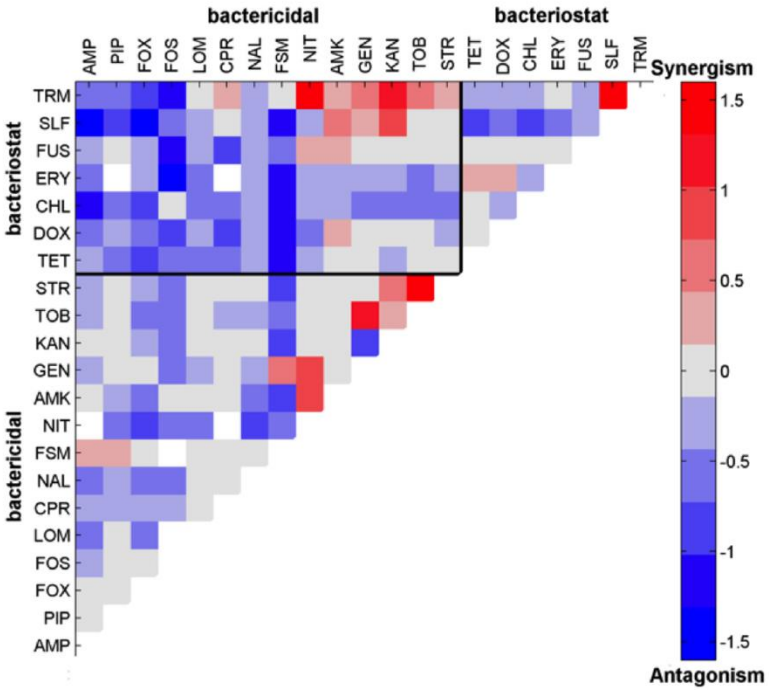
- uptake effects: the first pharmaceutical increases the permeability of the cell membrane for the second pharmaceutical,
- direct physical interaction: pharmaceuticals reciprocally stabilize their binding to the target site,
- targeting sequential metabolic steps: e.g., sulfonamides and trimethoprim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

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 additive (sulfonamides) but also synergistic effects (macrolides, tetracyclines, 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 (Figure 11). 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). Mixtures of antibiotics, exhibiting an antagonistic effect, are not recommended for combination products (Löscher et al., 2010; Schadewinkel-Scherkl and Scherkl, 1995). It is expected that such an antagonistic effect reduction will also occur in soil.

Figure 11: Heatmap showing pairwise interactions between 21 antibiotics measured systematically in *E. coli*.

Antibiotics are grouped according to their modes of action, and colors reflect interaction scores. Negative and positive scores correspond to antagonism (blue) and synergism (red), respectively, according to Loewe additivity criteria. White, missing data.



Abbreviation	Antibiotic	bact.static/ bact.cidal
AMP	Ampicillin	bact.cidal
PIP	Piperacillin	cidal
FOX	Cefoxitin	cidal
FOS	Fosfomycin	cidal
LOM	Lomefloxacin	cidal
CPR	Ciprofloxacin	cidal
NAL	Nalidixic acid	static-cidal
FSM	Fosmidomycin	cidal
NIT	Nitrofurantoin	static-cidal
AMK	Amikacin	cidal
GEN	Gentamicin	cidal
KAN	Kanamycin	static
TOB	Tobramycin	cidal
STR	Streptomycin	cidal
TET	Tetracycline	static
DOX	Doxycycline	static
CHL	Chloramphenicol	static
ERY	Erythromycin	static
FUS	Fusidic acid	static
SLF	Sulfamonomethoxine	static
TRM	Trimethoprim	static

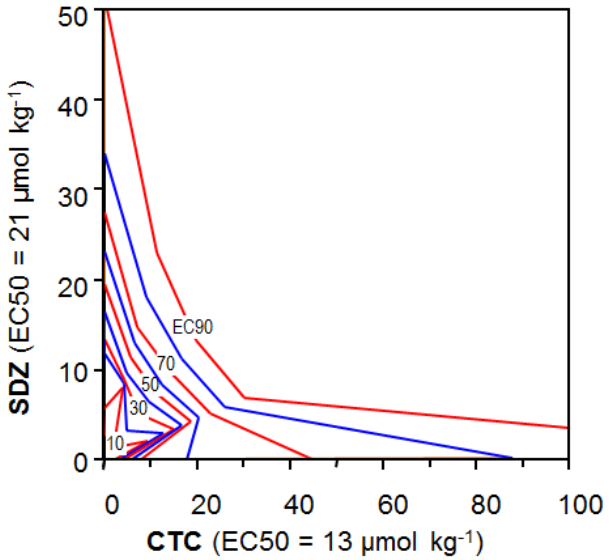
Source: Figure and text from Ocampo et al. (2014). Added table explaining abbreviations and with information on bacteriostatic (static) or bactericidal (cidal) mode of action of the antibiotics.

However, the general conclusion by Ocampo et al. (2014) was somewhat disproved by the findings of Christensen et al. (2006). They tested effects of binary mixtures of the antibiotics oxytetracycline, erythromycin, florfenicol (bacteriostatic), and oxolinic acid and flumequine (bactericidal) on freshwater algae (*Pseudokirchneriella subcapitata*) and activated sludge microorganisms. The finding that combinations of bactericidal and bacteriostatic antibiotics are mostly antagonistic was largely confirmed with the alga as test organism. However, in the presence of sludge bacteria numerous synergistic effects and in less cases concentration additivity was found even for combinations of bacteriostatic and bactericidal antibiotics (Christensen et al., 2006). Christensen et al. (2006) found synergistic effects or concentration additivity for combinations of bactericidal fluoroquinolones and benzimidazoles and bacteriostatic tetracyclines. Also combinations of bacteriostatic trimethoprim or sulfamonomethoxine with bactericidal aminoglycosides and a few more combinations resulted in synergistic effect enhancement (Ocampo et al., 2014). This even more emphasizes the possible and largely unknown higher risk of antibiotic mixtures compared to single compounds in the environment.

Effects of antibiotic mixtures may be largely modulated by the concentrations present. Vasquez et al. (2014) evaluated in a review 57 mixture toxicity studies from aqueous environments and human toxicology. The results largely confirm the before mentioned findings. Antagonistic effects occurred more often at low concentrations of the mixed chemicals while at higher concentrations even synergistic effects could occur, depending on the combined chemicals (González-Pleiter et al., 2013; Liu et al., 2012). In more detail, the joint effects of mixtures of two, four and five antibiotics, i.e. levofloxacin, norfloxacin (fluoroquinolones), tetracycline (tetracyclines), amoxicillin (ß-lactams), and erythromycin (macrolides), were tested using the bioluminescence and algal growth inhibition with the two aquatic test species cyanobacterium *Anabaena* CPB4337 and green alga *Pseudokirchneriella subcapitata*, respectively (González-Pleiter et al., 2013). The study showed that synergism largely predominates as joint effect. However, 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 as was reported for the effect of a combination of levofloxacin and norfloxacin on cyanobacteria (González-Pleiter et al., 2013).
Additionally, combinatory effects and their concentration related patterns very much depend on the investigated subject. For example, the same combination of levofloxacin and norfloxacin exhibited a clearly synergistic effect on green algae, while the effect on cyanobacteria was largely additive (González-Pleiter et al., 2013).
As it was shown by some of the before mentioned examples, it is not uncommon that combinations of antibiotics exert synergistic effects at higher concentrations but antagonistic effects at low doses. Such findings might be partly due to the fact that low-doses of antibiotics can cause increases of tested parameters, e.g. activities, exceeding the original homeostatic set point (Thiele-Bruhn, 2005). This effect can be attributed to hormesis or cryptic growth. Hormesis is defined as a biphasic dose–response phenomenon with low-dose stimulation and high-dose inhibition and results from a reparative process that slightly overshoots the original homeostatic set point, leading to the low-dose stimulatory response (Calabrese, 2008). It replaces the previous interpretation by the Arndt-Schulz effect that largely related such impact to homeopathic activities of compounds (Henschler, 2006). Cryptic growth occurs when parts of a mixed population are affected by a toxic impact and others can profit from it indirectly or even directly by utilizing lytic products as carbon, energy, or other nutrient sources (Chapman and Gray, 1986). Independent from the specific cause, low-dose activations by compounds or mixtures thereof with known adverse mode of action should not be misinterpreted as a positive effect on (soil) organisms (Malkomes, 1988).
Compared to clinical research and studies on aquatic environments, much less research was done on toxic effects of antibiotic mixtures on soil organisms. Studies found in the literature are reported in the following: A binary combination of chlortetracycline and sulfadiazine resulted in a synergistically increased effect on soil microbial Fe(III) reduction (Figure 12). The isolines deviate to the left from the 1:1 line, which is described as Loewe superadditivity. A synergistically increased effect was expected because tetracyclines and sulfonamides both are bacteriostatic but have different modes of action. The lowest combined EC50 concentrations of chlortetracycline and sulfadiazine were 8 µmol/kg soil, while of the single compounds 10 and 97 µmol/kg were necessary to cause the same effect (Thiele-Bruhn, 2005; Thiele-Bruhn, 2015). It might be additionally noted that the bending of the curves of EC_{50} and less at low contents of chlortetracycline and of the EC10 curve also at low contents of sulfadiazine are ascribed to an increase of the measured parameter at low-doses and was interpreted as hormesis and cryptic growth (Thiele-Bruhn, 2005; Thiele-Bruhn, 2015).

Figure 12: Isobologram of the effect of binary mixtures of chlortetracycline (CTC) and sulfadiazine (SDZ) on the microbial Fe(III) reduction in the Ap horizon of a Retisol.

Lines show concentration combinations causing the indicated effect level.



Source: Thiele-Bruhn (2015).

Cleary et al. (2016) added annually a mixture of the veterinary antibiotics tylosin, sulfamethazine and chlortetracycline to soil and after 10 years they investigated the effect on the soil bacterial composition by 16S rRNA gene sequencing. Significant alterations in the microbial community structural composition were identified. From 19 significantly ($p < 0.05$) affected operational taxonomic units (OTUs) identified, 16 were of the class Proteobacteria and their abundance was decreased compared to the control soils. Only one OTU, of the class Cyanobacteria, was shown to increase in abundance significantly; which is a bit surprising because this bacterial class is known for its susceptibility against antibiotics. Additionally, an increase in integron prevalence was determined. The authors conclude that these changes may represent a strong selective pressure on these taxa (Cleary et al., 2016).

Effects on microbial functions were demonstrated by David et al. (2016). Impacts of single compounds and mixtures of antibiotics from the four classes tetracyclines, sulfonamides, macrolides and fluoroquinolones on potential nitrification and denitrification of soil microbial communities were tested. Many mixtures of antibiotics from different classes, and thus with different modes of action, showed effects that deviated from those of the single compounds and were best predicted by the independent action concept. This went along with decreases in the abundance of several microbial groups related to the two functions potential nitrification and denitrification (David et al., 2016).

A combined contamination of soil with tetracycline, ciprofloxacin and sulfamonomethoxine yielded a substantially higher increase of the community level physiological profile (increase to 512% of control) compared to the samples that were contaminated with the single compounds (increase to 110 to 231%) (Ma et al., 2014). It must be noted, however, that the mixture contained 3×100 mg/kg while single antibiotics were added to soil at 1×100 mg/kg (Table 16). The mixture resulted in stronger accumulation of NO_3-N compared to soil contaminated with the single compounds, which was interpreted as an impact on the microbial N-cycling (Ma et al., 2014). This was further confirmed by stronger effects of the mixture on six functional genes covering the whole soil nitrogen turnover, i.e. *chiA* (ammonification), *amoA* (nitrification), *nifH* (N_2-fixation), *nirK* and *nirS* (denitrification) as well as *narG* (nitrite formation) (Ma et al., 2014).

Equal mass mixtures of three sulfonamide antibiotics, i.e. sulfadimethoxine, sulfamethoxazole, and sulfamethazine were tested at total concentrations of all three antibiotics of 0.9 to 900 mg/kg. Activities of urease and dehydrogenase enzymes as well as of the microbial biomass were reduced and a relative community shift towards gram-negative bacteria and towards fungi was determined (Gutiérrez et al., 2010).

When the earthworm *Eisenia fetida* was exposed to the individual tetracycline antibiotics tetracycline and chlortetracycline a dose-dependent, significant DNA damage in earthworm coelomocytes was observed (Dong et al., 2011). The combination of both antibiotics, however, resulted in slight antagonism at the maximum dose of 300 mg/L.

Not only combinations of several antibiotic compounds but also mixtures of antibiotics and other chemicals can exert different mixture toxicity. A contamination of agricultural soil with mixtures of antibiotics and other agrochemicals is rather the rule than the exemption (see sections 3 and 4). Pesticides, other non-antibiotic pharmaceuticals as well as heavy metals such as Cu and Zn enter agricultural soils through their use as agrochemicals or as contaminants in biosolids used as fertilizer and in untreated waste water used for irrigation (Blume et al., 2011; Jensen et al., 2016)

Various clinical and environmental studies found that copper (Cu) as well as other metals enhance the effects of antibiotics on soil microorganisms. An increased antibiotic effect on soil microorganisms (Biolog method) in the presence of Cu was reported for oxytetracycline (Kong et al., 2006). In another study, the interaction of the veterinary antibiotic sulfamethoxazole and Cu on soil microbial community composition and functions was investigated in a short-term microcosm experiment (Liu et al., 2016). Clear dose-dependent effects of sulfamethoxazole on microbial biomass and basal respiration were determined, and it was found that the interaction of sulfamethoxazole and Cu synergistically amplified adverse effects of sulfamethoxazole. This applied to the reduction of the soil microbial and especially bacterial biomass, structural composition as identified by phospholipid fatty acids analysis, and enzymatic functions (e.g., β-glucosidase, urease, protease) (Liu et al., 2016). The same applies to the antibiotics chloramphenicol, nalidixic acid, sulfanilamide, and tetracycline whose effective concentrations of 50% inhibition (EC_{50}) of bacterial growth increased up two fourfold in Cu contaminated soil compared to control soil (Berg et al., 2010). Yet, no such effect was found for ampicillin, olaquindox, and streptomycin.
Also altered effects on soil faunal species arise from the mixture of Cu and other heavy metals with antibiotics. The mixture effect of a combination of Cu and carbendazim on the soil nematode *Caenorhabditis elegans* was best described by the independent action model as well as by the additive reference model, despite the different modes of action (Jonker et al., 2004). This emphasizes the vagueness when conclusions with respect to modes of action are drawn from a model fit (Jonker et al., 2004). It is noted that carbendazim is used as a fungicide and not as an antibiotic, but due to molecular similarities to benzimidazoles, it has potential antimitotic (impeding the process of cell division) and antineoplastic (tumor inhibiting) activities. Combined contamination of soil with oxytetracycline and lead (Pb) affects lysosomal membrane stability and coelomocyte apoptosis of earthworm (Gao et al., 2014). The mixture of both exerted a combined effect that was synergistic at lower concentrations but antagonistic at higher concentrations of oxytetracycline and Pb. Furthermore, the joint toxicity of OTC and Pb decreased significantly with increasing OTC concentration (Gao et al., 2014), which again emphasizes the strong influence of the concentration of the individual compounds on the overall effect of the contaminant mixture, which substantially hinders a prognosis of effects.

Not only soil organisms but also plants are differently affected by mixtures compared to individual contaminants. A mixture of sulfamonomethoxine and cadmium (Cd) synergistically increased the effect on seed germination rate and especially shoot/root elongation of wheat (*Triticum aestivum*) and tomato (*Solanum lycopersicum*); again, the combined effect of the mixture varied with the (relative) concentrations of the two individual compounds (Jin et al., 2010). In contrast, a mixture of sulfadiazine and Cu showed an antagonistically reduced toxicity to wheat seedlings (growth, hydrogen peroxide, malondialdehyde, antioxidant enzyme activities) compared to the individual sulfadiazine and Cu alone (Xu et al., 2017b).
Chen et al. (2011) investigated single and joint toxicity of chloramphenicol and Hg acting on wheat (*Triticum aestivum* L.), Chinese cabbage (*Brassica campestris* L.) and corn (*Zea mays* L.). The results showed positive correlations between root elongation inhibition of three plants and concentrations of pollutants added to soil ($p \leq 0.01$) in the test concentration range. In terms of root elongation, wheat was the most sensitive to toxicity of chloramphenicol with an IC50 (concentration when 50% plants show inhibition) value as high as

26.8 mg/kg and also was the most sensitive one to the toxicity of Hg with the IC50 value as high as 300.8 mg/kg. The toxicity of chloramphenicol to the plants is stronger than that of Hg. Chloramphenicol and Hg had an antagonistic effect on the inhibition of root elongation of the three plants when the concentration of added Hg reached 30 mg/kg. Chloramphenicol and Hg had significantly synergistic effects on the inhibition of root elongation when Hg concentration was up to 200 mg/kg.

Also mixtures of pharmaceutical antibiotics with other organic contaminants lead to mixture toxicity. Combinations of the fungicide carbendazim and the antibiotic chloramphenicol (i) increased the inhibitory effect of carbendazim on the fungal:bacterial ratio, (ii) amplified the inhibitory effect of chloramphenicol on neutral phosphatase, and (iii) chloramphenicol partially diminished the increasing effect of carbendazim on soil catalase and urease activities (Yan et al., 2011). Also a combination of carbendazim and the antibiotic chlortetracycline had a stronger inhibitory effect on the average well color development (AWCD) in the Biolog ECO microplate test for community level physiological profiling (Fang et al., 2016).
Furthermore, the toxicity of microcystins, which toxins are produced by aquatic cyanobacteria, was investigated in combination with the antibiotics spiramycin and amoxicillin, using the bacterial luminescence test (Liu et al., 2012). After seven-day exposure to mixtures of microcycstins and the antibiotics, spiramycin-treated algal media and amoxicillin-treated algal media showed significantly (p<0.05) lower and higher inhibition on the luminescence of *Photobacterium phosphoreum*, respectively, compared with the untreated algal medium. It was concluded that the toxicity of microcystins was alleviated by spiramycin but enhanced by amoxicillin (Liu et al., 2012).
A complex mixture of the antibiotic sulfamethoxazole with other pharmaceuticals (propranolol, carbamazepine, ibuprofen, diclofenac) yielded an effect on luminescent bacteria (*Vibrio fischeri*) that was best decribed by the concentration additivity model (von Känel, 2002). Yet, the slope of the experimental dose response curve was clearly steeper than reflected by the model.
The uptake of antibiotics by higher organisms can vary, when different chemicals interact with each other. Examples given by Calabrese (1991) are the reduced uptake of the aminoglycoside neomycin in the presence of the antibiotic penicillin V as well as of the cardiac pharmaceutical digoxin, while uptake of tetracycline and pivamicillin is increased in the presence of the antiemetic pharmaceutical metoclopramine. However, these reports are not specific for soil organisms so that it remains unclear in how far they can be applied to soil.

Combinatory effects of antibiotic and pollutant mixtures might be further modulated by the addition of organic fertilizer such as manure, slurry or sludge (Thiele-Bruhn, 2003b). As is documented in chapter 3, these organic substrates may contain various contaminants such as pharmaceuticals, agrochemicals and heavy metals and are the typical entry pathway for antibiotics into the soil environment. The content and quality of the organic waste material added to soil that serves at the same time as a nutrient substrate for soil biota may also significantly modify the antibiotic effect (Hammesfahr et al., 2011a; Hammesfahr et al., 2011b). However, no respective studies have been published regarding the effects of mixtures of antibiotics.

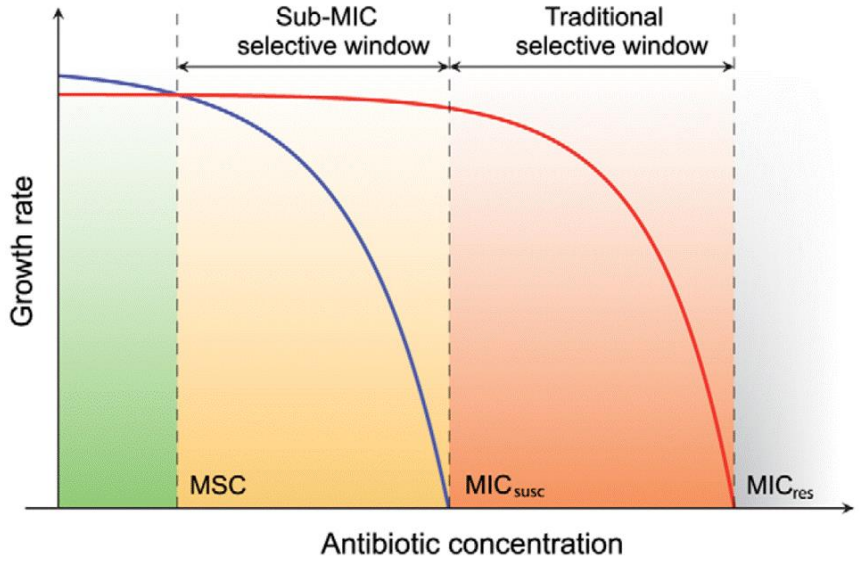
In total, this overview on published findings on mixture toxicity of antibiotics in soil clearly shows that the level of knowledge is rather fragmentary and far from a systematic understanding of the processes and factors of mixture toxicity. Anyhow, it makes clear that effects of antibiotic mixtures occur and are different from those of single contaminants. They can be varied by basic factors such as concentrations and other influences such as soil organic matter or additional contaminants. This strong variation may explain why in part even contradicting results are found in reports, e.g. antagonistic effects of a mixture in one and synergistic effects in another publication.

4.2 Effects of mixtures of antibiotics and/or other compounds on antibiotic resistance of microorganisms

The formation and mid-term establishment of increased resistance levels measured as higher abundance of antibiotic resistance genes in soils after application of antibiotics has been frequently determined (Agga et al., 2015; Binh et al., 2007; Byrne-Bailey et al., 2009; Chee-Sanford et al., 2001; Qiao et al., 2018; Williams-Nguyen et al., 2016). This is due to the survival and transfer with manure to soil of intestinal bacteria with increased resistance level and/or the building up of a higher resistance level in environmental bacteria under the impact of antibiotic contamination (Schmitt et al., 2017). Clear relations with the antibiotic load in soil have been found (Heuer et al., 2011a; Heuer et al., 2011b; Wang et al., 2016). The multiple effects of mixed contaminations are of specific relevance for the development of resistance in the environment. Cross resistance and multiple resistance are well known and especially a problem for the use of antibiotics in human and veterinary medicine (see chapter 1.1).

Figure 13: Schematic representation of growth rates as a function of antibiotic concentration.

Green area indicates a concentration interval where the susceptible strain (blue line) will outcompete the resistant strain (red line). Orange (sub-MIC selective window) and red (traditional mutant selective window) indicate concentration intervals where the resistant strain will outcompete the susceptible strain. MICsusc = minimal inhibitory concentration of the susceptible strain, MICres = minimal inhibitory concentration of the resistant strain and MSC = minimal selective concentration.



Source: Figure and text from Gullberg et al. (2011) doi: 10.1371/journal.ppat.1002158.g001, open access.

The selection of resistant bacteria occurs especially at low antibiotic concentrations, typically below the minimal inhibitory concentration (MIC) (Gullberg et al., 2011). This is depicted in Figure 13 as the 'Sub-MIC selective window'. It was shown on example of three clinically important antibiotics that pharmaceutical concentrations up to several hundred-fold below the MIC of susceptible bacteria could enrich resistant bacteria. De novo mutants can be selected at sub-MIC concentrations of antibiotics. This suggests that the low antibiotic concentrations found in many natural environments are important for enrichment and maintenance of resistance in bacterial populations (Gullberg et al., 2011).

Synergistic combinations can even 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 and 20 d to 1.7 to 11fold higher abundance of sulfamonomethoxine 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). However, this does not mean that resistance formation is fully inhibited by antagonistic pharmaceutical mixtures.

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 and of pharmaceutical 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). This is confirmed by other studies. High exposure of soil bacteria to Cu selects for Cu-tolerant bacterial communities and at the same time co-selects for an increased community-level tolerance to tetracycline and vancomycin (Berg et al., 2010). Furthermore, the frequency of resistance to the antibiotics ampicillin, chloramphenicol, nalidixic acid, olaquindox, streptomycin, sulfanilamide, and tetracycline was up to twofold and for most compounds significantly increased in Cu contaminated soil compared to control soil (Berg et al., 2010). Such co-selection of antibiotic resistance or co-tolerance against antibiotics in the presence of Cu contamination of soil was also reported for other antibiotics such as tylosin and triclosan (Gielen et al., 2016; Liu et al., 2017a). The abundance of individual antibiotic resistance genes and heavy metal resistance genes was found to be positively correlated (Xu et al., 2017a). Yet, again, also these interactions among compounds are modulated by the soil properties and co-selection of resistance might be absent in fine-textured soil as was found for Cu/triclosan and Zn/triclosan mixtures (Gielen et al., 2016), while in other studies an increased susceptibility to antibiotics (here vancomycin) in the presence of Cu in soil was reported (Wakelin et al., 2014).

It is not surprising that, together with the occurrence of mixed contaminations including antibiotics in agricultural soils, also different antibiotic resistance genes are abundant in these soils. Resistance genes against different classes of antibiotics and encoding different modes of action, e.g. ribosomal protection proteins, efflux pump proteins, antibiotic deactivation, are frequently found altogether in contaminated soils (Herrick et al., 2014; Wang et al., 2016; Zhu et al., 2013). All field studies targeting antibiotic resistance levels and diversity in soils consistently show that soil contamination with pharmaceutical antibiotics (and excreta such as manure or sewage sludge) significantly increases at least on a mid-term the abundance of genes encoding for resistance against classes of antibiotics such as ß-lactams, tetracyclines, erythromycin, aminoglycoside, macrolides, and sulfonamides (Agga et al., 2015; Heuer et al., 2011a; Knapp et al., 2010; Marti et al., 2013). Between 20 and more than 90 individual antibiotic resistance genes were discovered in single Chinese field sites (Zhu et al., 2013). The emergence of such numerous resistance genes results among others from mixed contaminations with antibiotics and heavy metals such as Cu, both can also co-select for resistance against other classes of antibiotics (Dang et al., 2006; Seiler and Berendonk, 2012).

Especially the altered and mostly increased formation of antibiotic resistance in contaminated soils and the risk that this large scale environmental contamination will directly or indirectly affect human and animal health via different routes are overriding problems (Westphal-Settele et al., 2018). This is further aggravated by the widespread contamination of the environment not only with single compounds but with mixtures of antibiotics as well as other chemicals. However, the state of knowledge is still incomplete and inconsistent so that the derivation of predicted no effect concentrations (PNEC) triggering increased abundance of microbial resistance genes in the soil environment is actually not feasible (Schönfeld et al., 2017).

5 Properties and conditions influencing the effects of antibiotics and mixtures

All **toxic effects are time-dependent**. This especially applies to bacteriostatic antibiotics that affect microbial growth. Hardly any acute effects can be expected (Schmitt et al., 2005; Thiele-Bruhn and Beck, 2005) but adverse effects increase over timescales of days and weeks before they decline again towards the original status (Chessa et al., 2016; Hammesfahr, 2011; Zielezny et al., 2006). Time dependence can be described by the empirically measured time dependence of toxic effects (TDT) (Dawson et al., 2014) and is calculated as:
$TDT = EC_{x,t1} - EC_{x,t2} / (EC_{t1} \times f_{t1:t2})$
with $EC_{x,ty}$ being the contaminant concentration, causing a reduction of the tested parameter by x% determined after a time y (e.g. days), and the time factor $f_{t1:t2}$, representing the quotient of different times t1/t2 (d/d) after which toxic effects have been measured (Dawson et al., 2014). This was tested for single (yet non-antibiotic) organic compounds and 25 sham (mix of compounds with similar mode of action) and 125 true combinations. Mixture TDT was well-predicted simply by averaging the TDT values of the individual components of binary mixtures (Dawson et al., 2014). Yet, this finding requires further confirmation, especially because it was only determined for acute (short-term) toxicity in aquatic test systems. Antibiotic effects in the soil environment much more result in mid- to long-term effects well beyond the life span of generations of soil microorganisms and small soil faunal species. Anyhow, TDT derived from single compounds might be used as a first estimate of the time-dependent effects of mixture toxicity. Based on the existing studies (see chapter 5.1) it can be assumed that **synergistic combinations will have a prolonged effect duration (smaller TDT) while antagonistic combinations will have a reduced duration of effect (larger TDT)**.

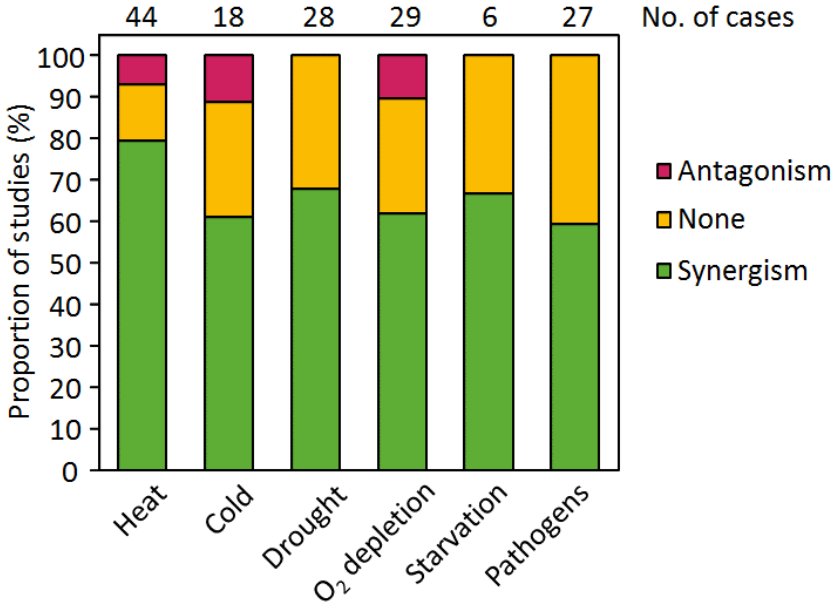
In studies with aquatic microcosms, mixtures of antibiotics differently affected the **pH of the solution** (Guo et al., 2016). Shifts in pH can alter the speciation of antibiotics, depending on their acid dissociation constant (p*K*a), and thus their fate in the environment and uptake into organisms (Tappe et al., 2008; Thiele-Bruhn, 2004; Zarfl et al., 2007). However, measurable changes in pH of typically well-buffered soils due to a contamination with micropollutants are unlikely.

Especially for tetracycline antibiotics it is known that they **interact with divalent ions**, forming chelate complexes (Oka et al., 2000). This may even lead to nutrient (Ca) deficiency of specific plants when they grow in heavily tetracycline contaminated soil as was shown by Batchelder (1981) and Batchelder (1982) in experiments with *Phaseolus vulgaris* plants. It is assumed that this indirect adverse effect of tetracyclines can be overcome by fertilization of the respective nutrient. **The interaction of antibiotic and nutrient can be understood as an antagonistic mixed effect.**

Not only interactions among different toxic chemicals but also **interactions with other boundary conditions** may add to a mixed effect in the environment. These are parameters that affect the fitness of the biota or - in other words – that cause additional stress for the organisms, such as heat or frost, drought or excess of water combined with oxygen depletion, starvation or nutrient substrate supply, and pathogens or predators. Holmstrup et al. (2010) reviewed 150 studies on the topic many of which dealing also with terrestrial, faunal species including soil organisms, i.e. earthworms, springtails and various insects. However, the researched studies did not cover (i) microorganisms, (ii) antibiotics (but heavy metals, polycyclic aromatic hydrocarbons, surfactants and pesticides), and (iii) mixtures of chemicals. Anyway, this review and a second study clearly showed that environmental stress mostly leads to an increased adverse effect of chemicals which the authors termed as synergism (Holmstrup et al., 2010; Laskowski et al., 2010). In 59.3 to 79.5% of all cases, an increased toxic effect of the regarded pollutant was determined under additional environmental stress (Figure 14). Synergism especially occurred in the case of heat stress. In contrast, only few cases were reported that showed a reduced toxic effect of the pollutant (antagonism) in the case of heat, cold and O_2 depletion.

Figure 14: Interacting influence (antagonistic, none, synergistic) of boundary conditions on the toxic effects of chemicals (heavy metals, polycyclic aromatic hydrocarbons, surfactants and pesticides) on faunal species.

Data compiled from 150 published studies.



Source: Figure redrawn from Holmstrup et al. (2010), modified.

The impact of **organic nutrient substrates such as manure** on the effects of pharmaceuticals on soil micro-organisms was reported by Hammesfahr et al. (2008). It was shown that antibiotic effects of sulfadiazine disproportionally increased with incremental liquid manure addition (Hammesfahr et al., 2011a). Impacts of manure even varied depending on whether fresh or stored (6 months) manure was used (Hammesfahr et al., 2011b) and occurred although extractable amounts of sulfadiazine declined with increasing liquid manure application. It must be stated that other combinations of manure and antibiotics increased the mobility of the antibiotics (Thiele-Bruhn and Aust, 2004; Zhou et al., 2016), which will most likely contribute to an increased effect as available (effective) concentrations increase.

However, the effect of non-contaminant impacts such as nutrient status and environmental stressors on the mixture toxicity of combined contaminations has hardly been tested and no such publications related to mixed antibiotics have been found with this literature research. It is assumed that the total effect of a contaminant mixture increases or decreases, as it was reported for single contaminants interacting with environmental stressors. Yet, no change in the type of the interacting effect (e.g. synergism, antagonism) of mixed chemicals is expected, unless the physicochemical properties and bioavailability (effective concentration) of one chemical changes substantially, e.g. through temperature increase. For example, the combined, synergistic effect of Cu and cyanobacterium *Microcystis aeruginosa* on *Daphnia magna* was not affected by temperature and total food concentration (Hochmuth et al., 2016).

6 Effects of mixtures of antibiotics and other pollutants on the fate in soil

Mixtures of chemicals in soil may not only exert effects on biota that are different to those of single substances, but also the fate of chemicals may be different in mixtures. This applies for the ad- and desorption as well as for the dissipation encompassing the sub-processes of immobilization and degradation. Sorption and degradation are two processes that largely define the fate of chemicals in soil. Significantly altered sorption and/or degradation of a compound in a mixture compared to the single substance will feed back on the effects. Consequently, determining overall rules of joint toxicity of a given antibiotic mixture is even more complicated for soils than for aquatic environments, where the dissolved phase largely dominates while the solid phase and sorption to it are less prominent.

Effects on sorption and immobilization

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 (Wang and Wang, 2015; Wegst-Uhrich et al., 2014). Examples are given in Table 19 in the Appendix. Averaged sorption coefficients (K_d; L/kg), taken from the review of Thiele-Bruhn (2003b) decline in the sequence FQs (7000) > TCs (850) > lipoglycosides (90) ≥ macrolides (80) = polypeptides (80) > SAs (4) > quinoxaline derivatives (1). The sequence shows that antibiotics from some structural classes exhibit rather low sorption coefficients. Consequently, there is a higher risk that these antibiotics are translocated in soil and may contaminate groundwater (Aust et al., 2010; Balzer et al., 2016; Hamscher, 2007; Höper et al., 2007). 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 (Figueroa-Diva et al., 2010; ter Laak et al., 2006b; Vasudevan et al., 2009). 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 (Figueroa-Diva et al., 2010; Schwarz et al., 2012; Thiele-Bruhn et al., 2004; Wang and Wang, 2015). Consequently, it must be assumed that different individual antibiotic compounds compete for such specific sorption sites.

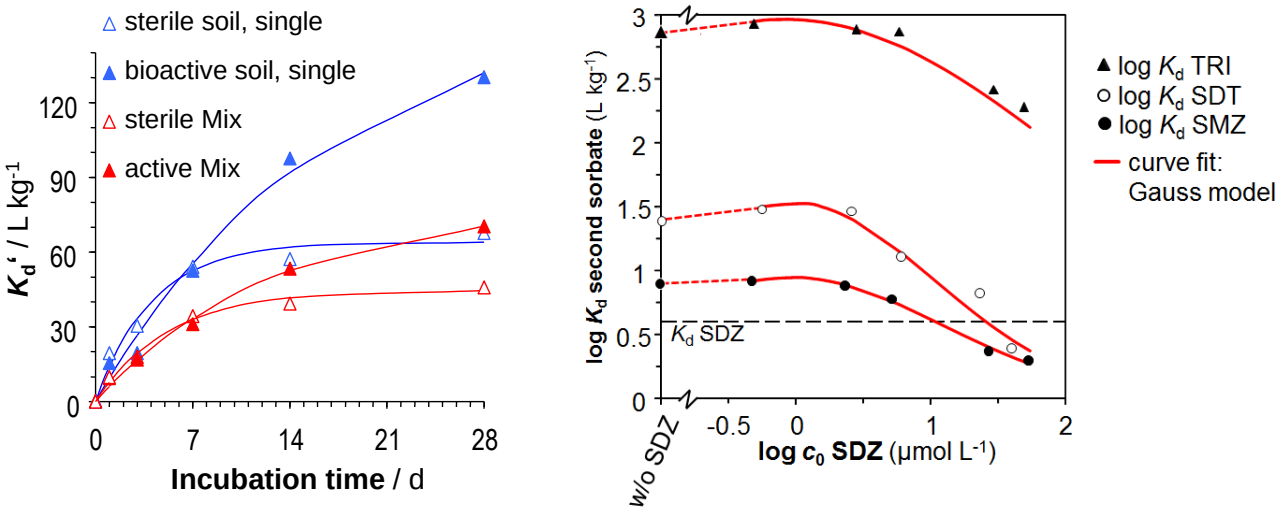
Competitive sorption among antibiotics from the same structural class has been reported, e.g. by Ahmed et al. (2017) and Conkle et al. (2010), who focused on the competition between several sulfonamides among each other, and of the fluoroquinolones ciprofloxacin, norfloxacin and ofloxacin, respectively. Results show a strongly reduced retardation in soil of antibiotics, whereby sorption competition increases with increasing concentrations. Resulting sorption coefficients were about 1/3 to 2/3 lower than for the single compounds (Ahmed et al., 2017; Conkle et al., 2010). It is concluded that sorption competition will especially occur with mixtures of antibiotics from the same compound class, competing for the same sorption sites in soil. Correspondingly, apparent sorption, being a combination of immobilization and degradation, and its temporal dynamics are substantially higher and faster, respectively, for a single sulfonamide compared to a mixture of three sulfonamides (Figure 15, left; Thiele-Bruhn, 2015).
There are also examples for **sorption competition among antibiotics from different structural classes**. One example is illustrated in Figure 15. It shows considerable sorption competition between sulfonamide antibiotics and the synergist trimethoprim (Thiele-Bruhn, 2015). Competition is clearly concentration dependent and results in lowest K_d values that equal 1/4 or less of the K_d of the single compound (Figure 15, left). Hence, mobility and bioavailability of these antibiotics is increased, which will contribute to the synergistically stronger effect of the antibiotic mixture. Liu et al. (2017b) demonstrated the sorption competition between the fluoroquinolone ciprofloxacin and the sulfonamide sulfamethoxazole, whereby ciprofloxacin was a stronger competitor to sulfamethoxazole than vice versa.

On the other hand, an antagonistic increase of the sorption of sulfamethoxazole in the presence of trimethoprim was found that was attributed to an elimination of repulsion between negatively charged molecules and particle surfaces due to cation sorption on soil particles (Kočárek et al., 2016). This contrasting result might be due to the **dependence of sorption competition on the speciation and charge** of the most often ionizing antibiotics. Competition between largely anionic sulfamethazine and trimethoprim resulted in considerably decreased sorption of sulfamethazine in neutral soil, while competition was negligible for the neutral antibiotic species in acidic soil (Peng et al., 2015).

All in all, it can be stated that sorption competition especially occurs among antibiotics from the same structural class, where single compounds compete for the same sorption sites (Ahmed et al., 2017). For compounds from different antibiotic classes, competitive sorption is largely restricted to the high concentration range, when specific high-energy sorption sites are already occupied and compounds compete for low-energy, unspecific sorption sites (Conkle et al., 2010).

Sorption competition may also occur between antibiotics and other organic contaminants. For example, sorption competition between antibiotics and non-antibiotic pharmaceuticals from different compound classes was reported, i.e. competition between trimethoprim, carbamazepine and atenolol (Kočárek et al., 2016). In the same study, an antagonistic increase of the sorption of sulfamethoxazole in the presence of atenolol and trimethoprim was found that was attributed to an elimination of repulsion between negatively charged molecules and particle surfaces due to cation sorption on soil particles (Kočárek et al., 2016). The fungicide carbendazim (benzimidazoles) affects the fate of the antibiotic chloramphenicol (fenicol antibiotics). In another study it was shown that sorption of sulfamethoxazole was reduced by the concurrent sorption of the surfactant linear alkylbenzene sulfonate (LAS) (Carrillo et al., 2016). However, this was only found for one soil with low soil organic matter content and no effect on ciprofloxacin sorption was determined (Carrillo et al., 2016). Combinations of surfactants and antibiotics typically derive from wastewater application to soil (Siemens et al., 2008).

Figure 15: **(Left) Immobilization (represented by the apparent sorption coefficient K_d') of sulfamethazine (SMZ) as single compound (single) and in an equimolar ternary mixture (Mix) with sulfadiazine (SDZ) and sulfadimethoxine (SDT) in biologically active and sterile topsoil, respectively. (Right) Alteration of sorption coefficient K_d of trimethoprim (Tri), SDT and SMZ applied at 10 µmol/L in the presence of different concentrations of SDZ.**



Source: Figure from Thiele-Bruhn (2015).

A mixed effect on sorption is not only restricted to the competitive sorption among antibiotics but also applies to the **competition between antibiotics and natural organic matter** as well as the interactions with nutrient ions and other metal ions. **Dissolved organic matter (DOM)** has strong impacts on the sorption of

antibiotics in soil. The DOM is either derived from soil or from soil amendment with organic waste material (manure, sewage sludge etc.) that is especially rich in DOM (Aust et al., 2009). Soil batch and column experiments showed that especially antibiotics that are only weakly retarded in soil such as sulfonamides are significantly mobilized by DOM (Boxall et al., 2002; Hou et al., 2010; Thiele-Bruhn and Aust, 2004). In comparison, impacts of DOM are much less on strongly sorbing antibiotics such as tetracyclines (Arenz-Leufen, 2012). However, also increased immobilization of antibiotics was found which can be explained by (i) the specific interplay between individual soils and manure materials (Aust, 2010), (ii) the impact of particulate material within the heterogeneous mixtures of organic waste materials (Aust et al., 2009), (ii) effects on pH and ion content of the soil (Aust, 2010), and (iii) the filtration of colloidal material in soil (Zhou et al., 2016). The influence of organic waste material and especially of DOM originating therefrom on the retardation and mobility of antibiotics in field soils was proven in different studies (Blackwell et al., 2009; Kreuzig and Höltge, 2005; Zhang et al., 2014).

Antibiotics and nutrient ions as well as heavy metals often coexist in soils due to land application of animal wastes and other sources of inputs (Xu et al., 2015). The presence of multivalent metal (Me) cations such as Ca^{2+} and Cu^{2+} typically enhances the sorption of antibiotics such as sulfonamides in slightly acidic and higher-pH soil (Xu et al., 2015). The sorption promoting effect increases with pH and is explained with the formation of cation bridges of the type sulfonamide-Me^{2+}-soil that are especially abundant in high-pH soil where antibiotics form anionic species. In contrast, no or even sorption inhibiting effects were found in acidic soils (Liu et al., 2017c), where largely cation species of the antibiotics exist. These compete with the metal cations for the same ion exchange sites (Pei et al., 2014). Also, soil sorption of tylosin (having a p*K*a of 7.7 so that it largely occurs in soil as neutral molecule) is suppressed by Ca^{2+} and Al^{3+} over the whole soil pH range tested (Pei et al., 2014). In summary this clearly shows that the impact of metal cations on antibiotic sorption depends on the antibiotic species occurring at the specific soil pH, with sorption of cationic species being reduced, that of neutral species being slightly reduced or unaffected, and that of anionic species being increased by metal cations. Furthermore, the effect of metal cations depends on concentrations and is largely restricted to higher concentrations of both, antibiotics and metal cations. At low concentrations both sorb to specific, high-energy sorption sites while only at high concentrations competition for low-energy, electrostatic attraction sorption sites occurs (Wu et al., 2014).

Also, **phosphate is known to compete with antibiotics for sorption sites**, which especially applies in soils with higher pH at which several antibiotics exist as anionic species. Such sorption competition was reported for phosphate and tetracycline (Munira and Farenhorst, 2017; Wang et al., 2010). Own results indicate that this applies not only to tetracyclines but also to sulfonamides (sulfamethazine), quinolones (ciprofloxacin), and fenicols (florfenicol) (Ngigi and Thiele-Bruhn, unpublished results).

Effects on dissipation

The dissipation of organic chemicals in soil is a somewhat vague term. It includes two processes, i.e. immobilization and degradation of chemicals, without precise assignment to one or the other process. It must be noted that in many studies no clear distinction between these processes has been done, even when it is stated that solely degradation or biodegradation had been investigated. Studies working with isotope labelled tracer compounds show that the true degradation of antibiotics through mineralization accounts for only a few percent of the initial content (Heise et al., 2006; Kreuzig et al., 2007; Kreuzig and Höltge, 2005; Kreuzig et al., 2003; Schmidt et al., 2008), while the formation of covalently bond residues is more important (Förster et al., 2008; Schwarz et al., 2015; Stoob et al., 2006). In comparison, the immobilization of antibiotics in soil and manure through strong, yet principally reversible sorption mechanisms is quantitatively much more relevant and faster (Müller et al., 2012; Stoob et al., 2007; Thiele-Bruhn and Peters, 2007). Additionally, transformation of antibiotics can lead to metabolites that are also antibiotic active. For example, Zhang et al.

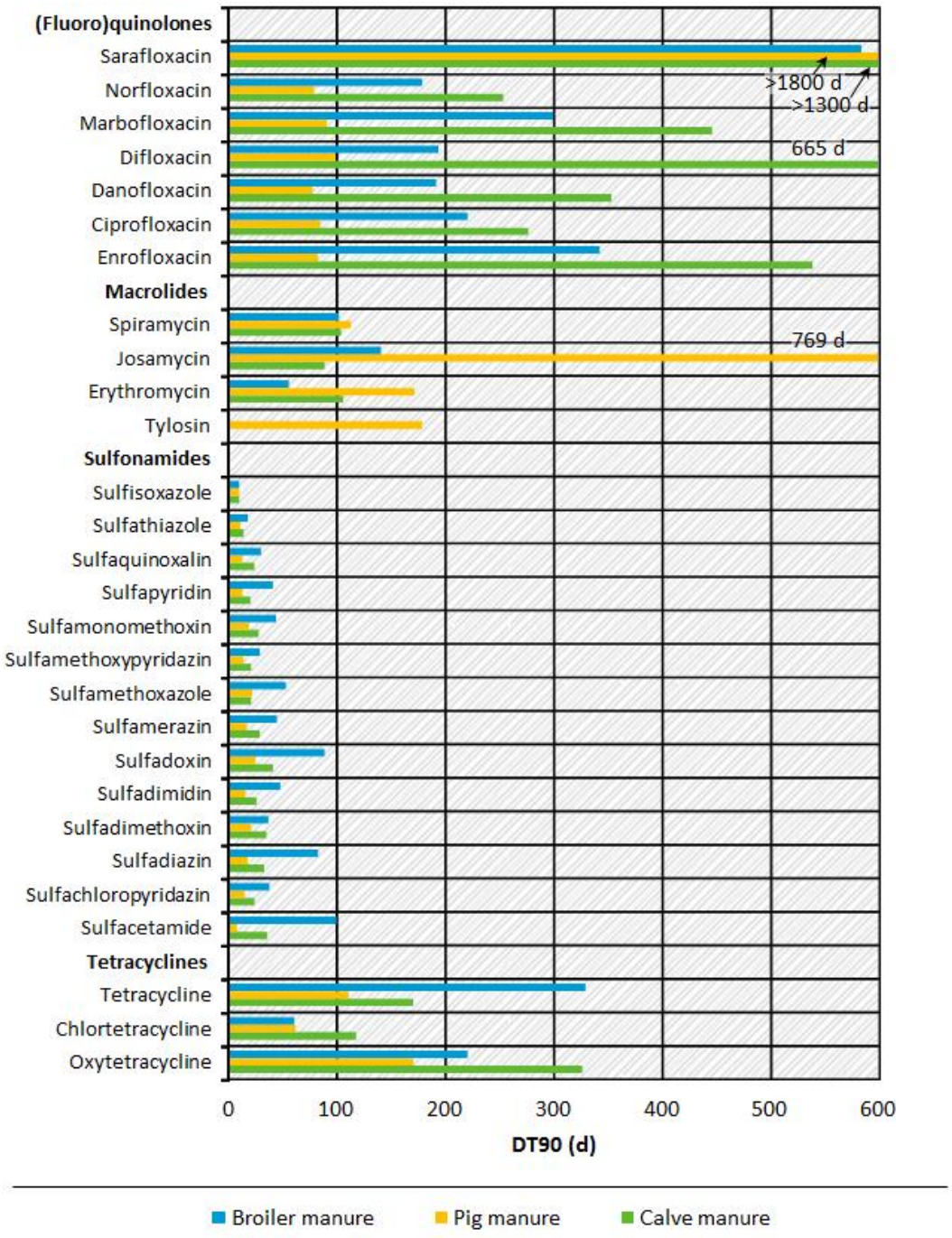
(2017) showed that sulfonamides are degraded in soil to different transformation products including oxidated compounds that are more toxic than the parent compound. The FQs ciprofloxacin and norfloxacin were detected as microbial biodegradation intermediates of enrofloxacin (Alexandrino et al., 2017).

Dissipation of antibiotics in manure: Although it is well known that mixed contaminations with antibiotics occur in manure (see section 3.1) hardly any publications could be identified through this literature research that deal with the dissipation of mixed antibiotics' contaminations in manure. The published studies focus on **single compounds** that were typically spiked to manure samples of different origin. In manure and related substrates such as sewage sludge, both immobilization of antibiotics through strong sorption (Kahle and Stamm, 2007; Thiele-Bruhn and Aust, 2004) as well as degradation with formation of various metabolites (Lamshöft et al., 2010; Wetzstein et al., 2006; Wetzstein et al., 1998) occur. Dissipation half-lives in manure vary between antibiotics of different structural classes, while the variation among antibiotics of the same structural class is much less. Reported half-lives (d) were compiled by Schmitt et al. (2017) and are in the following ranges: penicillins (5 d), macrolides (<2-130 d), SAs (<8-64 d), TCs (1-100 d) FQs (100-113 d). However, the given half-lives in part strongly disagree between authors, e.g. OTC 1 d (Wu et al., 2011) vs. 100 d (Chee-Sanford et al., 2009). It is assumed that this is – among other influencing factors that might be dissimilar between studies – due to the investigation of dissipation in one study or true and slower (bio)degradation in the other. A compilation of degradation times of different antibiotics in manure, added as mixed contamination, that was adopted from Berendsen et al. (2018) can be found in Table 21 (Appendix). It must be noted that many of the dissipation times reported in the literature are rather optimistic, because in most cases they have been measured at room temperature and at constant (optimum) soil moisture, while substantially lower and changing temperatures and highly variable moisture (from drought to supersaturation) occur in field soils. Furthermore, it must be stated that even strong immobilization on solid organic materials in manure and other organic waste materials is reversible and antibiotics can be released upon degradation of these organic materials, which will especially happen after addition of the organic waste materials as a fertilizer to soil.
Keeping in mind that even lowest residual concentrations of antibiotics are able to induce antibiotic resistance or may add up in mixed adverse effects on microbiota (see sections 4.1 and 4.2), it appears that the disappearance time of 90% of the parent compound (DT_{90}) are even more relevant than degradation half-lives (DT_{50}). The DT_{90} values of selected antibiotics that were determined by Berendsen et al. (2018) and also occurred in manure samples (see Table 14, page 60) are depicted in Figure 16. It can be seen that manure storage times of 3 months (90 d) are insufficient to reach a substantial reduction of the concentration of fluoroquinolones and of numerous macrolide and tetracycline antibiotics, while substantial reduction of sulfonamides can be reached. This agrees with the significant residual concentrations of antibiotics in organic waste materials and receiving soils (see Table 14 and Table 15, pg. 64).

Dissipation of antibiotics in soil: As it was found for manure and other organic waste materials, most reports on dissipation of antibiotics in soil are focused on **single substances** with experiments performed under standardized laboratory conditions. Compilations of respective results from literature can be found in Thiele-Bruhn (2003b) (see Table 22, Appendix) and Schmitt et al. (2017). From the publications it can be seen that dissipation half-lives of antibiotics from the structural classes of ß-lactams, macrolides sulfonamides, tetracyclines and quinolones are roughly in the range of 30 d. However, this can vary largely between individual compounds, soils and investigation conditions. For example, reported half-lives for sulfonamides range from as low as 3 d (Wang et al., 2006) to up to 139 d (Yang et al., 2009). Furthermore, it has to be noted that as for manure most of the dissipation is due to immobilization and related formation of non-extractable residues. Förster et al. (2009) and Rosendahl et al. (2011) showed that this residual fraction degrades much slower with half-lives of 23-330 d compared to 2-32 d required as DT_{50} for the not immobilized, easily extractable fraction of sulfadiazine., True mineralization is rather low with less than 1% of the parent compound within 64 d as was found for example for the sulfonamide sulfamethazine using ^{14}C-radiolabelled compounds (Langhammer et al., 1990). Consequently, residues of antibiotics can be found in soils even years after their

last application (Aust et al., 2008a; Aust et al., 2010; Schmidt et al., 2008). On the other hand, a slight increase of degradation rates was found when antibiotics had been repeatedly applied to the same soil (Goulas et al., 2018). This was related to the adaptation and accumulation of degrading microbial strains in soil. Nevertheless, it must be expected that repeated application of antibiotics through the periodic use of contaminated waste materials as fertilizer leads to the build-up of an apparently constant residue level, balanced between the input of additional antibiotics and the loss due to degradation and other processes (Hamscher et al., 2005; Hamscher et al., 2002; Höper et al., 2002).

Figure 16: Disappearance times of 90% of the parent compound (DT_{90}, d), measured at 20°C, of pharmaceutical antibiotics that were also detected in organic waste materials used for soil fertilization (see Table 14).



Source: Own figure with data from Berendsen et al. (2018).

Independent from that discussion, some reports exist on **altered dissipation of antibiotics in soil in the presence of mixed contaminations**. For mixtures of several antibiotics this was already shown in Figure 15-left (pg. 82), where the immobilization of sulfamethazine as single compound was faster and quantitatively stronger than in an equimolar ternary mixture with sulfadiazine and sulfadimethoxine. The further effect on the biodegradation of sulfamethazine became apparent from the parallel investigation of microbial active soil, in which the additional decline (termed in Figure 15, pg. 82, as apparent sorption) was clearly stronger for sulfamethazine alone compared to the antibiotic in a mixture with the other two sulfonamides (Thiele-Bruhn, 2015). The basic causes for these results are the before mentioned sorption competition (see "Effects on sorption") and the synergistically increased inhibition of microbial degradation activity by the antibiotic mixtures. Simple concentration additivity can be ruled out for that experiment because similar molar concentrations were tested for the single compound alone and the sum of all three antibiotics in the mixture.

Similar to sorption also the **dissipation of antibiotics is affected by multivalent cations** such as Cu^{2+}. The dissipation of sulfadiazine as well as of chlortetracycline in a loam soil was retarded by additional single and even more repeated application of Cu at a content of 2 g/kg (Liu et al., 2017a; Liu et al., 2012). Dissipation time ($DT_{50,\ 25°C}$) increased from 1.2 days to 3.3 days (single application of Cu) and 5.7 days (repeated application of Cu) and from 1.2 days to 1.3 days (single application of Cu) and 1.6 days (repeated application of Cu) for sulfadiazine and chlortetracycline, respectively (Liu et al., 2017a). These findings were confirmed by studies of Xu et al. (2015) and Xu et al. (2016) with clear consequences for the antibiotic and toxic effects, respectively, of both compounds on soil microbial activity, biomass and community structure.

A combination of the fungicide carbendazim with the antibiotic chloramphenicol caused little effect on carbendazim dissipation, whereas chloramphenicol dissipation was significantly retarded by the presence of carbendazim (Yan et al., 2011). Similarly, degradation times (DT_{50}) of chlortetracycline in soil were slightly but significantly decelerated from 10 days to 13 days in the in the presence of carbendazim (Fang et al., 2016).

All these examples show that combinations of antibiotics with other naturally occurring compounds and contaminants, respectively, may be seen as compound mixtures. These mixtures as well as compound mixtures in their strict sense, meaning combinations of different antibiotics, can be affected in their chemical fate and behavior in soil. The altered fate of the antibiotics and antibiotic mixtures will influence their toxic effects in the soil environment.

7 Conclusions on consequences for soil functioning and for further research

The existence of mixed contaminations with antibiotics in the soil environment and especially in agricultural soils is indisputable. As outlined in the previous sections of this report, the annual consumption of antibiotics is large and constantly increasing; the consumption of antibiotics for human and veterinary medicine in the EU in 2014 made up 3821 t and 8927 t, respectively (see Table 4, pg. 45). With a major percentage of each individual antibiotic being released from the medicated organisms in the unchanged active form, substantial contamination levels are reached in excreta. From the data reported in Table 14 (pg. 60) it can be seen that mean contents of single compounds are at 130 mg/kg with on average 12 antibiotic compounds detected in each sample and a total mean concentration of 1300 mg/kg (sum of all antibiotics). Additionally, co-contaminants such as Cu and Zn often are found in livestock manure which have been shown to enhance antibiotic resistance. Fertilizing soils with these substrates results in considerable contaminations. Analyzing the data listed in Table 15 (pg. 64) it turns out that mean contents of single compounds are at 100 µg/kg with on average eight antibiotic compounds detected in each sample and a total mean concentration of 1000 µg/kg (sum of all antibiotics). Furthermore, the retarded degradation of numerous antibiotics and repeated soil fertilization using contaminated organic waste materials lead to a continuing contamination level (apparent persistence).

The risks and adverse effects, however, arising from mixed contaminations with antibiotics are largely unknown and require to be identified, evaluated and regulated. Existing reports on pharmaceutical antibiotics in the soil environment are largely restricted to effects and fate of single antibiotic compounds. These publications clearly show the adverse, dose-related effects of single antibiotic compounds on soil microbial communities and activities, and thus on soil functioning (Ding and He, 2010; Grenni et al., 2018; Nesme and Simonet, 2015; Qiao et al., 2018). At the typical concentration level that is determined in contaminated soil, these effects are often in the range of lowest to no observable effect levels (LOEC, NOEC), meaning that they are at the border of being significant or occur only as a trend (see results on effect concentrations and environmental concentrations in Table 14, 15 and 16 (pg. 60, 64 and 67). However, this valuation might be different when it is considered that soils are not contaminated with single antibiotics but with a mixture of several antibiotics, each occurring at a low dose. Research on antibiotic mixtures in soils and usage of antibiotic mixtures in medicine clearly shows that effect additivity must be assumed and numerous mixtures even show a substantial effect amplification. In the sum, resulting effects of mixture toxicity in the soil environment may have significant adverse impacts on soil organisms.

Because most antibiotics are meant to affect microorganisms and especially bacteria, adverse effects on soil microorganisms are first of all expected and have been determined. This includes effects on enzymatic activities, and especially activities within the nitrogen cycle appear to be affected, respiration, biomass as well as shifts within the community composition (biodiversity) as well as the antibiotic resistance level. Such effects, even when they are small in a specific soil must be considered because of the superior relevance of microorganisms for soil ecosystem services and functioning. Soil microorganisms are indispensable for cycling of nutrients and carbon and carbon sequestration; pest control and plant growth promotion; greenhouse gas emissions; formation of soil structure affecting soil water, gas balance and filtration function; biodegradation of pollutants; food web support; and not last the vast contribution to biodiversity and genetic resources (Ockleford et al., 2017).

The effect on the antibiotic resistance level in soil is especially critical from a human health perspective because there are strongest indications that the altered resistance level in the soil environment considerably contributes to health problems associated with the increase of infections of humans by antibiotic-multi-resistant pathogens that cannot be treated with most antibiotic pharmaceuticals (Forsberg et al., 2012; Smith et al., 2005; Xie et al., 2018).

However, the current regulatory guidelines of pharmaceuticals do not consider the impact of antibiotic mixtures but regard only the risks of single compounds, nor do they consider the impact on antibiotic resistance formation and spread in the environment.

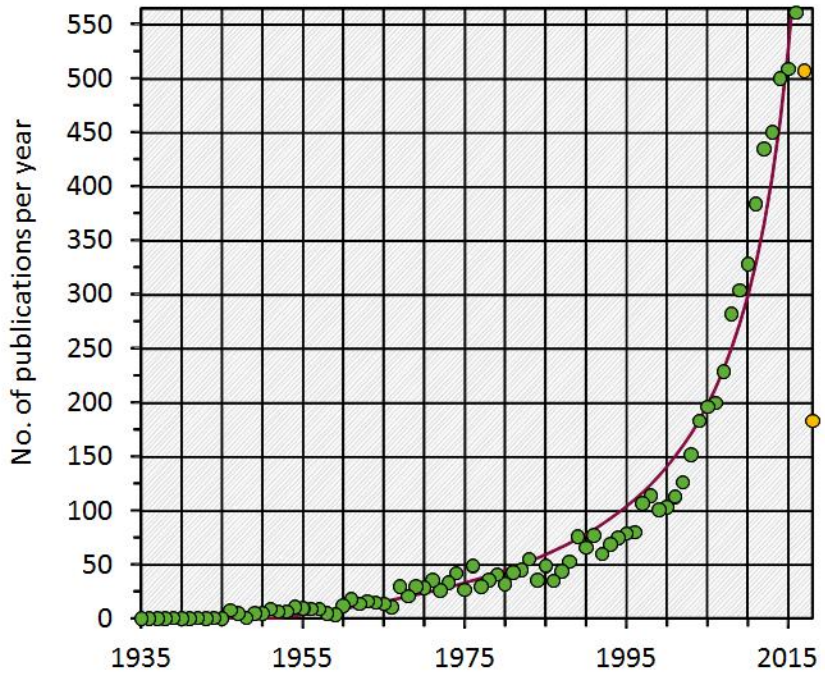
7.1 Knowledge gaps

A detailed literature review done in this study revealed that, in general, there is a steady increase of the number of publications on pharmaceuticals and pharmaceutical antibiotics in soils and the environment. While at the turn of the millennium 1,924 studies on “antibiotics” and “soil” had been published, the number increased until the end of 2017 to 7,669 publications (Figure 17). While no publications were recorded for several years between 1935 and 1945, the number of publications grew since then to now >500 publications per year. The first publications focused on natural antibiosis and the identification of natural antibiotics in soil, though (Lal, 1939; Metzger et al., 1942). It took until the 1970s that possible environmental problems related to the massive consumption of antibiotics such as environmental pollution (Strauch, 1974) and increasing resistance levels (Tessi, 1974) were first mentioned.

Especially in the past 20 years, the knowledge on the input, fate and effects of antibiotics in soil, excreta and organic fertilizers largely expanded. However, the work is still very much focused on specific structural classes of antibiotics, i.e. tetracyclines and sulfonamides, while the level of knowledge is still fragmentary for classes such as benzimidazoles, lincosamides and cephalosporins (Table 17). Additionally, many of the 7669 studies have been published in medicinal journals, where the term ‘soil’ might occur but most often the research presented is in fact not related to environmental issues. Among the publications researched in Table 17 none contained the search terms “mixture”, “joint toxicity”, “joint effects”, “binary mixtures”, “ternary mixtures”, “toxicity interactions” (compare Table 1).

Figure 17: Number of scientific journal articles published in each year from 1935 until April 2018.

Hits from a literature search using the Scopus data base and the search terms “antibiotics” and “‘soil”. The numbers for 2017 and 2018 were not complete at the date of the literature search, which is indicated by ochre-colored symbols.



Source: Own figure from S. Thiele-Bruhn (Univ. Trier).

Summary of knowledge gaps on antibiotic mixtures in soil

It must be stated that still many knowledge gaps exist, which is further confirmed by other authors which are summarized below.

- Reports on the occurrence of antibiotics in environmental substrates such as manure, sewage sludge and soils are largely limited to compounds from the structural classes of tetracyclines and sulfonamides and a minor number of reports on fluoroquinolones, macrolides and lincosamides (Łukaszewicz et al., 2017). Respective knowledge on compound classes such as benzimidazoles, quinoxalines, cephalosporins, phenoxyphenols, streptogramins and pleuromutilines is substantially lacking.
- The knowledge on the environmental inputs, fate and effects of antibiotics of other structural classes is fragmented and incomplete or fully missing.
- Even less information is available on the occurrence, composition and concentrations of mixed antibiotic contaminations as well as their fate in soil.
- Soils are heterogeneous with different soil horizons as macrostructure and aggregates, rhizosphere etc. as microstructure. The distribution of antibiotics and antibiotic mixtures within soils is largely unknown.

Table 17: Numbers of references since 1935 that investigated structural classes of antibiotics and single compounds.

Literature search under the search terms "antibiotics" and "soil" that yielded 7669 records on Scopus (search on April 16, 2018). Double entries may occur.

Structural classes		Single compounds	
Tetracyclines	1040		
Sulfonamides	342	Trimethoprim	217
Macrolides	243		
Aminoglycosides	176		
Quinolones	119 [a]	Nalidixic acid	212
Fluoroquinolones	75	Ciprofloxacin	384
ß-Lactams	62	Penicillin	519
Polyethers	33 [b]		
Benzimidazoles	19		
Avermectines	11		
Quinoxalines	7		
Imidazoles	7 [c]		
Lincosamides	5		
Cephalosporins	2		
Phenicols	2		
Polypeptides		Bacitracin	92
Polychlorinated phenoxyphenols		Triclosan	31
Streptogramins		Virginiamycin	28
Pleuromutilines		Thiamulin	10

[a] 71 of these references also covered "fluoroquinolones"; [b] 19 of these references also covered "polyether ionophores"; [c] one of these references also covered "benzimidazoles".

- Other than in the aqueous environment, in soil also the fate of antibiotics can change in the presence of mixed contaminations, for example through altered (competitive) sorption, which will feed-back on effects exerted by the antibiotics. However, knowledge on this topic is scarce.
- There are too few data on toxic effects of pharmaceuticals in soil and even less in particular on effects of mixtures (Backhaus, 2014; Brandt et al., 2015).
- The underlying processes of mixture effects such as antagonism and synergism are only rudimentary understood. It should be aimed to overcome empirical description but identify (and further model) principles. This especially applies to the lacking understanding of the ecological roles of antibiotics in nature and possible adverse effects of environmental pollution arising from that (Brandt et al., 2015). A long-term goal will be the development of a systematic approach for unraveling the underlying causes of any given pharmaceutical interaction (Bollenbach, 2015).
- The existing knowledge on toxic effects of pharmaceuticals in soil (not to speak of mixtures) is based on a rather broad, non-systematic number of different test methods and endpoints (see Table 16, pg. 67) that hinders the integration of the existing knowledge. It must be expected that effects and effect concentrations largely vary between different tested subjects and endpoints (Jonker et al., 2010).
- In this regard it is further necessary to investigate the factors that influence mixture toxicity such as number of contaminants, their (relative) concentrations, and environmental conditions such as soil moisture and temperature, chemical and physical soil properties, status and composition of soil (microbial) community.
- A specific aspect is the time dependence of antibiotic effects (Jonker et al., 2010); especially from mixtures, long-term exposure and chronic effects are expected.
- A specific problem in the assessment of toxicity is hormesis. Seemingly positive, because increasing effects at low doses can be part of a toxicity response and can occur in the presence of pharmaceuticals (Thiele-Bruhn, 2005) and their mixtures (Backhaus et al., 2011; Zou et al., 2013). It remains unclear how to valuate a hormetic increase and even more so in the presence of mixtures with single compounds having different effects in this regard (Vasquez et al., 2014).
- Lastly, viable concepts for cumulative exposure assessment strategies need to be developed (Kortenkamp and Altenburger, 2010).
- All this is flanked by the need for uniform and at best standardized methods to determine total contents and bioavailable fractions of antibiotics in soil and to reliably determine antibiotic effects on soil organisms. It is assumed that a bioavailable fraction will best represent effect concentrations (see Eq. 1, section7.2).

7.2 General approaches and research strategies to tackle mixture toxicity in soil

Systematic research to address mixture toxicity

Systematic research is needed to deal with and to answer the open questions listed in chapter 7.1. It should be aimed to identify and prove in how far the existing, fragmented and patchy findings can be used and combined to give a clearer idea of how the overall image looks. Targeted research is needed to find out in how far research gaps can be bridged by model calculations, which data can be used as surrogate for missing other information, which factors might be negligible and if necessary to fill major gaps with additional specific experimental research and data, respectively. It will be the overall goal to come to a practical solution how the toxicity of antibiotic mixtures in the soil environment can be assessed. Backhaus (2014) stated that for a holistic, mixture-aware environmental risk assessment of pharmaceuticals at least three interlinked but distinct purposes have to be tackled: (i) to quantify and assess the hazard and risk that a given pharmaceutical

mixture poses for the environment; (ii) to predict which pharmaceutical mixtures, in terms of composition and concentration, can be tolerated at a given site or in a given environmental compartment; (iii) to identify which compounds are the ecotoxicological drivers at a given site.

Considering mixture toxicity in environmental risk assessment requires therefore

a) to identify mixed contaminations with antibiotics in soils representing typical input situations, starting from the existing information on the input side and going to a multi-targeted, analytical approach (not restricted to only a few selected compounds and compound classes) for a complete identification of mixed contaminations in agricultural soils;
b) to further research and combine the existing information on fate, effects and interactions of antibiotics with other chemicals from different scientific disciplines, i.e. pharmacology, medicine, environmental sciences, and to even better identify existing knowledge on one hand and research gaps on the other;
c) to combine the existing knowledge using evaluation models to calculate expected mixed toxicity and
d) to validate and/or calibrate modelled estimates, e.g. of effect concentrations, with data from selected experiments that were specifically designed for that purpose;
e) to verify if existing results on other tested parameters can be used as surrogate for missing data in order to integrate as much existing knowledge as possible.
f) All this should be done with the additional aim to identify as best as possible f.1) underlying mechanisms or at least modes of mixture toxicity and f.2) influencing factors, i.e. the influence of relative concentrations, soil moisture, temperature, etc. (see bullet points in chapter 7.1).

Standard methods to test mixture toxicity in soil microorganisms

Because antibiotics first of all act on bacteria, the environmental risk assessment (ERA) of antibiotics should be focused on ecotoxicological tests targeting microorganisms and especially bacteria (Brandt et al., 2015). To this end, microbial community-based tests should be used, assessing functions and the structural diversity of microbial communities (Brandt et al., 2015; Grenni et al., 2018). Changes in community composition have been found to occur at sub-inhibitory levels, even before effects on microbial functions appear (Hammesfahr et al., 2008; Hammesfahr et al., 2011). Respective methods are thus considered to be more sensitive and less affected by microbial resilience (Grenni et al., 2018; Hammesfahr et al., 2008). Proposed methods are listed in Table 18.

For experimental testing of the effects of mixtures it is recommended to use standard test methods for soil (ISO, OECD) that address major ecosystem services of the soil organisms (Brandt et al., 2015; Thiele-Bruhn et al., 2018). Respective methods are listed in Table 18; some of them can be seen as alternative methods such as the determination of the microbial biomass using either the respiration (ISO 14240-1) or the fumigation extraction method (ISO 12240-2), as well as the determination of enzyme activities using either fluorogenic substrates (ISO/TS 22939) or colorimetric substrates (ISO 20130). All these methods were first of all established to test toxic effects of xenobiotic (synthetic) chemicals in soils such as pesticides and persistent organic pollutants. More and more they are additionally used to determine natural levels of (micro)biotic activity and abundance in soils and the effects of immaterial influences, e.g. of soil use or climate change. Test methods to investigate effects on the antibiotic resistance level and possible formation of tolerance against antibiotics have been evaluated by Schmitt et al. (2017). Proposed methods (Schmitt et al., 2017) are those for real-time qPCR identification and quantification of antibiotic resistance genes as well as tests for minimum selective concentrations (Gullberg et al., 2011) and pollution-induced community tolerance (PICT) (Rutgers et al., 1998; Van Beelen et al., 2001).

Table 18 Proposed standard methods for the testing of mixture toxicity of mixed antibiotic contaminations in soils.

Method (year released)	Microbial biodiversity and resistance level	
ISO 11063 (2012)	Method to directly extract DNA from soil samples	
ISO 17601 (2016)	Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil	
----	Determination of functional genes and resistance genes by qPCR	
ISO/TS 29843-1 (2010)	Phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis for the determination of soil microbial diversity	
ISO/TS 29843-2 (2011)	Simplified PLFA extraction method for the determination of soil microbial diversity	
	Microbial biomass and respiration	
ISO 14240-1 (1997)	Determination of soil microbial biomass – Part 1: Substrate induced respiration method	
ISO 14240-2 (1997)	Determination of soil microbial biomass – Part 2: Fumigation – extraction method	
ISO 16072 (2002)	Laboratory method for determination of microbial soil respiration	
ISO 17155 (2012)	Determination of the activity of the soil microflora using respiration curves	
	Microbial enzymatic activities: carbon, nitrogen and phosphorus turnover	
ISO/TS 22939 (2010)	Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates	UEnzymes measured:U β-xylosidase EC 3.2.1.37; cellobiosidase EC 3.2.1.91; phosphomonoesterase EC 3.1.3.2; leucine-aminopeptidase EC 3.4.11.1; alanine-aminopeptidase EC 3.4.11.12
	UEnzymes measured in both ISO/TS 22939 and ISO 20130:U Arylsulfatase EC 3.1.6.1; α-glucosidase EC 3.2.1.20; β-glucosidase EC 3.2.1.21; N-acetylglucosaminidase EC 3.2.1.52; phosphodiesterase EC 3.1.4.1	
ISO 20130 (2017)	Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates	UEnzymes measured:U Arylamidase EC 3.4.11.2; ß-galactosidase EC 3.2.1.22; urease EC 3.5.1.5
ISO 23753-1 (2005) ISO 23753-2 (2005)	Determination of dehydrogenase activity in soils — Part 1: Method using triphenyltetrazolium chloride (TTC) — Part 2: Method using iodotetrazolium chloride (INT)	
EN ISO 14238 (2012)	Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes	
EN ISO 15685 (2012)	Determination of potential nitrification and inhibition of nitrification — Rapid test by ammonium oxidation	
OECD 216 (2000)	Soil microorganisms: Nitrogen transformation test	
OECD 217 (2000)	Soil microorganisms: Carbon transformation test	
	Degradative activities of soil microorganisms	
ISO/NP 23265 (2018a)	Test for measuring organic matter (cellulose) decomposition in contaminated soil	

[a] Under development.

It is assumed that a combination of different tests (test battery) will be suited to determine adverse effects of antibiotics from different structural classes and of mixed antibiotics' contaminations. However, this needs further investigation to identify the most suited combination of methods and endpoints, because, as was stated previously, no systematic use of methods was done in the existing studies (see Table 16, pg. 67). On the other hand, it seems not necessary and it will not be manageable to measure all possible subjects and endpoints described in Table 18 for analyzing the effect of mixtures in soil. From a set of various test methods

on soil microorganisms, a toxicity threshold can be derived that can be used for all possible endpoints of an investigated organismic group (here soil microbiota). This applies because it is assumed that multiple effect measures of species are log-normally distributed and a distribution determined with sufficient accuracy contains all possible endpoints (Hanson and Solomon, 2002).

Strategies to model mixture toxicity

For modelling of mixture toxicity, first of all the standard models of concentration additivity (CA) and of independent action (IA) can be used as default (Coors et al., 2018; Yang et al., 2017). Both models have often been proposed, the CA to be used for mixtures of chemicals with similar mode of action, the IA for chemicals with different modes of action (de Zwart and Posthuma, 2005).

Concentration addition is calculated based on an effect concentration (*EC*) resulting in an inhibition/reduction of the tested subject by x%, for which the following equation is used (Jonker et al., 2010):

$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i}\right)^{-1} \quad \text{Eq. 1}$$

In that equation p_i denotes the relative fraction of a chemical i within a mixture with $p_1 + p_2 + \ldots + p_n = 1$.

Independent action is also known as response addition, Bliss independence, or effect multiplication (Vasquez et al., 2014) and is calculated as

$$E(c_{mix}) = 1 - \prod_{i=1}^{n}[1 - E(c_i)] \quad \text{Eq. 2}$$

with $E(c_{mix})$ the proportional effect that the total mixture causes at a concentration c_i of the individual compounds and with $c_1 + c_2 + \ldots + c_n = c_{mix}$. $E(c_i)$ represents the proportional effects that the individual compounds would cause when applied alone at the respective concentration.

The two models are not free from criticism and alternatives have been proposed (Escher et al., 2005; González-Pleiter et al., 2013). For example, the Combination Index (CI) method has the advantage to better predict antagonism, additive effects and synergism, respectively (Rodea-Palomares et al., 2010). Coming back to the deficits of the two models, IA does not allow accumulation of effects lower than NOEC to eventually exceed as a sum the NOEC level (Backhaus, 2014). This restriction strongly limits the applicability of the IA model because the addition of many compounds, each at a sub-effective concentration level, leading to a summed effect is a striking feature of mixture toxicity (see Figure 1, pg. 32). Cedergreen et al. (2008) assessed 158 data sets using the models of IA and CA; they found that 50% of the data sets were not correctly described. In contrast, Kortenkamp et al. (2009) reported the broad usability of the CA model and deviations between model prognosis and measured effects were for most studies vastly below an acceptable factor of 5. Even more, Kortenkamp and Altenburger (2010) emphasized that a disregard of mixture effects may lead to considerable underestimates of hazards from chemicals. Hence, it is much preferred to use the proven concepts of IA and CA, even though they might be not fully applicable, instead of ignoring mixture effects (Kortenkamp and Altenburger, 2010).

The pragmatic decision to use the models of CA and IA as a default, does not question the need to come to a more mechanistic understanding of the processes involved in mixture toxicity (Ragas et al., 2010). Understanding fundamental mechanisms and relations among factors of mixture toxicity will enable to strive for improved models as well. To that end, it is advantageous to know the mode of action of the investigated chemicals (Escher et al., 2005; Escher and Hermens, 2002). It is a substantial advantage that the mode of action against target organisms of every antibiotic is well known and documented in pharmaceutical sciences. This information can be used for the assessment of environmental pollution and effects on non-target organisms as well.

Additionally it must be noted that, much more than in the aquatic environment, chemicals in soil may become immobilized so that total and bioavailable (effective) concentrations of pollutants may largely differ. In addition to the total content (quantity) it is needed to determine the bioavailable content (intensity), for which some operationally defined methods have been proposed for some groups of organic chemicals, especially for hydrophobic organic chemicals (Bernhardt et al., 2013; Cachada et al., 2014; Thiele-Bruhn and Brümmer, 2004). Yet, such methods are missing for antibiotic pharmaceuticals. Here, additional work is needed.

In completion to the above mentioned CA and IA concepts, toxicity units (*TU*) can be calculated

$TU_i = C_i / ECx_i$

with C_i as concentration of each compound i in the mixture. It can be used as a measure for the relative contribution of each component to the overall toxicity of a mixture (Coors et al., 2018). The related sum of toxic units is termed as toxicity index (TI):

$$TI = \sum_{i=1}^{n}(C_i/ECx_i) \quad \text{Eq. 3.}$$

The TI is very similar to the CA model, using the quantitative contribution of each chemical in a mixture and the toxicity that the chemical alone would exert, to assess the relative contributions to and overall toxicity of a mixture (Azarbad et al., 2013; Khorshid and Thiele-Bruhn, 2016).

Furthermore, risk quotients (RQ) are used in order to estimate the probability of an adverse effect of a mixed contamination. The ratio between Predicted Environmental Concentrations (PEC) or Measured Environmental Concentrations (MEC) and Predicted No Effect Concentrations (PNEC) is calculated as (von der Ohe et al., 2011):

$$RQ = PEC/PNEC \text{ or } RQ = MEC/PNEC \quad \text{Eq. 4.}$$

To this end, it is proposed to combine the lowest PNECs for single compounds and mixtures with the highest available MECs from the literature (González-Pleiter et al., 2013). For aquatic environments, González-Pleiter et al. (2013) reported ratios of measured environmental concentrations and predicted no effect concentrations (MEC/PNEC) higher than 1. Hence, they concluded that combinations of antibiotics already exist in surface waters that may pose a potential ecologic risk to the environment.

Because PNECs of mixtures are not available, it is here proposed to calculate a weighted PNEC for each specific mixture ($PNEC_{mix}$) that depends on the relative contribution of the individual compounds and their individual PNECs to the overall mixture:

$$PNEC_{mix} = \sum_{i=1}^{n}\left(PNEC_i \times {}^{c_i}/_{c_{mix}}\right) \quad \text{Eq. 5.}$$

Correspondingly RQ of the mixture (RQ_{mix}) is calculated as

$$RQ_{mix} = \sum_{i=1}^{n}\left({}^{c_i}/_{PNEC_{mix}}\right) \quad \text{Eq. 6.}$$

7.3 Proposal of successive research projects and their use in regulation

A major obstacle in exploring the effects of pharmaceutical combinations is the huge number of samples that needs to be analyzed in case all possible combinations of a set of pharmaceuticals has to be systematically investigated. Testing all pairwise combinations of n pharmaceuticals at one fixed concentration requires n^2 experiments; investigating large numbers of pharmaceuticals, combinations of more than two compounds or different concentrations rapidly becomes unmanageable due to a 'combinatorial explosion' (Bollenbach, 2015). At the same time, research in ecotoxicology has not only the task to elucidate the scientific fundamentals but also (or even more) to provide a scientific basis for a practical implementation in risk assessment, precautionary measures, cleanup, and decision support for policy and management (Escher and Hermens, 2002; Ragas et al., 2010).

In order to react to these practical issues and the existing mixed contaminations with antibiotics in the environment, research should be quickly implemented to deliver first estimates on mixtures and mixture toxicity of antibiotics in soils on a short-term before extensive experimental research delivers precise analytical data to fill the existing knowledge gaps as best as possible on a mid- to long-term.

1. *Contamination status and effects of mixtures of pharmaceutical antibiotics in the terrestrial environment – a meta-analysis*

For a first, short-term approach, it is proposed to do a meta-analysis based on an extended literature research through

- collecting existing quantitative data on consumption of antibiotics and their (possible) dissemination into the soil environment, and combine and verify this with the existing analytical data on residual levels in waste materials and waste water, used for soil amendment, and in soils,
- in order to derive PEC and MEC of mixed contaminations of antibiotics in soils,
- combining these data with existing information on effect concentrations (EC) and PNEC, at best from soil related tests or as a surrogate from other tests (e.g. aquatic toxicology, pharmacology) and
- assessing these data using the concepts of CA, IA, TI and RQ.

With this approach it will be possible a) to identify risks from mixture toxicity of antibiotics in soils and b) to refine and improve the state-of-knowledge about soil contamination with antibiotic mixtures and their ecotoxicological significance and consequences. First recommendations for action could be given to stakeholders and regulatory authorities based on this approach and further experimental work could be designed.

2. *A general suite of methods for testing adverse effects of mixtures of pharmaceutical antibiotics in soil*

In a second, subsequent (mid-term) project, experimental work based on the previous findings should be carried out. The proposed aim should be i) to identify suitable methods to indicate adverse effects of pharmaceutical antibiotics on soil microbial abundance and functioning. Testing of the methods summarized in Table 18 and eventually of further methods aimed to determine relevant functions of soil microorganisms could be done in a dose-response approach with a suite of soils and a set of selected, representative antibiotics from the structural classes most often used in veterinary and human medicine, i.e. penicillins, tetracyclines, polypeptide antibiotics, sulfonamides, macrolides, aminoglycosides, lincosamides, pleuromutilines, fluoroquinolones, folic acid antagonists, fenicols, cephalosporines. ii) A test battery should be arranged with a subset of the identified, suitable methods. This set should at best comprise methods and endpoints representing parameters of microbial abundance and indicators of relevant functions.

3. *Mixture toxicity of mixed contaminations with pharmaceutical antibiotics and other pollutants in agricultural soils*

A third study (executed on a longer-term) should be aimed to investigate effects of mixtures of antibiotics by using the test battery (second study). Typical mixtures should be derived from information on application of antibiotics and mixtures recovered in waste materials and soils (first study). The research would start from binary mixtures and proceed with ternary and more complex mixtures. This would include antibiotics used in the second study as well as Cu and Zn as antibiotic active metals that often occur in contaminated waste materials and soils. Research tasks and questions will be:

- Which effects, i.e. antagonistic, synergistic, additive, occur when compounds of the same structural class are combined?
- What effects are caused by mixtures of antibiotics from different structural classes? Do effects match with combination effects reported from veterinary and human medicine, respectively?
- Are effects, being either antagonistic, synergistic or additive, similar for all combinations of two antibiotics from different structural classes?
- Can combinations with different proportions of the individual compounds sufficiently be modelled using the described concepts (e.g. CA, IA)?

The overall aim should be to identify underlying principles of antibiotic action of mixtures in soil to enable further modelling and prognosis, which knowledge would also better enable to define regulatory standards and thresholds.

This research would be ideally combined with scientific workshops in order to share the latest state-of-the-art scientific knowledge, to discuss the results of the proposed projects and to define further strategies on how to address the topic in research and regulation.

The proposed research is aimed to support regulation. In general, the overall aim should be to reduce and minimize the dissemination of pharmaceutical antibiotics and related heavy metals (Cu, Zn) in the environment in order to prevent adverse effects on soil function and fertility and to impede the increase and spread of antibiotic resistance in the environment with its increasing health risks for animals (livestock) and humans. Regulatory measures could cover the analytical determination of antibiotic contamination of waste materials such as manure used as soil fertilizer and/or the determination of the resulting contamination level in soils. This could be especially relevant for manure exports and imports between farms and countries. Acceptable contamination levels (regulatory limit values) could be oriented to no-effect concentrations determined in soil toxicity studies and concentration levels inducing increased antibiotic resistance. Knowledge about and the consideration of environmental issues would enable to better plan and organize the use of pharmaceutical antibiotics on the farm level. With no doubt, the priority should still be the curing of infectious diseases of humans and animals. This, however, requires much more than before the consideration of environmental issues, especially to avoid the increasing ineffectiveness of antibiotics due to the formation of antibiotic resistance in the environment. There is strong conviction that this will also contribute to an improved sustainable soil use and protection of water resources.

8 References

Agga, G.E., Arthur, T.M., Durso, L.M., Harhay, D.M., Schmidt, J.W. (2015): Antimicrobial-resistant bacterial populations and antimicrobial resistance genes obtained from environments impacted by livestock and municipal waste. PLoS ONE, 10.

Ahmed, M.B. et al. (2017): Single and competitive sorption properties and mechanism of functionalized biochar for removing sulfonamide antibiotics from water. Chemical Engineering Journal, 311:348-358.

Alexandrino, D.A.M. et al. (2017): Biodegradation of the veterinary antibiotics enrofloxacin and ceftiofur and associated microbial community dynamics. Science of the Total Environment, 581-582:359-368.

Alexy, R., Längin, A., Schöll, A., Sütterlin, H., Kümmerer, K. (2006): Pharmazeutische Wirkstoffe, Desinfektionsmittel und Diagnostika mit besonderen strukturellen Eigenschaften in der Umwelt: Untersuchung bisher nicht beachteter Aspekte hinsichtlich Verhalten und Wirkung mittels Experiment, Arbeitsbericht. Institut für Umweltmedizin und Krankenhaushygiene, Freiburg, pp. 200.

An, J., Chen, H., Wei, S., Gu, J. (2015): Antibiotic contamination in animal manure, soil, and sewage sludge in Shenyang, northeast China. Environmental Earth Sciences, 74:5077-5086.

Arenz-Leufen, M. (2012): Manure composition and its influence on the input and transfer of veterinary antibiotics in soil. Universitäts-Druckerei, Trier.

Armstrong, A., Philips, K. (1998): A strategic review of sheep dipping. Environment Agency Research and Develpoment Technical Report P170. Environment Agency, Bristol, UK.

Aust, M.-O. (2010): Retention and transport of sulfonamide antibiotics applied to soil with manure. Universitäts-Druckerei, Trier.

Aust, M.O. et al. (2008a): Distribution of sulfamethazine, chlortetracycline and tylosin in manure and soil of Canadian feedlots after subtherapeutic use in cattle. Environmental Pollution, 156:1243-1251.

Aust, M.O., Thiele-Bruhn, S., Eckhardt, K.U., Leinweber, P. (2009): Composition of organic matter in particle size fractionated pig slurry. Bioresource Technology, 100:5736-5743.

Aust, M.O., Thiele-Bruhn, S., Jandl, G., Leinweber, P. (2008b): Determination of six membered ring sulfonamides used in veterinary medicine in manure, soil and soil leachates using LC-ESI mass spectrometry. Fresenius Environmental Bulletin, 17:331-340.

Aust, M.O. et al. (2010): Sulfonamides leach from sandy loam soils under common agricultural practice. Water, Air, and Soil Pollution, 211:143-156.

Awad, Y.M. et al. (2014): Veterinary antibiotics contamination in water, sediment, and soil near a swine manure composting facility. Environmental Earth Sciences, 71:1433-1440.

Awad, Y.M. et al. (2016): Sulphamethazine in poultry manure changes carbon and nitrogen mineralisation in soils. Chemistry and Ecology, 32:899-918.

Azarbad, H. et al. (2013): Microbial community structure and functioning along metal pollution gradients. Environmental Toxicology and Chemistry, 32:1992-2002.

Backhaus, T. (2014): Medicines, shaken and stirred: a critical review on the ecotoxicology of pharmaceutical mixtures. Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 369.

Backhaus, T., Froehner, K., Altenburger, R., Grimme, L.H. (1997): Toxicity testing with Vibrio fischeri: A comparison between the long term (24 h) and the short term (30 min) bioassay. Chemosphere, 35:2925-2938.

Backhaus, T. et al. (2011): Single-substance and mixture toxicity of five pharmaceuticals and personal care products to marine periphyton communities. Environmental Toxicology and Chemistry, 30:2030-2040.

Backhaus, T., Scholze, M., Grimme, L.H. (2000): The single substance and mixture toxicity of quinolones to the bioluminescent bacterium Vibrio fischeri. Aquatic Toxicology, 49:49-61.

Baguer, A.J., Jensen, J., Krogh, P.H. (2000): Effects of the antibiotics oxytetracycline and tylosin on soil fauna. Chemosphere, 40:751-757.

Baker-Austin, C., Wright, M.S., Stepanauskas, R., McArthur, J.V. (2006): Co-selection of antibiotic and metal resistance. Trends in Microbiology, 14:176-182.

Balzer, F., Zöhlke, S., Hannappel, S. (2016): Antibiotics in groundwater under locations with high livestock density in Germany. Water Science and Technology: Water Supply, 16:1361-1369.

Batchelder, A.R. (1981): Chlortetracycline and oxytetracycline effects on plant growth and development in liquid cultures. J.Environ.Qual., 10:515-518.

Batchelder, A.R. (1982): Chlortetracycline and oxytetracycline effects on plant growth and development in soil systems. J.Environ.Qual., 11:675-678.

Baym, M., Stone, L.K., Kishony, R. (2016): Multidrug evolutionary strategies to reverse antibiotic resistance. Science, 351.

Berendsen, B.J.A. et al. (2018): The persistence of a broad range of antibiotics during calve, pig and broiler manure storage. Chemosphere, 204:267-276.

Berg, J. et al. (2010): Cu exposure under field conditions coselects for antibiotic resistance as determined by a novel cultivation-independent bacterial community tolerance assay. Environmental Science and Technology, 44:8724-8728.

Bernhardt, C., Derz, K., Kördel, W., Terytze, K. (2013): Applicability of non-exhaustive extraction procedures with Tenax and HPCD. Journal of Hazardous Materials, 261:711-717.

Bewick, M.W.M. (1979): The adsorption and release of tylosin by clays and soils. Plant Soil, 51:363-372.

Białk-Bielińska, A. et al. (2012): Sulfadimethoxine and sulfaguanidine: Their sorption potential on natural soils. Chemosphere, 86:1059-1065.

Binh, C.T.T. et al. (2007): Short-term effects of amoxicillin on bacterial communities in manured soil. Fems Microbiology Ecology, 62:290-302.

Blackwell, P.A., Boxall, A.B.A., Kay, P., Noble, H. (2005): Evaluation of a lower tier exposure assessment model for veterinary medicines. Journal of Agricultural and Food Chemistry, 53:2192-2201.

Blackwell, P.A., Kay, P., Ashauer, R., Boxall, A.B.A. (2009): Effects of agricultural conditions on the leaching behaviour of veterinary antibiotics in soils. Chemosphere, 75:13-19.

Blair, J.M.A., Webber, M.A., Baylay, A.J., Ogbolu, D.O., Piddock, L.J.V. (2015): Molecular mechanisms of antibiotic resistance. Nature Reviews Microbiology, 13:42-51.

Blume, H.-P., Horn, R., Thiele-Bruhn, S. (2011): Handbuch des Bodenschutzes. 4 ed. Wiley, Weinheim.

BMELV (2008): Strategie zur Bekämpfung des Feuerbranderregers im Obstbau ohne Antibiotika: 2008 - 2012. Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (BMELV), Bonn.

Bollenbach, T. (2015): Antimicrobial interactions: mechanisms and implications for drug discovery and resistance evolution. Current opinion in microbiology, 27:1–9.

Boreen, A.L., Arnold, W.A., McNeill, K. (2005): Triplet-sensitized photodegradation of sulfa drugs containing six-membered heterocyclic groups: Identification of an SO2 extrusion photoproduct. Environmental Science and Technology, 39:3630-3638.

Borgman, O., Chefetz, B. (2013): Combined effects of biosolids application and irrigation with reclaimed wastewater on transport of pharmaceutical compounds in arable soils. Water Research, 47:3431-3443.

Bottoni, P., Caroli, S. (2018): Presence of residues and metabolites of pharmaceuticals in environmental compartments, food commodities and workplaces: A review spanning the three-year period 2014–2016. Microchemical Journal, 136:2-24.

Bourdat-Deschamps, M. et al. (2017): Fate and impacts of pharmaceuticals and personal care products after repeated applications of organic waste products in long-term field experiments. Science of the Total Environment, 607-608:271-280.

Boxall, A.B.A. (2010): Veterinary medicines and the environment, Handbook of Experimental Pharmacology, pp. 291-314.

Boxall, A.B.A., Blackwell, P., Cavallo, R., Kay, P., Tolls, J. (2002): The sorption and transport of a sulphonamide antibiotic in soil systems. Toxicol.Lett., 131:19-28.

Boxall, A.B.A. et al. (2004): Veterinary medicines in the environment. Reviews of Environmental Contamination and Toxicology, 180:1-91.

Boxall, A.B.A. et al. (2006): Uptake of veterinary medicines from soils into plants. Journal of Agricultural and Food Chemistry, 54:2288-2297.

Boxall, A.B.A. et al. (2012): Pharmaceuticals and personal care products in the environment: What are the big questions? Environmental Health Perspectives, 120:1221-1229.

Brandt, K.K. et al. (2015): Ecotoxicological assessment of antibiotics: A call for improved consideration of microorganisms. Environment International, 85:189-205.

Brandt, K.K., Sjøholm, O.R., Krogh, K.A., Halling-Sørensen, B., Nybroe, O. (2009): Increased pollution-induced bacterial community tolerance to sulfadiazine in soil hotspots amended with artificial root exudates. Environmental Science and Technology, 43:2963-2968.

Braschi, I. et al. (2013): Persistence and degradation of new β-lactam antibiotics in the soil and water environment. Chemosphere, 93:152-159.

Bundestierärztekammer (2015): Bundestierärztekammer (BTK): Leitlinien für den sorgfältigen Umgang mit antibakteriell wirksamen Tierarzneimitteln – mit Erläuterungen. Deutsches Tierärzteblatt, 3, supplement:24.

Burkhardt, M., Stamm, C., Waul, C., Singer, H., Müller, S.R. (2005): Surface runoff and transport of sulfonamide antibiotics and tracers on manured grassland. Journal of Environmental Quality, 34:1363-1371.

BVL (2016a): Bundesamt für Verbraucherschutz und Lebensmittelsicherheit - Menge der abgegebenen Antibiotika in der Tiermedizin halbiert. BVL, Berlin.

BVL (2016b): Bundesamt für Verbraucherschutz und Lebensmittelsicherheit and Paul-Ehrlich-Gesellschaft für Chemotherapie e.V. GERMAP 2015 – Antibiotika-Resistenz und -Verbrauch. Bericht über den Antibiotikaverbrauch und die Verbreitung von Antibiotikaresistenzen in der Human- und Veterinärmedizin in Deutschland. Antiinfectives Intelligence, Rheinbach.

Byrne-Bailey, K.G. et al. (2009): Prevalence of sulfonamide resistance genes in bacterial isolates from manured agricultural soils and pig slurry in the United Kingdom. Antimicrobial Agents and Chemotherapy, 53:696-702.

Cachada, A., Pereira, R., da Silva, E.F., Duarte, A.C. (2014): The prediction of PAHs bioavailability in soils using chemical methods: State of the art and future challenges. Science of the Total Environment, 472:463-480.

Calabrese, E.J. (1991): Chapter 4: Drugs; chapter 5: The drug-pollutant interface, Multiple chemical interactions. Lewis Publishers, Chelsea, MI, pp. 389-578.

Calabrese, E.J. (2008): Hormesis: Why it is important to toxicology and toxicologists. Environmental Toxicology and Chemistry, 27:1451-1474.

Caminero, J.A., Sotgiu, G., Zumla, A., Migliori, G.B. (2010): Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis. The Lancet Infectious Diseases, 10:621–629.

Carrillo, M., Siebe, C., Dalkmann, P., Siemens, J. (2016): Competitive sorption of linear alkylbenzene sulfonate (LAS) surfactants and the antibiotics sulfamethoxazole and ciprofloxacin in wastewater-irrigated soils of the Mezquital Valley, Mexico. Journal of Soils and Sediments, 16:2186-2194.

Cedergreen, N. et al. (2008): A review of independent action compared to concentration addition as reference models for mixtures of compounds with different molecular target sites. Environmental Toxicology and Chemistry, 27:1621-1632.

Čermák, L. et al. (2008): Bacterial communities of two contrasting soils reacted differently to lincomycin treatment. Applied Soil Ecology, 40:348-358.

Chander, K., Ulrich, E., Joergensen, R.G., Klein, T. (2002): Decomposition of ^{14}C labelled wheat straw in repeatedly fumigated and non-fumigated soils with different levels of heavy metal contamination. Biol Fertil Soils, 35, 86-91.

Chander, Y., Kumar, K., Goyal, S.M., Gupta, S.C. (2005): Antibacterial activity of soil-bound antibiotics. Journal of Environmental Quality, 34, 1952-1957.

Chapman, S.J., Gray, T.R.G. (1986): Importance of cryptic growth, yield factors and maintenance energy in models of microbial growth in soil. Soil Biology and Biochemistry, 18:1-4.

Chee-Sanford, J.C., Aminov, R.I., Krapac, I.J., Garrigues-Jeanjean, N., Mackie, R.I. (2001): Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. Applied and Environmental Microbiology, 67:1494-1502.

Chee-Sanford, J.C. et al. (2009): Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. Journal of Environmental Quality, 38:1086-1108.

Chefetz, B., Mualem, T., Ben-Ari, J. (2008): Sorption and mobility of pharmaceutical compounds in soil irrigated with reclaimed wastewater. Chemosphere, 73:1335-1343.

Chen, Q.Y., Wu, Z.H., Liu, J.L. (2011): Ecotoxicity of chloramphenicol and Hg acting on the root elongation of crops in North China. International Journal of Environmental Research, 5:909-916.

Chessa, L., Pusino, A., Garau, G., Mangia, N.P., Pinna, M.V. (2016): Soil microbial response to tetracycline in two different soils amended with cow manure. Environmental Science and Pollution Research, 23:5807-5817.

Christensen, A.M., Ingerslev, F., Baun, A. (2006): Ecotoxicity of mixtures of antibiotics used in aquacultures. Environmental Toxicology and Chemistry, 25:2208.

Christoph-Schulz, I. et al. (2018): SocialLab – Nutztierhaltung im Spiegel der Gesellschaft. Journal of Consumer Protection and Food Safety, 13:145-236.

Christou, A. et al. (2017): The potential implications of reclaimed wastewater reuse for irrigation on the agricultural environment: The knowns and unknowns of the fate of antibiotics and antibiotic resistant bacteria and resistance genes – A review. Water Research, 123:448-467.

Clara, M., Scheffknecht, C., Weiß, S. (2013): Eintrag von Arzneimittelwirkstoffen in die Umwelt. Umweltbundesamt, Umweltinstitut des Landes Vorarlberg, Bregenz.

Cleary, D.W. et al. (2016): Long-term antibiotic exposure in soil is associated with changes in microbial community structure and prevalence of class 1 integrons. FEMS microbiology ecology, 92.

Colinas, C., Ingham, E., Molina, R. (1994): Population responses of target and non-target forest soil-organisms to selected biocides. Soil Biol.Biochem., 26:41-47.

Conkle, J.L., Lattao, C., White, J.R., Cook, R.L. (2010): Competitive sorption and desorption behavior for three fluoroquinolone antibiotics in a wastewater treatment wetland soil. Chemosphere, 80:1353-1359.

Coors, A. et al. (2018): Prospective environmental risk assessment of mixtures in wastewater treatment plant effluents – Theoretical considerations and experimental verification. Water Research, 140:56-66.

Cui, H. et al. (2014): Influence of ciprofloxacin on microbial community structure and function in soils. Biology and Fertility of Soils, 50:939-947.

Dang, H., Song, L., Chen, M., Chang, Y. (2006): Concurrence of cat and tet genes in multiple antibiotic-resistant bacteria isolated from a sea cucumber and sea urchin mariculture farm in China. Microbial Ecology, 52:634-643.

Daughton, C.G., Ternes, T.A. (1999): Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ.Health Perspect., 107 Suppl 6:907-938.

David, V. et al. (2016): Mixture effects of antibiotics on soil microbial nitrogen processes. Presentation at 1st International Conference on Risk Assessment of Pharmaceuticals in the Environment - ICRAPHE; Paris, France; 8-9 September 2016, http://prodinra.inra.fr/record/382231.

Dawson, D.A. et al. (2014): Time-dependence in mixture toxicity prediction. Toxicology, 326:153–163.

de Zwart, D., Posthuma, L. (2005): Complex mixture toxicity for single and multiple species: proposed methodologies. Environmental Toxicology and Chemistry, 24:2665.

Demoling, L.A., Bååth, E. (2008): No long-term persistence of bacterial pollution-induced community tolerance in tylosin-polluted soil. Environmental Science and Technology, 42:6917-6921.

Demoling, L.A., Bååth, E., Greve, G., Wouterse, M., Schmitt, H. (2009): Effects of sulfamethoxazole on soil microbial communities after adding substrate. Soil Biology & Biochemistry, 41:840-848.

Ding, C., He, J.Z. (2010): Effect of antibiotics in the environment on microbial populations. Applied Microbiology and Biotechnology, 87:925-941.

Dolliver, H., Kumar, K., Gupta, S. (2007): Sulfamethazine uptake by plants from manure-amended soil. Journal of Environmental Quality, 36:1224-1230.

Dong, L., Xie, X., Zhou, Q. (2011): The DNA damage and joint effects of two typical veterinary drugs on the earthworm *Eisenia foetida*. Huanjing Kexue Xuebao/Acta Scientiae Circumstantiae, 31:2798-2803.

Du, L., Liu, W. (2012): Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. Agronomy for Sustainable Development, 32:309-327.

DWA-AG GB-7.4 (2017): Arbeitsblatt DWA-A 920-2; Bodenfunktionsansprache. Teil 2: Filter und Puffer für organische Chemikalien. Authors: Thiele-Bruhn S. and Wilke B.-M., Hennef.

ECDC, EFSA, EMA (2017): ECDC (European Centre for Disease Prevention and Control), EFSA (European Food Safety Authority), and EMA (European Medicines Agency) second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals – Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA) Report. EFSA Journal, 15:135.

Ejim, L. et al. (2011): Combinations of antibiotics and nonantibiotic drugs enhance antimicrobial efficacy. Nature Chemical Biology, 7:348-350.

EMEA (1997): Note for guidance: Environmental risk assessment for veterinary medicinal products other than GMO-containing and immunological products. Committee for Veterinary and Medicinal Products, London.

Escher, B.I., Bramaz, N., Eggen, R.I.L., Richter, M. (2005): In vitro assessment of modes of toxic action of pharmaceutical in aquatic life. Environmental Science and Technology, 39:3090-3100.

Escher, B.I., Hermens, J.L.M. (2002): Modes of action in ecotoxicology: Their role in body burdens, species sensitivity, QSARs, and mixture effects. Environmental Science and Technology, 36:4201-4217.

Fang, H. et al. (2016): Changes in soil microbial community structure and function associated with degradation and resistance of carbendazim and chlortetracycline during repeated treatments. Science of the Total Environment, 572:1203-1212.

FDA (1987): Environmental assessment technical assistance handbook. FDA Center for Food Safety and Applied Nutrition, Environmental Impact Section.

Figueroa-Diva, R.A., Vasudevan, D., Mackay, A.A. (2010): Trends in soil sorption coefficients within common antimicrobial families. Chemosphere, 79:786-793.

Figueroa, R.A., Leonard, A., Mackay, A.A. (2004): Modeling tetracycline antibiotic sorption to clays. Environmental Science & Technology, 38:476-483.

Figueroa, R.A., Mackay, A.A. (2005): Sorption of oxytetracycline to iron oxides and iron oxide-rich soils. Environmental Science & Technology, 39:6664-6671.

Forsberg, K.J. et al. (2012): The shared antibiotic resistome of soil bacteria and human pathogens. Science, 337:1107-1111.

Förster, M. et al. (2009): Sequestration of manure-applied sulfadiazine residues in soils. Environmental Science & Technology, 43:1824-1830.

Förster, M., Laabs, V., Lamshöft, M., Pütz, T., Amelung, W. (2008): Analysis of aged sulfadiazine residues in soils using microwave extraction and liquid chromatography tandem mass spectrometry. Analytical and Bioanalytical Chemistry, 391:1029-1038.

Frankenberger Jr, W.T., Tabatabai, M.A. (1982): Amidase and urease activities in plants. Plant and Soil, 64:153-166.

Freitag, M., Yolcu, D.H., Hayen, H., Betsche, T., Grote, M. (2008): Screening of antibiotic transfer from soil into cereals in areas with high livestock concentration in Northrhine Westphalia (Germany). Screening zum antibiotika-transfer aus dem boden in getreide in regionen Nordrhein-Westfalens mit großen viehbeständen, 3:174-184.

Füssle, R. (2011): Prinzipien der Antibiotikatherapie, Deutsche Akademie für Anästhesiologische Fortbildung: Refresher course. Ebelsbach : Aktiv Druck und Verl., Nürnberg, Germany, pp. 16.

Gao, J.A., Pedersen, J.A. (2005): Adsorption of sulfonamide antimicrobial agents to clay minerals. Environmental Science & Technology, 39:9509-9516.

Gao, M., Zhou, Q., Song, W., Ma, X. (2014): Combined effects of oxytetracycline and Pb on earthworm *Eisenia fetida*. Environmental Toxicology and Pharmacology, 37:689-696.

García-Galán, M.J., Díaz-Cruz, S., Barceló, D. (2013): Multiresidue trace analysis of sulfonamide antibiotics and their metabolites in soils and sewage sludge by pressurized liquid extraction followed by liquid chromatography-electrospray-quadrupole linear ion trap mass spectrometry. Journal of Chromatography A, 1275:32-40.

Gavalchin, J., Katz, S.E. (1994): The persistence of fecal-borne antibiotics in soil. J.AOAC Int., 77:481-485.

Gielen, G.J.H.P., van Schaik, A.P., Northcott, G., Horswell, J. (2016): Effect of copper and zinc on microbial tolerance to triclosan in two soil types. Journal of Soils and Sediments, 16:1944-1959.

Gieringer, J.H., Wenz, A.F., Just, H.-M., Daschner, F.D. (1986): Effect of 5-fluorouracil, mitoxantrone, methotrexate, and vincristine on the antibacterial activity of ceftriaxone, ceftazidime, cefotiam, piperacillin, and netilmicin. Chemotherapy, 32:418–424.

Gilbertson, T.J. et al. (1990): Environmental fate of ceftiofur sodium, a cephalosporin antibiotic - role of animal excreta in its decomposition. Journal of Agricultural and Food Chemistry, 38:890-894.

Gildemeister, D., Tauchnitz, N., Berkner, S. (2011): Tierarzneimittelmischungen in landwirtschaftlich genutzten Böden. Poster presentation at conference: Böden verstehen - Böden nutzen – Böden fit machen. DBG, 03.09.–09.09.2011, Berlin. Mitteilungen der Deutschen Bodenkundlichen Gesellschaft.

Girardi, C. et al. (2011): Biodegradation of ciprofloxacin in water and soil and its effects on the microbial communities. Journal of Hazardous Materials, 198:22-30.

González-Pleiter, M. et al. (2013): Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: Implications for environmental risk assessment. Water Research, 47:2050-2064.

Goulas, A. et al. (2018): Explaining the accelerated degradation of ciprofloxacin, sulfamethazine, and erythromycin in different soil exposure scenarios by their aqueous extractability. Environmental Science and Pollution Research, 25:16236-16245.

Gräfe, U. (1992): Biochemie der Antibiotika: Struktur - Biosynthese - Wirkmechanismus. Spektrum Akademischer Verlag, Heidelberg.

Grenni, P., Ancona, V., Barra Caracciolo, A. (2018): Ecological effects of antibiotics on natural ecosystems: A review. Microchemical Journal, 136:25-39.

Grimme, L.H. et al. (2000): Combined effects of environmental pollutants in ecotoxicology: Biometrical models as concepts for prediction and their experimental proof (Review). Umweltwissenschaften und Schadstoff-Forschung, 12:226-234.

Grønvold, J., Svendsen, T.S., Kraglund, H.O., Bresciani, J., Monrad, J. (2004): Effect of the antiparasitic drugs fenbendazole and ivermectin on the soil nematode Pristionchus maupasi. Veterinary Parasitology, 124:91-99.

Grossberger, A., Hadar, Y., Borch, T., Chefetz, B. (2014): Biodegradability of pharmaceutical compounds in agricultural soils irrigated with treated wastewater. Environmental Pollution, 185:168-177.

Grote, M. et al. (2007): Incorporation of veterinary antibiotics into crops from manured soil. Landbauforschung Völkenrode, 57:25-32.

Gruber, V.F., Halley, B.A., Hwang, S.C., Ku, C.C. (1990): Mobility of avermectin B 1a in soil. J.Agric.Food Chem., 38:886-890.

Gullberg, E. et al. (2011): Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathogens, 7.

Guo, J., Selby, K., Boxall, A.B.A. (2016): Assessment of the risks of mixtures of major use veterinary antibiotics in european surface waters. Environmental Science & Technology, 50:8282–8289.

Gutiérrez, I.R., Watanabe, N., Harter, T., Glaser, B., Radke, M. (2010): Effect of sulfonamide antibiotics on microbial diversity and activity in a Californian Mollic Haploxeralf. Journal of Soils and Sediments, 10:537-544.

Haeili, M. et al. (2014): Copper complexation screen reveals compounds with potent antibiotic properties against methicillin-resistant *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy, 58:3727-3736.

Haham, H., Oren, A., Chefetz, B. (2012): Insight into the role of dissolved organic matter in sorption of sulfapyridine by semiarid soils. Environmental Science and Technology, 46:11870-11877.

Halling-Sørensen, B. (2000): Algal toxicity of antibacterial agents used in intensive farming. Chemosphere, 40:731-739.

Halling-Sørensen, B. (2001): Inhibition of aerobic growth and nitrification of bacteria in sewage sludge by antibacterial agents. Arch.Environ.Contam Toxicol., 40:451-460.

Halling-Sørensen, B., Lützhøft, H.C., Andersen, H.R., Ingerslev, F. (2000): Environmental risk assessment of antibiotics: comparison of mecillinam, trimethoprim and ciprofloxacin. J.Antimicrob.Chemother., 46 Suppl 1:53-58.

Halling-Sørensen, B., Nielsen, S.N., Jensen, J. (2002a): Environmental Assessment of Veterinary Medicinal Products in Denmark. Environmental Project No. 659 2002. Danish Environmental Protection Agency, Copenhagen.

Halling-Sørensen, B. et al. (1998): Occurrence, fate and effects of pharmaceutical substances in the environment - a review. Chemosphere, 36:357-393.

Halling-Sørensen, B., Sengeløv, G., Tjørnelund, J. (2002b): Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. Arch.Environ.Contam Toxicol., 42:263-271.

Hammesfahr, U. (2011): Alterations in soil microbial structure and function due to different manure and antibiotic loads. Universitäts-Druckerei, Trier.

Hammesfahr, U., Bierl, R., Thiele-Bruhn, S. (2011a): Combined effects of the antibiotic sulfadiazine and liquid manure on the soil microbial-community structure and functions. Journal of Plant Nutrition and Soil Science, 174:614-623.

Hammesfahr, U., Heuer, H., Manzke, B., Smalla, K., Thiele-Bruhn, S. (2008): Impact of the antibiotic sulfadiazine and pig manure on the microbial community structure in agricultural soils. Soil Biology & Biochemistry, 40:1583-1591.

Hammesfahr, U. et al. (2011b): Effects of sulfadiazine-contaminated fresh and stored manure on a soil microbial community. European Journal of Soil Biology, 47:61-68.

Hammesfahr, U., Bierl, R., Thiele-Bruhn, S. (2011): Combined effects of the antibiotic sulfadiazine and liquid manure on the soil microbial-community structure and functions. J Plant Nutr Soil Sci, 175.

Hamscher, G. (2007): Tierarzneimittel in Boden, Sicker- und Grundwasser: Langzeituntersuchungen an Dauerbeobachtungsflächen in Niedersachsen 2000 - 2006. in: Röder, R., Weiß, K., Sengl, M. (Eds.). Oldenbourg Industrieverlag, Munich, pp. 61-82.

Hamscher, G. (2008): Review: Tierarzneimittel in der Umwelt unter besonderer Berücksichtigung von Stallstäuben. Journal für Verbraucherschutz und Lebensmittelsicherheit, 3:165-173.

Hamscher, G., Mohring, S.A.I. (2012): Tierarzneimittel in Böden und in der aquatischen Umwelt. Chemie Ingenieur Technik, 84:1052-1061.

Hamscher, G., Pawelzick, H.T., Höper, H., Nau, H. (2005): Different behavior of tetracyclines and sulfonamides in sandy soils after repeated fertilization with liquid manure. Environmental Toxicology and Chemistry, 24:861-868.

Hamscher, G., Pawelzick, H.T., Sczesny, S., Nau, H., Hartung, J. (2003): Antibiotics in dust originating from a pig-fattening farm: A new source of health hazard for farmers? Environmental Health Perspectives, 111:1590-1594.

Hamscher, G., Sczesny, S., Abu-Qare, A., Höper, H., Nau, H. (2000): Stoffe mit pharmakologischer Wirkung einschließlich hormonell aktiver Substanzen: Nachweis von Tetracyclinen in güllegedüngten Böden. Dtsch. Tierärztl. Wochenschr., 107:332-334.

Hamscher, G., Sczesny, S., Höper, H., Nau, H. (2001): Tierarzneimittel als persistente organische Kontaminanten in Böden. in: Bodenforschung, N.L.f. (Ed.), 10 Jahre Boden-Dauerbeobachtung in Niedersachsen, Hannover.

Hamscher, G., Sczesny, S., Höper, H., Nau, H. (2002): Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Analytical Chemistry, 74:1509-1518.

Han, S.M. et al. (2016): Antibacterial activity and antibiotic-enhancing effects of honeybee venom against methicillin-resistant *Staphylococcus aureus*. Molecules (Basel, Switzerland), 21:79.

Hannappel, S., Köpp, C., Rejman-Rasinska, E. (2017): Regionaler Themenbericht: Ermittlung der Ursachen des Eintrages von Tierarzneimitteln in das oberflächennahe Grundwasser; Datenauswertung 2012 bis 2016. Niedersächsischer Landesbetrieb für Wasserwirtschaft, Küsten- und Naturschutz (NLWKN), Norden.

Hanson, M.L., Solomon, K.R. (2002): New technique for estimating thresholds of toxicity in ecological risk assessment. Environmental Science and Technology, 36:3257-3264.

Hegreness, M., Shoresh, N., Damian, D., Hartl, D., Kishony, R. (2008): Accelerated evolution of resistance in multidrug environments. Proceedings of the National Academy of Sciences of the United States of America, 105:13977-13981.

Heise, J., Höltge, S., Schrader, S., Kreuzig, R. (2006): Chemical and biological characterization of non-extractable sulfonamide residues in soil. Chemosphere, 65:2352-2357.

Hektoen, H., Berge, J.A., Hormazabal, V., Ynestad, M. (1995): Persistence of antibacterial agents in marine sediments. Aquaculture, 133:175-184.

Hembrock-Heger, A., Nießner, M., Reupert, R. (2011): Tierarzneimittel in landwirtschaftlich genutzten Böden und oberflächennahem Grundwasser in Nordrhein-Westfalen. Bodenschutz, 4:109-113.

Henschler, D. (2006): The origin of hormesis: Historical background and driving forces. Human and Experimental Toxicology, 25:347-351.

Herrick, J.B., Haynes, R., Heringa, S., Brooks, J.M., Sobota, L.T. (2014): Coselection for resistance to multiple late-generation human therapeutic antibiotics encoded on tetracycline resistance plasmids captured from uncultivated stream and soil bacteria. Journal of Applied Microbiology, 117:380-389.

Heuer, H., Schmitt, H., Smalla, K. (2011a): Antibiotic resistance gene spread due to manure application on agricultural fields. Current Opinion in Microbiology, 14:236-243.

Heuer, H., Smalla, K. (2007): Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. Environ Microbiol., 9:657-666.

Heuer, H. et al. (2011b): Accumulation of sulfonamide resistance genes in arable soils due to repeated application of manure containing sulfadiazine. Applied and Environmental Microbiology, 77:2527-2530.

Hochmuth, J.D., Janssen, C.R., de Schamphelaere, K.A.C. (2016): Temperature and food concentration have limited influence on the mixture toxicity of copper and Microcystis aeruginosa to Daphnia magna. Environmental Toxicology and Chemistry, 35:742-749.

Holm, J.V., Rugge, K., Bjerg, P.L., Christensen, T.H. (1995): Occurrence and distribution of pharmaceutical organic-compounds in the groundwater downgradient of a landfill (Grindsted, Denmark). Environmental Science & Technology, 29:1415-1420.

Holmstrup, M. et al. (2010): Interactions between effects of environmental chemicals and natural stressors: A review. Science of the Total Environment, 408:3746-3762.

Holten Lützhøft, H.C., Halling-Sørensen, B., Jørgensen, S.E. (1999): Algal toxicity of antibacterial agents applied in Danish fish farming, pp. 1-6.

Höper, H., Kues, J., Nau, H., Hamscher, G. (2002): Eintrag und Verbleib von Tierarzneimittelwirkstoffen in Böden. Bodenschutz, 4:141-148.

Höper, H., Schäfer, W., Hamscher, G. (2007): Zur Stoffverlagerung von Tierarzneimittelwirkstoffen mit dem Sickerwasser. in: Röder, R., Weiß, K., Sengl, M. (Eds.), Tierarzneimittel in der Umwelt. Oldenbourg Industrieverlag, München, pp. 83-104.

Hou, J., Pan, B., Niu, X., Chen, J., Xing, B. (2010): Sulfamethoxazole sorption by sediment fractions in comparison to pyrene and bisphenol A. Environmental Pollution, 158:2826-2832.

Hu, X.G., Luo, Y., Zhou, Q.X., Xu, L. (2008): Determination of thirteen antibiotics residues in manure by solid phase extraction and high performance liquid chromatography. Fenxi Huaxue/ Chinese Journal of Analytical Chemistry, 36:1162-1166.

Hurtado, C., Montano-Chávez, Y.N., Domínguez, C., Bayona, J.M. (2017): Degradation of emerging organic contaminants in an agricultural soil: decoupling biotic and abiotic processes. Water, Air, and Soil Pollution, 228.

Ingerslev, F., Halling-Sørensen, B. (2001): Biodegradability of metronidazole, olaquindox, and tylosin and formation of tylosin degradation products in aerobic soil-manure slurries. Ecotoxicol.Environ.Saf, 48:311-320.

Ingerslev, F., Torang, L., Loke, M.L., Halling-Sørensen, B., Nyholm, N. (2001): Primary biodegradation of veterinary antibiotics in aerobic and anaerobic surface water simulation systems. Chemosphere, 44:865-872.

Ingham, E.R., Coleman, D.C. (1984): Effects of streptomycine, cycloheximide, fungizone, captan, carbofuran, cygon, and PCNB on soil microorganisms. Microbial Ecology, 10:345-358.

Inyinbor, A.A., Bello, O.S., Fadiji, A.E., Inyinbor, H.E. (2018): Threats from antibiotics: A serious environmental concern. Journal of Environmental Chemical Engineering, 6:784-793.

Jagnow, G. (1977): Mikrobieller Abbau der Futtermittelantibiotika Zink-Bacitracin, Flavophospholipol, Spiramycin und von Tetracyclin in feucht gelagertem und in mit Boden vermischtem H• hnerkot. Landwirtsch.Forsch.Kongreáband, Teil 1:227-234.

Jahnke, A. et al. (2016): Strategies for transferring mixtures of organic contaminants from aquatic environments into bioassays. Environmental Science & Technology, 50:5424–5431.

Jensen, J., Larsen, M.M., Bak, J. (2016): National monitoring study in Denmark finds increased and critical levels of copper and zinc in arable soils fertilized with pig slurry. Environmental Pollution, 214:334-340.

Jensen, J., Scott-Fordsmand, J.J. (2012): Ecotoxicity of the veterinary pharmaceutical ivermectin tested in a soil multi-species (SMS) system. Environmental Pollution, 171:133-139.

Jin, C.X., Liu, J.J., Bao, L.L., Zhou, Q.X., Zhou, Q.X. (2010): Joint toxicity of sulfamonomethoxine and Cd on seed germination and root elongation of crops in soil. Zhongguo Huanjing Kexue/China Environmental Science, 30:839-844.

Joergensen, R.G., Wichern, F. (2018): Alive and kicking: Why dormant soil microorganisms matter. Soil Biology and Biochemistry, 116:419-430.

Jonker, M., Gerhardt, A., Backhaus, T., van Gestel, C.A.M. (2010): Test design, mixture characterization, and data evaluation. in: van Gestel, C.A.M., Jonker, M., Kammenga, J.E., Laskowski, R., Svendsen, C. (Eds.), Mixture Toxicity: Linking Approaches from Ecological and Human Toxicology. CRC Press, Boca Raton, FL, pp. 121-156.

Jonker, M.J., Sweijen, R.A.J.C., Kammenga, J.E. (2004): Toxicity of simple mixtures to the nematode *Caenorhabditis elegans* in relation to soil sorption. Environmental Toxicology and Chemistry, 23:480.

Kahle, M., Stamm, C. (2007): Sorption of the veterinary antimicrobial sulfathiazole to organic materials of different origin. Environ Sci Technol, 41:132-138.

Karci, A., Balcioğlu, I.A. (2009): Investigation of the tetracycline, sulfonamide, and fluoroquinolone antimicrobial compounds in animal manure and agricultural soils in Turkey. Science of the Total Environment, 407:4652-4664.

Kay, P., Blackwell, P.A., Boxall, A.B.A. (2004): Fate of veterinary antibiotics in a macroporous tile drained clay soil. Environmental Toxicology and Chemistry, 23:1136-1144.

Kay, P., Blackwell, P.A., Boxall, A.B.A. (2005a): A lysimeter experiment to investigate the leaching of veterinary antibiotics through a clay soil and comparison with field data. Environmental Pollution, 134:333-341.

Kay, P., Blackwell, P.A., Boxall, A.B.A. (2005b): Transport of veterinary antibiotics in overland flow following the application of slurry to arable land. Chemosphere, 59:951-959.

Keith, C.T., Borisy, A.A., Stockwell, B.R. (2005): Multicomponent therapeutics for networked systems. Nature reviews. Drug discovery, 4:71–78.

Kemper, N. (2008): Veterinary antibiotics in the aquatic and terrestrial environment. Ecological Indicators, 8:1-13.

Khalid, S. et al. (2018): A review of environmental contamination and health risk assessment of wastewater use for crop irrigation with a focus on low and high-income countries. International Journal of Environmental Research and Public Health, 15.

Khorshid, M.S.H., Thiele-Bruhn, S. (2016): Contamination status and assessment of urban and non-urban soils in the region of Sulaimani City, Kurdistan, Iraq. Environmental Earth Sciences, 75:1-15.

Knapp, C.W., Dolfing, J., Ehlert, P.A.I., Graham, D.W. (2010): Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. Environmental Science and Technology, 44:580-587.

Kočárek, M. et al. (2016): Simultaneous sorption of four ionizable pharmaceuticals in different horizons of three soil types. Environmental Pollution, 218:563-573.

Kong, W.D., Zhu, Y.G., Fu, B.J., Marschner, P., He, J.Z. (2006): The veterinary antibiotic oxytetracycline and Cu influence functional diversity of the soil microbial community. Environmental Pollution, 143:129-137.

Kortenkamp, A., Altenburger, R. (2010): Toxicity from combined exposure to chemicals. in: van Gestel, C.A.M., Jonker, M., Kammenga, J.E., Laskowski, R., Svendsen, C. (Eds.), Mixture Toxicity: Linking Approaches from Ecological and Human Toxicology. CRC Press, Boca Raton, FL, pp. 95-120.

Kortenkamp, A., Backhaus, T., Faust, M. (2009): State of the Art Report on MixtureToxicity - Executive Summary.

Kostich, M.S., Batt, A.L., Lazorchak, J.M. (2014): Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in the US and implications for risk estimation. Environmental Pollution, 184:354-359.

Kotzerke, A. et al. (2010): Influence of difloxacin-contaminated manure on microbial community structure and function in soils. Biology and Fertility of Soils.

Kotzerke, A. et al. (2008): Alterations in soil microbial activity and N-transformation processes due to sulfadiazine loads in pig-manure. Environmental Pollution, 153:315-322.

Kreuzig, R., Blümlein, K., Höltge, S. (2007): Fate of the benzimidazole antiparasitics flubendazole and fenbendazole in manure and manured soils. Clean - Soil, Air, Water, 35:488-494.

Kreuzig, R., Höltge, S. (2005): Investigations on the fate of sulfadiazine in manured soil: Laboratory experiments and test plot studies. Environmental Toxicology and Chemistry, 24:771-776.

Kreuzig, R. et al. (2005): Test-plot studies on runoff of sulfonamides from manured soils after sprinkler irrigation. Environmental Toxicology and Chemistry, 24:777-781.

Kreuzig, R., Kullmer, C., Matthies, B., Höltge, S., Dieckmann, H. (2003): Fate and behaviour of pharmaceutical residues in soils. Fresenius Environmental Bulletin, 12:550-558.

Kues, J., Höper, H., Pawelzick, H.T., Pluquet, E., Hamscher, G. (2002): Gehalte von Tierarzneimitteln in Wirtschaftsdüngern, Eintrag in Böden und Abbauverhalten. in: BMVEL, B. (Ed.), KTBL-Schrift: Landwirtschaftliche Verwertung von Klärschlamm, Gülle und anderen Düngern unter Berücksichtigung des Umwelt- und Verbraucherschutzes. KTBL, pp. 317-322.

Kümmerer, K. (2001): Pharmaceuticals in the environment - sources, fate, effects and risks. 1st ed., Springer Verlag, Berlin.

Kümmerer, K. (2003): Significance of antibiotics in the environment. Journal of Antimicrobial Chemotherapy, 52:5-7.

Kümmerer, K. (2008): Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks. 2nd ed., Springer, Heidelberg.

Kümmerer, K. (2009): Antibiotics in the aquatic environment - A review - Part I. Chemosphere, 75:417-434.

Kuppusamy, S. et al. (2018): Veterinary antibiotics (VAs) contamination as a global agro-ecological issue: A critical view. Agriculture, Ecosystems & Environment, 257:47-59.

Lal, A. (1939): Interaction of soil microorganisms with *Ophiobolus graminis Sacc.*, the fungus causing the take-all disease of wheat. Annals of Applied Biology, 26:247-261.

Lamshöft, M., Sukul, P., Zühlke, S., Spiteller, M. (2007): Metabolism of ^{14}C-labelled and non-labelled sulfadiazine after administration to pigs. Analytical and Bioanalytical Chemistry, 388:1733-1745.

Lamshöft, M., Sukul, P., Zühlke, S., Spiteller, M. (2010): Behaviour of ^{14}C-sulfadiazine and ^{14}C-difloxacin during manure storage. Science of the Total Environment, 408:1563-1568.

Langhammer, J.P. (1989): Untersuchungen zum Verbleib antimikrobiell wirksamer Arzneistoffe als Rückstände in Gülle und im landwirtschaftlichen Umfeld, pp. 138.

Langhammer, J.P., Büning-Pfaue, H. (1989): Bewertung von Arzneistoff-Rückständen aus der Gülle im Boden. Lebensmittelchem.Gerichtl.Chem., 43:103-113.

Langhammer, J.P., Führ, F., Büning-Pfaue, H. (1990): Verbleib von Sulfonamid-Rückständen aus der Gülle in Boden und Nutzpflanze. Lebensmittelchemie, 44:93.

Lapen, D.R. et al. (2008): Pharmaceutical and personal care products in tile drainage following land application of municipal biosolids. Science of the Total Environment, 399:50-65.

Laskowski, R. et al. (2010): Interactions between toxic chemicals and natural environmental factors - A meta-analysis and case studies. Science of the Total Environment, 408:3763-3774.

LAVES (2012): Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit - Tätigkeitsbericht 2011. Niedersächsisches Ministerium für Ernährung, Landwirtschaft, Verbraucherschutz und Landesentwicklung, Oldenburg.

Li, W., Shi, Y., Gao, L., Liu, J., Cai, Y. (2013): Occurrence, distribution and potential affecting factors of antibiotics in sewage sludge of wastewater treatment plants in China. Science of the Total Environment, 445-446:306-313.

Li, Y.W. et al. (2011): Investigation of sulfonamide, tetracycline, and quinolone antibiotics in vegetable farmland soil in the pearl river delta area, Southern China. Journal of Agricultural and Food Chemistry, 59:7268-7276.

Liu, A., Cao, H., Yang, Y., Ma, X., Liu, X. (2016): Combinational effects of sulfomethoxazole and copper on soil microbial community and function. Environmental Science and Pollution Research, 23:4235-4241.

Liu, B., Li, Y., Gao, S., Chen, X. (2017a): Copper exposure to soil under single and repeated application: Selection for the microbial community tolerance and effects on the dissipation of antibiotics. Journal of Hazardous Materials, 325:129-135.

Liu, B., Li, Y., Zhang, X., Wang, J., Gao, M. (2014): Combined effects of chlortetracycline and dissolved organic matter extracted from pig manure on the functional diversity of soil microbial community. Soil Biology and Biochemistry, 74:148-155.

Liu, F. et al. (2009a): Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. Environmental Pollution, 157:1636-1642.

Liu, X., Lu, S., Liu, Y., Meng, W., Zheng, B. (2017b): Adsorption of sulfamethoxazole (SMZ) and ciprofloxacin (CIP) by humic acid (HA): Characteristics and mechanism. RSC Advances, 7:50449-50458.

Liu, X.Z., Zhang, L.M., Prosser, J.I., He, J.Z. (2009b): Abundance and community structure of sulfate reducing prokaryotes in a paddy soil of southern China under different fertilization regimes. Soil Biology and Biochemistry, 41:687-694.

Liu, Y. et al. (2012): Influences of two antibiotic contaminants on the production, release and toxicity of microcystins. Ecotoxicology and Environmental Safety, 77:79-87.

Liu, Z., Han, Y., Jing, M., Chen, J. (2017c): Sorption and transport of sulfonamides in soils amended with wheat straw-derived biochar: effects of water pH, coexistence copper ion, and dissolved organic matter. Journal of Soils and Sediments, 17:771-779.

Loke, M.L., Tjørnelund, J., Halling-Sørensen, B. (2002): Determination of the distribution coefficient (log K d) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure. Chemosphere, 48:351-361.

Löscher, W., Ungemach, F.R., Kroker, R. (2010): Pharmakotherapie bei Haus- und Nutztieren. Enke, Stuttgart.

Łukaszewicz, P. et al. (2017): Impact of veterinary pharmaceuticals on the agricultural environment: A re-inspection, Reviews of Environmental Contamination and Toxicology, pp. 89-148.

Lumsden, R.D., Locke, J.C., Adkins, S.T., Walter, J.F., Rideout, C.J. (1992): Isolation and localization of the antibiotic gliotoxin produced by *Gliocladium virens* from alginate prill in soil and soilless media. Phytopathol., 82:230-235.

Ma, J. et al. (2014): Soil microbial systems respond differentially to tetracycline, sulfamonomethoxine, and ciprofloxacin entering soil under pot experimental conditions alone and in combination. Environmental Science and Pollution Research, 21:7436-7448.

Ma, T. et al. (2016): Effects of different concentrations and application frequencies of oxytetracycline on soil enzyme activities and microbial community diversity. European Journal of Soil Biology, 76:53-60.

Malik, M. et al. (2014): Lethal synergy involving bicyclomycin: An approach for reviving old antibiotics. Journal of Antimicrobial Chemotherapy, 69:3227-3235.

Malkomes, H.P. (1988): Effect of environmental chemicals on soil microorganisms and their activities with and without fertilizers and plant growth. Zentralbl. Mikrobiol., 143:511-521.

Marti, R. et al. (2013): Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. Applied and Environmental Microbiology, 79:5701-5709.

Martínez-Carballo, E., González-Barreiro, C., Scharf, S., Gans, O. (2007): Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. Environmental Pollution, 148:570-579.

McClellan, K., Halden, R.U. (2010): Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA national sewage sludge survey. Water Research, 44:658-668.

McManus, P.S., Stockwell, V.O., Sundin, G.W., Jones, A.L. (2002): Antibiotic use in plant agriculture. Annu.Rev.Phytopathol., 40:443-465.

Metzger, H.J., Waksman, S.A., Pugh, L.H. (1942): In vivo activity of streptothricin against Brucella abortus. Proceedings of the Society for Experimental Biology and Medicine, 51:251-252.

Meyer, M.T., Kolpin, D.W., Bumgarner, J.E., Varns, J.L., Daughtridge, J.V. (2000): Detection of antibiotics in surface and ground water near confined animal feeding operations and wastewater-treatment plants by using radioimmunoassay and liquid chromatography/mass spectrometry. ACS Division of Environmental Chemistry, Preprints, 40:106-107.

Michelini, L. (2012): Sulfonamide accumulation and effects on herbaceous and woody plants and microorganisms. Università degli Studi di Padova, Padua.

Michelini, L., La Rocca, N., Rascio, N., Ghisi, R. (2013): Structural and functional alterations induced by two sulfonamide antibiotics on barley plants. Plant Physiology and Biochemistry, 67:55-62.

Michelini, L. et al. (2015): Sulfadiazine uptake and effects in common hazel (*Corylus avellana* L.). Environmental Science and Pollution Research, 22:13362-13371.

Michelini, L., Reichel, R., Werner, W., Ghisi, R., Thiele-Bruhn, S. (2012): Sulfadiazine uptake and effects on *Salix fragilis* L. and *Zea mays* L. plants. Water, Air, and Soil Pollution, 223:5243-5257.

Miller, G.C., Donaldson, S.G. (1994): Factors affecting photolysis of organic compounds on soils. in: Helz, G.R., Zepp, R.G., Currier, R.W. (Eds.), Aquatic and Surface Photochemistry. Lewis Publishers, Boca Raton, FL, pp. 97-109.

Misato, T., Ko, K., Yamaguchi, I. (1977): Use of antibiotics in agriculture, Advances in Applied Microbiology, pp. 53-88.

Mitscher, L.A. (1978): The chemistry of the tetracycline antibiotics. Marcel Dekker, Basel.

Molaei, A. et al. (2017): Assessment of some cultural experimental methods to study the effects of antibiotics on microbial activities in a soil: An incubation study. PLoS ONE, 12.

Monteiro, S.C., Boxall, A.B.A. (2010): Occurrence and fate of human pharmaceuticals in the environment. Reviews of Environmental Contamination and Toxicology, 202:53-154.

Müller, A.K., Westergaard, K., Christensen, S., Sørensen, S.J. (2002): The diversity and function of soil microbial communities exposed to different disturbances. Microbial Ecology, 44:49-58.

Müller, S.R. et al. (2003): Occurence and fate of antibiotics in manure, soil and water. Mitteilungen aus Lebensmittelwissenschaften und Hygiene, 94:574-578.

Müller, T. et al. (2012): Short-term extractability of sulfadiazine after application to soils. Environmental Pollution, 172:180-185.

Munira, S., Farenhorst, A. (2017): Sorption and desorption of glyphosate, MCPA and tetracycline and their mixtures in soil as influenced by phosphate. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes, 52:887-895.

Natal-da-Luz, T., Ojeda, G., Pratas, J., Van Gestel, C.A.M., Sousa, J.P. (2011): Toxicity to Eisenia andrei and Folsomia candida of a metal mixture applied to soil directly or via an organic matrix. Ecotoxicology and Environmental Safety, 74:1715-1720.

Nesme, J., Simonet, P. (2015): The soil resistome: A critical review on antibiotic resistance origins, ecology and dissemination potential in telluric bacteria. Environmental Microbiology, 17:913-930.

Nkanga, E.J., Hagedorn, C. (1978): Detection of antibiotic producing Streptomyces inhabiting forest soils. Antimicrobial Agents and Chemotherapy, 14:51-59.

Nowara, A., Burhenne, J., Spiteller, M. (1997): Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. J.Agric.Food Chem., 45:1459-1463.

Ocampo, P.S. et al. (2014): Antagonism between bacteriostatic and bactericidal antibiotics is prevalent. Antimicrobial Agents and Chemotherapy, 58:4573-4582.

Ockleford, C. et al. (2017): EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues) Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA Journal, 15:255.

Ok, Y.S. et al. (2011): Monitoring of selected veterinary antibiotics in environmental compartments near a composting facility in Gangwon Province, Korea. Environmental Monitoring and Assessment, 174:693-701.

Oka, H., Ito, Y., Matsumoto, H. (2000): Chromatographic analysis of tetracycline antibiotics in foods. J.Chromatogr.A, 882:109-133.

Pan, M., Chu, L.M. (2017): Fate of antibiotics in soil and their uptake by edible crops. Science of the Total Environment, 599-600:500-512.

Paut Kusturica, M., Tomas, A., Sabo, A. (2017): Disposal of unused drugs: Knowledge and behavior among people around the world, Reviews of Environmental Contamination and Toxicology, pp. 71-104.

Pei, Z. et al. (2014): Effects of copper and aluminum on the adsorption of sulfathiazole and tylosin on peat and soil. Environmental Pollution, 184:579-585.

Peng, F.J., Ying, G.G., Liu, Y.S., Su, H.C., He, L.Y. (2015): Joint antibacterial activity of soil-adsorbed antibiotics trimethoprim and sulfamethazine. Science of the Total Environment, 506-507:58-65.

Pfeiffer, C., Emmerling, C., Schröder, D., Niemeyer, J. (1998): Antibiotika (Ivermectin, Monensin) und endokrine Umweltchemikalien (Nonylphenol, Ethinylöstradiol) im Boden: Mögliche Auswirkungen von synthetischen Umweltchemikalien auf mikrobielle Eigenschaften eines landwirtschaftlich genutzten Bodens. Umweltwissenschaften und Schadstoff-Forschung, 10:147-153.

Pollard, A.T., Morra, M.J. (2018): Fate of tetracycline antibiotics in dairy manure-amended soils. Environmental Reviews, 26:102-112.

Poole, K. (2017): At the nexus of antibiotics and metals: The impact of Cu and Zn on antibiotic activity and resistance. Trends in Microbiology, 25:820-832.

Posthuma, L., Eijsackers, H.J.P., Koelmans, A.A., Vijver, M.G. (2008): Ecological effects of diffuse mixed pollution are site-specific and require higher-tier risk assessment to improve site management decisions: A discussion paper. Science of the Total Environment, 406:503-517.

Puckowski, A. et al. (2017): Mixture toxicity of flubendazole and fenbendazole to Daphnia magna. International Journal of Hygiene and Environmental Health, 220:575-582.

Qiao, M., Ying, G.G., Singer, A.C., Zhu, Y.G. (2018): Review of antibiotic resistance in China and its environment. Environment International, 110:160-172.

Raaijmakers, J.M., Mazzola, M. (2012): Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. Annual Review of Phytopathology, 50:403-424.

Rabølle, M., Spliid, N.H. (2000): Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. Chemosphere, 40:715-722.

Ragas, A.M.J., Teuschler, L.K., Posthuma, L., Cowan, C.E. (2010): Human and ecological risk assessment of chemical mixtures. in: van Gestel, C.A.M., Jonker, M., Kammenga, J.E., Laskowski, R., Svendsen, C. (Eds.), Mixture Toxicity: Linking Approaches from Ecological and Human Toxicology. CRC Press, Boca Raton, FL, pp. 157-212.

Rahube, T.O. et al. (2014): Impact of fertilizing with raw or anaerobically digested sewage sludge on the abundance of antibiotic-resistant coliforms, antibiotic resistance genes, and pathogenic bacteria in soil and on vegetables at harvest. Applied and Environmental Microbiology, 80:6898-6907.

Ratsak, C., Guhl, B., Zühlke, S., Delschen, T. (2013): Veterinary antibiotic residues in manure and digestates in Northrhine-Westfalia. Environmental Sciences Europe, 25:7.

Reddersen, K., Heberer, T., Dunnbier, U. (2002): Identification and significance of phenazone drugs and their metabolites in ground- and drinking water. Chemosphere, 49:539-544.

Rehman, M.S.U. et al. (2015): Global risk of pharmaceutical contamination from highly populated developing countries. Chemosphere, 138:1045-1055.

Riaz, L. et al. (2018): Fluoroquinolones (FQs) in the environment: A review on their abundance, sorption and toxicity in soil. Chemosphere, 191:704-720.

Riviere, J.E. (2011): Comparative Pharmacokinetics: Principles, Techniques and Applications. 2 ed. Wiley-Blackwell, Hoboken, NJ.

Rodea-Palomares, I. et al. (2010): Application of the combination index (CI)-isobologram equation to study the toxicological interactions of lipid regulators in two aquatic bioluminescent organisms. Water Research, 44:427-438.

Römbke, J., Knacker, T., Stahlschmidt-Allner, P. (1996): Umweltprobleme durch Arzneimittel - Literaturstudie. UBA Texte, Berlin.

Rosendahl, I. et al. (2011): Dissipation and sequestration of the veterinary antibiotic sulfadiazine and its metabolites under field conditions. Environmental Science and Technology, 45:5216-5222.

Russell, A.D., Hugo, W.B. (1994): Antimicrobial activity and action of silver, Progress in Medicinal Chemistry, pp. 351-370.

Rutgers, M., Van't Verlaat, I.M., Wind, B., Posthuma, L., Breure, A.M. (1998): Rapid method for assessing pollution-induced community tolerance in contaminated soil. Environmental Toxicology and Chemistry, 17:2210-2213.

Sachverständigenrat für Umweltfragen (2007): Arzneimittel in der Umwelt - Stellungnahme.

Sanders, S.M. et al. (2008): Sorption of the veterinary antimicrobials sulfadimethoxine and ormetoprim in soil. Journal of Environmental Quality, 37:1510-1518.

Sarmah, A.K., Meyer, M.T., Boxall, A.B.A. (2006): A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere, 65:725-759.

Sattelberger, R. (1999): Drug residues in the environment. Berichte des Umweltbundesamtes, Umweltbundesamt GmbH, Wien, Österreich.

Schadewinkel-Scherkl, A.M., Scherkl, R. (1995): Antibiotika und Chemotherapeutika in der tierärztlichen Praxis. Gustav Fischer, Jena, Stuttgart.

Schatz, A., Bugle, E., Waksman, S.A. (1944): Streptomycin, a Substance Exhibiting Antibiotic Activity Against Gram-Positive and Gram-Negative Bacteria. Proceedings of the Society for Experimental Biology and Medicine, 55:66-69.

Schmidt, B. et al. (2008): Fate in soil of ^{14}C-sulfadiazine residues contained in the manure of young pigs treated with a veterinary antibiotic. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes, 43:8-20.

Schmitt, H., Haapakangas, H., Van Beelen, P. (2005): Effects of antibiotics on soil microorganisms: time and nutrients influence pollution-induced community tolerance. Soil Biology & Biochemistry, 37:1882-1892.

Schmitt, H., Stoob, K., Hamscher, G., Smit, E., Seinen, W. (2006): Tetracyclines and tetracycline resistance in agricultural soils: microcosm and field studies. Microb.Ecol., 51:267-276.

Schmitt, H., ter Laak, T., Duis, K. (2017): Development and dissemination of antibiotic resistance in the environment under environmentally relevant concentrations of antibiotics and its risk assessment. Umweltbundesamt, Dessau-Roßlau.

Schönfeld, J., Konradi, S., Berkner, S., Westphal-Settele, K. (2017): Antimikrobielle Resistenzen in der Umwelt – Gibt es Neues zum bekannten Phänomen? UMID – Umwelt + Mensch Informationsdienst, 2:7-19.

Schwabe, U., Paffrath, D. (2001): Arzneiverordnungs-Report 2001. Springer Verlag, Berlin, Heidelberg.

Schwabe, U., Paffrath, D., Ludwig, W.-D., Klauber, J. (2017): Arzneiverordnungs-Report 2017. Springer-Verlag, Berlin, Heidelberg.

Schwarz, J., Aust, M.O., Thiele-Bruhn, S. (2010): Metabolites from fungal laccase-catalysed transformation of sulfonamides. Chemosphere, 81:1469-1476.

Schwarz, J., Knicker, H., Schaumann, G.E., Thiele-Bruhn, S. (2015): Enzymatic transformation and bonding of sulfonamide antibiotics to model humic substances. Journal of Chemistry, 2015.

Schwarz, J., Thiele-Bruhn, S., Eckhardt, K.-U., Schulten, H.-R. (2012): Sorption of sulfonamide antibiotics to soil organic sorbents: Batch experiments with model compounds and computational chemistry. ISRN Soil Science, 2012:10.

Scott Wells, J. et al. (1982): Distribution of β-lactam and β-lactone producing bacteria in nature. The Journal of Antibiotics, 35:814-821.

Seiler, C., Berendonk, T.U. (2012): Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Frontiers in Microbiology, 3.

Sengeløv, G. et al. (2003): Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. Environment International, 28:587-595.

Shanahan, P., Borro, A., O'Gara, F., Glennon, J.D. (1992): Isolation, trace enrichment and liquid chromatographic analysis of diacetylphloroglucinol in culture and soil samples using UV and amperometric detection. J.Chromatogr.A, 606:171-177.

Siemens, J., Huschek, G., Siebe, C., Kaupenjohann, M. (2008): Concentrations and mobility of human pharmaceuticals in the world's largest wastewater irrigation system, Mexico City-Mezquital Valley. Water Research, 42:2124-2134.

Siemens, J. et al. (2010): Transport of pharmaceuticals in columns of a wastewater-irrigated Mexican clay soil. Journal of Environmental Quality, 39:1201-1210.

Singh, N., Yeh, P.J. (2017): Suppressive drug combinations and their potential to combat antibiotic resistance. Journal of Antibiotics, 70:1033-1042.

Smith, D.L., Dushoff, J., Morris Jr, J.G. (2005): Agricultural antibiotics and human health: Does antibiotic use in agriculture have a greater impact than hospital use? PLoS Medicine, 2:0731-0735.

Smith, P., Samuelsen, O.B. (1996): Estimates of the significance of out-washing of oxytetracycline from sediments under Atlantic salmon sea-cages. Aquaculture, 144:17-26.

Soulides, D.A. (1965): Antibiotics in soils. VII. Production of streptomycin and tetracyclines in soils. Soil Science, 100:200-206.

Stamm, C., Burkhardt, M., Vollmer, T., Müller, S. (2003): Gülle-Einfluss auf Abschwemmungsprozesse von Grünland. Posterbeitrag bei der Jahrestagung der Deutschen Bodenkundlichen Gesellschaft, 30.08.-07.09.2003, Frankfurt (Oder).

Steinfeld, H. (2004): The livestock revolution—a global veterinary mission. Veterinary Parasitology, 125:19-41.

Stoob, K., Singer, H.P., Mueller, S.R., Schwarzenbach, R.P., Stamm, C.H. (2007): Dissipation and transport of veterinary sulfonamide antibiotics after manure application to grassland in a small catchment. Environmental Science and Technology, 41:7349-7355.

Stoob, K. et al. (2006): Exhaustive extraction of sulfonamide antibiotics from aged agricultural soils using pressurized liquid extraction. J.Chromatogr.A, 1128:1-9.

Straub, J.O. (2002): Environmental risk assessment for new human pharmaceuticals in the European Union according to the draft guideline/discussion paper of January 2001. Toxicol.Lett., 131:137-143.

Strauch, D. (1974): Hygienic problems of large scale animal breeding. ZBL.BAKT.REIHE B, 159:309-323.

Stuer-Lauridsen, F., Birkved, M., Hansen, L.P., Lutzhoft, H.C., Halling-Sorensen, B. (2000): Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use. Chemosphere, 40:783-793.

Sun, J., Zeng, Q., Tsang, D.C.W., Zhu, L.Z., Li, X.D. (2017): Antibiotics in the agricultural soils from the Yangtze River Delta, China. Chemosphere, 189:301-308.

Takahashi, K., Takita, T., Umezawa, H. (1987): Activation of bleomycin-Fe(III) by bleomycin-Cu(II) and cysteine. The Journal of Antibiotics, 40:542-546.

Tamtam, F. et al. (2011): Assessing the fate of antibiotic contaminants in metal contaminated soils four years after cessation of long-term waste water irrigation. Science of the Total Environment, 409:540-547.

Tappe, W. et al. (2008): Growth-inhibitory effects of sulfonamides at different pH: Dissimilar susceptibility patterns of a soil bacterium and a test bacterium used for antibiotic assays. Chemosphere, 72:836-843.

ter Laak, T.T., Gebbink, W., Tolls, J. (2006a): Estimation of soil sorption coefficients of veterinary pharmaceuticals from soil properties. Environmental Toxicology and Chemistry, 25:933-941.

ter Laak, T.T., Gebbink, W.A., Tolls, J. (2006b): The effect of pH and ionic strength on the sorption of sulfachloropyridazine, tylosin, and oxytetracycline to soil. Environmental Toxicology and Chemistry, 25:904-911.

Ternes, T. (2000): Abbau und Verhalten von Pharmaka in aquatischen Systemen. Schriftenreihe Wasserforschung, 6:23-33.

Tessi, M.A. (1974): Factors of resistance to antibiotics and sulfa drugs of Enterobacteriaceae isolated from waters and sediments of the middle Parana River (Santa Fe Parana). Revista de la Associacion Argentina de Microbiologia, 6:62-74.

Thiele-Bruhn, S. (2003a): Antibiotika. in: Blume, H.P. et al. (Eds.), Handbuch der Bodenkunde. ecomed Verlagsgesellschaft, Landsberg/Lech, pp. 1-19.

Thiele-Bruhn, S. (2003b): Pharmaceutical antibiotic compounds in soils - a review. Journal of Plant Nutrition and Soil Science, 166:145-167.

Thiele-Bruhn, S. (2004): Antibiotische Pharmazeutika. in: Blume, H.P. (Ed.), Handbuch des Bodenschutzes. Ecomed Verlagsgesellschaft, Landsberg/Lech, pp. 483-492.

Thiele-Bruhn, S. (2005): Microbial inhibition by pharmaceutical antibiotics in different soils - Dose-response relations determined with the iron(III) reduction test. Environmental Toxicology and Chemistry, 24:1835-1835.

Thiele-Bruhn, S. (2015): Das Umweltverhalten und Effekte pharmazeutischer Antibiotika in Böden unterscheiden sich zwischen Einzelsubstanzen und Gemischen. Oral presentation. Jahrestagung der Deutschen Bodenkundlichen Gesellschaft, Munich, 7-9 September 2015

Thiele-Bruhn, S., Aust, M.-O. (2014): Sulfonamide. in: Wilcke, W., Wilke, B.-M., Litz, N. (Eds.), Bodengefährdende Stoffe: Bewertung - Stoffdaten - Ökotoxikologie - Sanierung. Wiley.

Thiele-Bruhn, S., Aust, M.O. (2004): Effects of pig slurry on the sorption of sulfonamide antibiotics in soil. Archives of Environmental Contamination and Toxicology, 47:31-39.

Thiele-Bruhn, S., Beck, I.C. (2005): Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. Chemosphere, 59:457-465.

Thiele-Bruhn, S., Brümmer, G.W. (2004): Fractionated extraction of polycyclic aromatic hydrocarbons (PAHs) from polluted soils: estimation of the PAH fraction degradable through bioremediation. European Journal of Soil Science, 55:567-578.

Thiele-Bruhn, S., Macovei, L., Puff, H., Riepert, F. (2006): Effects of the antiparasitic fenbendazole on soil microorganisms and faunal species. . SETAC Europe 16th Annual Meeting, 7. - 11.05.2005, The Hague, The Netherlands.

Thiele-Bruhn, S., Mogk, A., Freitag, D. (2003): Einsatz von Tierarzneimitteln zur Anwendung bei landwirtschaftlichen Nutztieren in Mecklenburg-Vorpommern. Berichte über Landwirtschaft, 81:374-391.

Thiele-Bruhn, S., Peters, D. (2007): Photodegradation of pharmaceutical antibiotics on slurry and soil surfaces. Landbauforschung Völkenrode, 57:13-23.

Thiele-Bruhn, S. et al. (2018): Identification of new standards for the analysis of microbial functions in soil to assess soil quality. in preparation.

Thiele-Bruhn, S., Seibicke, T., Schulten, H.R., Leinweber, P. (2004): Sorption of sulfonamide pharmaceutical antibiotics on whole soils and particle-size fractions. Journal of Environmental Quality, 33:1331-1342.

Thiele, S. (2000): Adsorption of the antibiotic pharmaceutical compound sulfapyridine by a long-term differently fertilized loess Chernozem. J.Plant Nutr.Soil Sci., 163:589-594.

Thiele, S., Leinweber, P. (2000): Bedeutung der Huminstoffe für Bindung und Umsatz organischer Fremdstoffe - am Beispiel ausgewählter Veterinärantibiotika. Rostocker Agrar- und Umweltwissenschaftliche Beitr„ge, 8:265-273.

Thomashow, L.S., Bonsall, R.F., Weller, D.M. (1997): Antibiotic production by soil and rhizosphere microbes in situ. in: Hurst, C.J., Knudson, G.R., McInerney, M.J., Stetzenbach, L.D., Walter, M.V. (Eds.), Manual of environmental microbiology. ASM Press, Washington, D.C., pp. 493-499.

Tolls, J. (2001): Sorption of veterinary pharmaceuticals in soils: a review. Environ.Sci.Technol., 35:3397-3406.

Tolls, J., Gebbink, W., Cavallo, R. (2002): pH-dependence of sulfonamide antibiotic sorption: data and model evaluation. SETAC Europe 12th Annual Meeting, 12-16 May, 2002:Vienna, Austria.

Topp, E. et al. (2008): Runoff of pharmaceuticals and personal care products following application of biosolids to an agricultural field. Science of the Total Environment, 396:52-59.

Topp, W. (1981): Biologie der Bodenorganismen. Quelle & Meier - UTB, Heidelberg.

Toth, J.D., Feng, Y., Dou, Z. (2011): Veterinary antibiotics at environmentally relevant concentrations inhibit soil iron reduction and nitrification. Soil Biology and Biochemistry, 43:2470-2472.

Ueda, Y. et al. (1983): Interactions of beta-lactam antibiotics and antineoplastic agents. Antimicrobial agents and chemotherapy, 23:374–378.

Ungemach, F.R. (2000): Figures on quantities of antibacterials used for different purposes in the EU countries and interpretation. Acta Vet.Scand.Suppl, 93:89-97.

Van Beelen, P., Fleuren-Kemila, A.K., Aldenberg, T. (2001): The relation between extrapolated risk, expressed as potentially affected fraction, and community effects, expressed as pollution-induced community tolerance. Environmental Toxicology and Chemistry, 20:1133-1140.

Van Boeckel, T.P. et al. (2014): Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. The Lancet Infectious Diseases, 14:742-750.

Van Dijck, P., van de Voorde, H. (1976): Sensitivity of environmental microorganisms to antimicrobial agents. Applied and Environmental Microbiology, 31:332-336.

van Gool, S. (1993): Possible effects on the environment of antibiotic residues in animal manure. Tijdschr.Diergeneeskd., 118:8-10.

Vasquez, M.I., Lambrianides, A., Schneider, M., Kümmerer, K., Fatta-Kassinos, D. (2014): Environmental side effects of pharmaceutical cocktails: What we know and what we should know. Journal of Hazardous Materials, 279:169-189.

Vasudevan, D., Bruland, G.L., Torrance, B.S., Upchurch, V.G., MacKay, A.A. (2009): pH-dependent ciprofloxacin sorption to soils: Interaction mechanisms and soil factors influencing sorption. Geoderma, 151:68-76.

Velagaleti, R., Burns, P.K., Gill, M., Prothro, J. (2002): Impact of current good manufacturing practices and emission regulations and guidances on the discharge of pharmaceutical chemicals into the environment from manufacturing, use, and disposal. Environmental Health Perspectives, 110:213-220.

Verlicchi, P., Al Aukidy, M., Zambello, E. (2012): Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment-A review. Science of the Total Environment, 429:123-155.

Verlicchi, P., Zambello, E. (2015): Pharmaceuticals and personal care products in untreated and treated sewage sludge: Occurrence and environmental risk in the case of application on soil - A critical review. Science of the Total Environment, 538:750-767.

Versorgungsatlas (2016): Versorgungsatlas.de - Die medizinische Versorgung regional – Daten Fakten Analysen. Newsletter, 2.

von der Ohe, P.C. et al. (2011): A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive. Science of the Total Environment, 409:2064-2077.

von Känel, C. (2002): Extraktion von Pharmazeutika aus Urin und deren Toxizitätsmessung mithilfe von zwei biologischen Testsystemen. Departement für Umweltnaturwissenschaften der Eidgenössischen Technischen Hochschule Zürich (D-UMNW-ETHZ), Zurich, Switzerland.

Wakelin, S. et al. (2014): Mechanisms of pollution induced community tolerance in a soil microbial community exposed to Cu. Environmental Pollution, 190:1-9.

Wang, N. et al. (2016): A comprehensive analysis on spread and distribution characteristic of antibiotic resistance genes in livestock farms of Southeastern China. PLoS ONE, 11.

Wang, Q., Guo, M., Yates, S.R. (2006): Degradation kinetics of manure-derived sulfadimethoxine in amended soil. J.Agric.Food Chem., 54:157-163.

Wang, S., Wang, H. (2015): Adsorption behavior of antibiotic in soil environment: a critical review. Frontiers of Environmental Science and Engineering, 9:565-574.

Wang, Y.J., Sun, R.J., Xiao, A.Y., Wang, S.Q., Zhou, D.M. (2010): Phosphate affects the adsorption of tetracycline on two soils with different characteristics. Geoderma, 156:237-242.

Watanabe, N., Bergamaschi, B.A., Loftin, K.A., Meyer, M.T., Harter, T. (2010): Use and environmental occurrence of antibiotics in freestall dairy farms with manured forage fields. Environmental Science and Technology, 44:6591-6600.

Wegst-Uhrich, S.R., Navarro, D.A.G., Zimmerman, L., Aga, D.S. (2014): Assessing antibiotic sorption in soil: A literature review and new case studies on sulfonamides and macrolides. Chemistry Central Journal, 8.

Weiß, K., Schüssler, W., Porzelt, M. (2007): Auswaschung von Sulfadimidin, Enrofloxazin und Flubendazol in das Sickerwasser. in: Röder, R., Weiß, K., Sengl, M. (Eds.), Münchener Beiträge zur Abwasser-, Fischerei- und Flussbiologie. Bayerisches Landesamt für Umwelt, Oldenbourg Verlag, Munich, pp. 37-60.

Westphal-Settele, K., Konradi, S., Balzer, F., Schönfeld, J., Schmithausen, R. (2018): Die Umwelt als Reservoir für Antibiotikaresistenzen – ein wachsendes Problem für die öffentliche Gesundheit? Bundesgesundheitsblatt, 61:533-542.

Wetzstein, H., Schneider, J., Karl, W. (2006): Patterns of metabolites produced from the fluoroquinolone enrofloxacin by basidiomycetes indigenous to agricultural sites. Applied Microbiology and Biotechnology, 71:90-100.

Wetzstein, H.G. (2001): Chinolone in der Umwelt: Biologische Abbaubarkeit der Gyrasehemmer. Pharmazie in unserer Zeit, 30:450-457.

Wetzstein, H.G., Schmeer, N., Karl, W. (1998): Degradation of the fluoroquinolone enrofloxacin by the brown rot fungus *Gloeophyllum striatum*: Identification of metabolites. Applied and Environmental Microbiology, 64:1166.

Williams-Nguyen, J. et al. (2016): Antibiotics and antibiotic resistance in agroecosystems: State of the science. Journal of Environmental Quality, 45:394-406.

Winckler, C. et al. (2004): Verhalten von Tetrazyklinen und anderen Veterinärantibiotika in Wirtschaftsdünger und Boden. Umweltbundesamt, Berlin.

Wolfson, J.S., Hooper, D.C. (1985): The fluoroquinolones: Structures, mechanisms of action and resistance, and spectra of activity in vitro. Antimicrobial Agents and Chemotherapy, 28:581-586.

Wolters, A., Steffens, M. (2005): Photodegradation of antibiotics on soil surfaces: Laboratory studies on sulfadiazine in an ozone-controlled environment. Environmental Science and Technology, 39:6071-6078.

Wood, K., Nishida, S., Sontag, E.D., Cluzel, P. (2012): Mechanism-independent method for predicting response to multidrug combinations in bacteria. Proceedings of the National Academy of Sciences of the United States of America, 109:12254-12259.

Wright, G.D. (2010): Q&A: Antibiotic resistance: Where does it come from and what can we do about it? BMC Biology, 8.

Wu, D. et al. (2014): Co-sorption of ofloxacin and Cu(II) in soils before and after organic matter removal. Science of the Total Environment, 481:209-216.

Wu, X., Wei, Y., Zheng, J., Zhao, X., Zhong, W. (2011): The behavior of tetracyclines and their degradation products during swine manure composting. Bioresource Technology, 102:5924-5931.

Xie, W.-Y., Shen, Q., Zhao, F.J. (2018): Antibiotics and antibiotic resistance from animal manures to soil: a review. European Journal of Soil Science, 69:181-195.

Xing, Y., Chen, X., Zhuang, J., Chen, X. (2015): What happens when pharmaceuticals meet colloids. Ecotoxicology, 24:2100-2114.

Xu, Y., Xu, J., Mao, D., Luo, Y. (2017a): Effect of the selective pressure of sub-lethal level of heavy metals on the fate and distribution of ARGs in the catchment scale. Environmental Pollution, 220:900-908.

Xu, Y. et al. (2016): The combined effect of sulfadiazine and copper on soil microbial activity and community structure. Ecotoxicology and Environmental Safety, 134:43-52.

Xu, Y., Yu, W., Ma, Q., Zhou, H. (2015): Interactive effects of sulfadiazine and Cu(II) on their sorption and desorption on two soils with different characteristics. Chemosphere, 138:701-707.

Xu, Y., Yu, W., Ma, Q., Zhou, H., Jiang, C. (2017b): Toxicity of sulfadiazine and copper and their interaction to wheat (*Triticum aestivum* L.) seedlings. Ecotoxicology and Environmental Safety, 142:250-256.

Yan, H. et al. (2011): Dissipation of carbendazim and chloramphenicol alone and in combination and their effects on soil fungal:bacterial ratios and soil enzyme activities. Chemosphere, 84:634-641.

Yang, G. et al. (2017): Mixture toxicity of four commonly used pesticides at different effect levels to the epigeic earthworm, *Eisenia fetida*. Ecotoxicology and Environmental Safety, 142:29-39.

Yang, J.F. et al. (2012): Biological degradation and microbial function effect of norfloxacin in a soil under different conditions. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes, 47:288-295.

Yang, J.F. et al. (2009): Degradation behavior of sulfadiazine in soils under different conditions. Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 44:241-248.

Yang, L.H. et al. (2008): Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*. Environmental Toxicology and Chemistry, 27:1201-1208.

Yeager, R.L., Halley, B.A. (1990): Sorption/desorption of [^{14}C]efrotomycin with soils. J. Agric. Food Chem., 38:883-886.

Zarfl, C., Matthies, M., Klasmeier, J. (2007): A mechanistical model for the uptake of sulfonamides by bacteria. Chemosphere, 70:753-760.

Zhang, Y. et al. (2017): Degradation kinetics and mechanism of sulfadiazine and sulfamethoxazole in an agricultural soil system with manure application. Science of the Total Environment, 607-608:1348-1356.

Zhang, Y.L., Lin, S.S., Dai, C.M., Shi, L., Zhou, X.F. (2014): Sorption-desorption and transport of trimethoprim and sulfonamide antibiotics in agricultural soil: effect of soil type, dissolved organic matter, and pH. Environmental Science and Pollution Research:1-9.

Zhou, D. et al. (2016): Impact of manure-related DOM on sulfonamide transport in arable soils. Journal of Contaminant Hydrology, 192:118-128.

Zhou, L.J. et al. (2012): Simultaneous determination of human and veterinary antibiotics in various environmental matrices by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry. Journal of Chromatography A, 1244:123-138.

Zhu, Y.G. et al. (2013): Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proceedings of the National Academy of Sciences of the United States of America, 110:3435-3440.

Zielezny, Y., Groeneweg, J., Vereecken, H., Tappe, W. (2006): Impact of sulfadiazine and chlorotetracycline on soil bacterial community structure and respiratory activity. Soil Biology and Biochemistry, 38:2372-2380.

Zou, X., Lin, Z., Deng, Z., Yin, D. (2013): Novel approach to predicting hormetic effects of antibiotic mixtures on *Vibrio fischeri*. Chemosphere, 90:2070-2076.

Appendix

Table 19: Adsorption coefficients of pharmaceutical antibiotics in soils and sediments

Antibiotic class / Antibiotic	Concentration µg/g	Soil sample Texture / pH_{CaCl_2} / OC %	K_d L/kg	K_{oc} L/kg	Reference
Sulfonamides					
Sulfamethazine	0.05 - 20	sandy loam / 6.8 /	0.9		(Tolls et al., 2002)
	0.2 - 25	sand / 5.2 / 0.9	1.3 [a]	139	(Langhammer and Büning-Pfaue, 1989)
	0.2 - 25	loamy sand / 5.6 / 2.3	3.5 [a]	151	
	0.2 - 25	sandy loam / 6.3 / 1.2	2.0 [a]	170	
	0.2 - 25	clay-silt / 6.9 / 1.1	0.9 [a]	80	
	0.2 - 25	sand / 5.2 / 0.9	1.2	174	(Langhammer, 1989)
	0.2 - 25	loamy sand / 5.6 / 2.3	3.1	125	
	0.2 - 25	sandy loam / 6.3 / 1.2	2.0	208	
	0.2 - 25	clay-silt / 6.9 / 1.1	1.0	82	
		clay-loam / 6.2/ 3.1	3 [a]	97	(Tolls et al., 2002)
	1.0 - 10	silt-loam / 7.0 / 1.6	2.4	149	(Thiele-Bruhn et al., 2004)
Sulfapyridine	0.1 - 500	silt-loam / 6.9 / 2.4	7.4	308	
	1.0 - 10	silt-loam / 7.0 / 1.6	3.5	217	
Sulfapyridine	0.1 - 500	silt-loam / 7.0 / 1.6	1.6	101	Thiele 2000
– " –	5	clay / 7.7 / 0.145	4.47		(Haham et al., 2012)
– " –	5	– " – ; DOM covered	2.03		
– " –	5	sandy loam / 8.0 / 0.078	5.62		
– " –	5	– " – ; DOM covered	2.77		
– " –	5	sandy loam / 7.2 / 0.081	0.44		
– " –	5	– " – ; DOM covered	1.18		
Sulfadiazine	1.0 - 10	silt-loam / 7.0 / 1.6	2.0	124	
		clay-loam / 6.2/ 3.1	2.5 [a]	81	(Tolls et al., 2002)
Sulfanilamide	1.0 - 10	silt-loam / 7.0 / 1.6	1.7	106	(Thiele-Bruhn et al., 2004)
Sulfadimethoxine	1.0 - 10	silt-loam / 7.0 / 1.6	2.3	143	
		clay-loam / 6.2/ 3.1	10 [a]	323	(Tolls et al., 2002)
	14.1-1800	[b] silty clay / 5.6 / 24.5	108		(Białk-Bielińska et al., 2012)
	2.8-360	[b] sandy loam / 6.9 / 19.4	4.83		
	1.1-144	[b] sand / 7.4 / 0.14	0.31		
	0.1-10	loamy sand / 5.0 / 1.5	10.4		(Sanders et al., 2008)
	– " –	– " –; cosorbate or-methoprim	12.5		
	– " –	loam / 4.7 / 2.1	25.8		
	– " –	– " –; cosorbate or-methoprim	22.1		
	– " –	sand / 7.0 / 0	0.4		

Antibiotic class / Antibiotic	**Concentration** µg/g	**Soil sample** Texture / pH_{CaCl_2} / OC %	K_d L/kg	K_{oc} L/kg	**Reference**
Sulfadimethoxine	– " –	sand / 7.0 / 0; cosorbate ormethoprim	2.5		
Sulfachloro-		clay-loam / 6.2/ 3.1	4 [a]	129	(Tolls et al., 2002)
pyridazine	0.05 - 20	clay loam / 6.5 /	1.8		(Boxall et al., 2002)
Sulfaisoxazole		clay-loam / 6.2/ 3.1	1.5 [a]	48	(Tolls et al., 2002)
Sulfathiazole		clay-loam / 6.2/ 3.1	3 [a]	97	
Sulfaguanidine	14.1-1800	[b] silty clay / 5.6 / 24.5	31.0		(Białk-Bielińska et al., 2012)
	2.8-360	[b] sandy loam / 6.9 / 19.4	2.26		
	1.1-144	[b] sand / 7.4 / 0.14	1.03		
Tetracyclines					
Oxytetracycline	2.5 - 50	loamy sand / 6.1 / 1.6	680	425,000	(Rabølle and Spliid, 2000)
	2.5 - 50	sand / 5.6 / 1.4	670	47,900	
	2.5 - 50	sandy loam / 5.6 / 1.1	1026	93,300	
	2.5 - 50	sand / 6.3 / 1.5	417	27,800	
	285	organic marine sediment	0.7		(Smith and Samuelsen, 1996)
	10.9	organic marine sediment	2.6		
Macrolides					
Tylosin	50 mg/g	kaolinite	0.7		(Bewick, 1979)
	50 mg/g	illite	3.9		
	500 mg/g	montmorillonite	0.7		
	500 mg/g	bentonite	3.1		
	1.25 - 25	loamy sand / 6.1 / 1.6	128	7990	(Rabølle and Spliid, 2000)
	1.25 - 25	sand / 5.6 / 1.4	10.8	771	
	1.25 - 25	sandy loam / 5.6 / 1.1	62.3	5660	
	1.25 - 25	sand / 6.3 / 1.5	8.3	553	
Fluoroquinolones					
Ciprofloxacin	2 - 200	loamy sand / 5.3/ 0.70	427	61,000	(Nowara et al., 1997)
Enrofloxacin	2 - 200	clay / 4.9/ 1.63	3037	186,300	
	2 - 200	loam / 5.3/ 0.73	5612	768,700	
	2 - 200	loamy sand / 6.0/ 1.23	1230	100,000	
	2 - 200	loam / 7.5/ 1.58	260	16,500	
	2 - 200	loamy sand / 5.3/ 0.70	496	70,900	
Antibiotic class / Antibiotic	**Concentration** µg/g	**Soil sample** Texture / pH_{CaCl_2} / OC %	K_d L/kg	K_{oc} L/kg	**Reference**
decarboxylated Enrofloxacin	2 - 200	loamy sand / 5.3/ 0.70	7.7	1100	
Ofloxacin	2 - 200	loamy sand / 5.3/ 0.70	309	44,100	
Imidazoles					
Metronidazole	1.25 - 25	loamy sand / 6.1/ 1.6	0.67	42	(Rabølle and Spliid, 2000)
	1.25 - 25	sand / 5.6/ 1.4	0.54	39	

	1.25 - 25	sandy loam / 5.6/ 1.1	0.62	56	
	1.25 - 25	sand / 6.3/ 1.5	0.57	38	
Fenbendazole	0.5 - 100	silt loam / 7.0/ 1.6	0.91	57.2	(Thiele and Leinweber, 2000)
	0.5 - 100	silt loam / 6.9/ 2.4	0.84	35.1	
Polypeptides					
Avermectin	0.006 - 2.17	clay loam / 6.6/ 4.8	147	5300	(Gruber et al., 1990)
	0.006 - 2.17	sand / 7.5/ 0.1	17.4	30,000	
	0.006 - 2.17	silt loam /7.5/ 2.1	80.2	6600	
Quinoxaline derivatives					
Olaquindox	1.25 - 25	loamy sand / 6.1/ 1.6	1.67	104	(Rabølle and Spliid, 2000)
	1.25 - 25	sand / 5.6/ 1.4	1.21	86	
	1.25 - 25	sandy loam / 5.6/ 1.1	1.27	116	
	1.25 - 25	sand / 6.3/ 1.5	0.69	46	
Lipoglycosides					
Efrotomycin		silt loam / 7.5/ 2.1	18	1460	(Yeager and Halley, 1990)
		loam / 6.7/ 2.5	8.3	580	
	1.0 - 135	sandy loam / 7.5/ 1.1	51	8000	
	1.0 - 135	clay loam / 5.0/ 4.6	290	11,000	

[a] Data derived from figures.

[b] Soil texture derived from general information.

; adopted from Thiele-Bruhn (2003b) and Thiele-Bruhn and Aust (2014).

Table 20: Adsorption coefficients of pharmaceutical antibiotics in pig manure and sewage sludge.

Antibiotic Class	Antibiotic Compound	Concentration	Sample	K_d L/kg	K_{oc} L/kg	Reference
Tetracyclines	oxytetracycline		sewage sludge / 6.5 / 37[a]	3020	8160	Holten-Lützhøft and Halling-Sørensen [c]
		33-2000 mg/g	pig manure 6 h / 24 h [b]	83.2 / 77.6	195	(Loke et al., 2002)
Macrolides		100-2000 mg/g	pig manure 6 h / 24 h [b]	45.7 / 240	110	
Fluoroquinolones	ciprofloxacin	250 µg/L	sewage sludge/ 6.5 / 37 [a]	417	1127	(Halling-Sørensen et al., 2000)
Quinoxaline deriv.	olaquindox	100-2000 mg/g	pig manure 6 h / 24 h [b]	20.4 / 9.77	50	(Loke et al., 2002)
Diaminopyrimidines	trimethoprim	500 µg/L	sewage sludge/ 6.5 / 37 [a]	76	205	(Halling-Sørensen et al., 2002a)

[a] Substrate / pH_{CaCl_2} / OC (%).

[b] Degradation after repeated addition of the antibiotic.

[b] Cited in Stuer-Lauridsen et al. (2000).

Table 21: Dissipation of antibiotics in different types of manure.

Dissipation was measured for 24 days and dissipation half lives DT50 and the time for dissipation of 90% of parent compound (DT90 20°C) were calculated using kinetic models. Antibiotics highlighted in blue were detected in organic waste materials (see Table 14, pg. 60).

	Calve manure semi-solid		Pig manure		Broiler manure	
Compound class / compound	**DT_{50} in d**	**DT_{90} in d**	**DT_{50} in d**	**DT_{90} in d**	**DT_{50} in d**	**DT_{90} in d**
Tetracyclines						
Oxytetracycline	98	327	16	171	30	221
Chlortetracycline	35	118	19	62	18	61
Doxycycline	44	147	10	98	20	268
Tetracycline	52	171	12	111	62	330
Sulfonamides						
Dapsone	1.2	15	1.2	11	2.3	20
Sulfacetamide	11	36	1.5	8	4.9	100
Sulfachloropyridazine	2.4	24	1.6	15	2.9	38
Sulfadiazine	4.4	33	2.2	18	4.4	83
Sulfadimethoxine	4.6	35	3.2	21	3.4	37
Sulfadimidine	2.5	26	1.8	16	3.7	48
Sulfadoxine	7	41	3	25	5	89
Sulfamerazine	3.4	29	1.8	17	3.7	45
Sulfamethizole	2.2	21	1	9	2.4	23
Sulfamethoxazole	3.2	21	2.6	22	2.5	53
Sulfamethoxypyridazine	1.7	21	1.6	14	2.5	29
Sulfamonomethoxine	3.4	28	2.1	19	3.3	44
Sulfamoxole	0.4	1.3	0.7	3.5	0.4	3
Sulfaphenazole	1.8	19	1.6	13	0.7	3.2
Sulfapyridine	1.6	20	1.4	13	3.2	41
Sulfaquinoxaline	1.6	24	3.8	13	2.2	30
Sulfathiazole	1.1	14	1.2	11	2	18
Sulfisoxazole	2	10	1.3	10	0.7	10
Macrolides						
Tylosin	no data	no data	42	179	no data	no data
Aivlosin	2.8	54	35	159	0.4	37
Erythromycin	32	106	52	172	17	56
Gamithromycin	61	203	50	239	53	177
Josamycin	27	89	231	769	43	141
Natamycin	2.6	22	5	17	0.7	18
Spiramycin	31	104	20	113	31	102
Tildipyrosin	71	236	5	78	16	106
Tilmicosin	104	346	47	220	71	235
Tulathromycin	92	304	6	89	317	1053

	Calve manure semi-solid		Pig manure		Broiler manure	
Compound class / compound	**DT_{50} in d**	**DT_{90} in d**	**DT_{50} in d**	**DT_{90} in d**	**DT_{50} in d**	**DT_{90} in d**
Lincosamides						
Lincomycin	175	581	269	892	>2000[a]	>2000[a]
Pirlimycin	142	473	125	414	443	1473
Pleuromutilins						
Tiamulin	338	1124	101	335	280	930
Valnemulin	13	96	42	179	7	70
(Fluoro)quinolones						
Enrofloxacin	162	540	6	83	103	343
Ciprofloxacin	61	277	6	85	23	221
Danofloxacin	106	354	7	78	58	192
Difloxacin	200	665	11	99	41	194
Flumequin	259	860	44	146	197	655
Marbofloxacin	134	447	4.6	91	90	300
Nalidixic acid	614	2040	70	295	388	1290
Norfloxacin	60	254	5	79	18	179
Oxolinic acid	268	889	36	181	116	387
Sarafloxacin	398	1322	562	1867	176	585

[a] >2000: largely above the maximum extrapolated time of 2000 d.

Source: Data adopted from Berendsen et al. (2018).

Table 22: Degradation of pharmaceutical antibiotics in soil.

Compound class / compound	Concen-tration µg/g [a]	Sample soil: texture / pH / OC%	Degra-dation %	Degrad. time d	Reference
Aminoglycosides					
Streptomycin	5.6	sandy loam+manure / 6.1	0	30	(Gavalchin and Katz, 1994)
ß-Lactams					
Penicillin	5.6	sandy loam+manure / 6.1	0	30	
Ceftiotur		clay loam	50 [b]	22.2	(Gilbertson et al., 1990)
		sand	50 [b]	49.0	
		silty clay loam	50 [b]	41.4	
Macrolides					
Erythromecin	5.6	sandy loam+manure / 6.1	25	30	(Gavalchin and Katz, 1994)
Tylosin	5.6	sandy loam+manure / 6.1	0	30	
	100	sand+slurry / 6.3 / 1.4	50	4.2	(Ingerslev and Halling-Sørensen, 2001)
	100	sandy loam+slurry /6.8/1.6	50	5.7	

Compound class / compound	Concen-tration µg/g [a]	Sample soil: texture / pH / OC%	Degra-dation %	Degrad. time d	Reference
Sulfonamides					
Sulfanilamide		diverse soils	0	14	(Frankenberger Jr and Tabatabai, 1982)
	500	loamy sand / 6,6 / 0,8	0	28	(Thiele-Bruhn and Peters, 2007)
Sulfadiazine	10	three soils, aerobic	50	12-18	(Yang et al., 2009)
	10	three soils, anaerobic	50	57-237	
	500	loamy sand / 6,6 / 0,8	0	28	(Thiele-Bruhn and Peters, 2007)
Sulfamethazine	1.0	loamy sand / 5.6 / 2.3	0.2/0.3 [b,c]	64	(Langhammer et al., 1990)
	1.0	clay-silt / 6.9 / 1.1	0.3/0.7 [b,c]	64	
	250	loamy sand / 6.6 / 0.8	0	28	(Thiele-Bruhn and Peters, 2007)
Sulfadimethoxine	500	loamy sand / 6.6 / 0.8	0	28	
Sulfapyridine	250	loamy sand / 6.6 / 0.8	0	28	
	250	silt-loam / 7.0 / 1.6	0	28	
	250	sand / 6.0 / 0.05	50	139	
Sulfachloro-	1.6	sandy loam / 6.6 / 1.3	50	2.8	(Blackwell et al., 2005)
pyridazine	1.6	clay-loam / 6.8 / 2.2	50	3.5	
Sulfamethoxazole		three soils, aerobic	50	20	Wu et al. 2012
Tetracyclines					
Chlortetracycline	5.6	sandy loam+manure / 6.1	88	30	(Gavalchin and Katz, 1994)
	4.7 µg/kg	soil	0	ca. 180	(Hamscher et al., 2001)
Tetracycline	50-300 µg/kg	soil	0	ca. 180	
	10	soil+manure	100[b]	14	(Jagnow, 1977)
Oxytetracycline		soil+manure	0	180	(van Gool, 1993)
		sediment slurry, aerobic	50	43.8	(Ingerslev et al., 2001)

[a] If not indicated otherwise.
[b] Dissipation at 10°C and 10°C / 20°C, respectively.
[c] Mineralization determined with ^{14}C-radioactive labelled compounds.

Source: Adopted from Thiele-Bruhn (2003b).