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Main sources and emission pathways of micropollutants released in surface water at a catchment scale: from a theoretical approach to a practical application on a case-study

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Table of contents

List of acronyms

AOX	Adsorbable Organic Halides
BHT	Butylated hydroxytoluene
BNR	Biological Nutrient Reactor
CAS	Conventional Activated Sludge
CBZ	Carbamazepine
CEC	Cation Excange Capacity
CFU	Colony-Forming Unit
CSO	Combined Sewer Overflow
CV	Coefficient of Variation
DM	Dry Matter
DMB	Dewatered Municipal Biosolid
D_{OW}	Octanol-water distribution coefficient
DT	Disappearance Time
E1	Estrone
E2	Estradiol
EE2	Ethinylestradiol
EC	Effective concentration
EHMC	Ethylhexyl methoxycinnamate
f_{OC}	Fraction of Organic Carbon
HM	Heavy Metal
HRT	Hydraulic Retention Time
k_{biol}	Biodegradation rate constant
K_d	Partition Coefficient
K_H	Henry’s law volatility constant
K_{OC}	Carbon-water partition coefficient
K_{OW}	Octanol-water partition coefficient
LC	Lethal concentration

LMB	Liquid Municipal Biosolid
LOD	Limit of Detenction
LOQ	Limit of Quantification
MBR	Membrane Bioreactor
MPN	Most Probable Number
M_W	Molecular Weight
PCB	Polychlorinated biphenyl
PCDD/F	Dioxins and furans
PhAC	Pharmaceutical Active Compound
pK_a	Dissociation constant
PPCP	Pharmaceuticals and Personal Care Products
PSD	Passive sampling device
SC	Sub-Catchment
SFA	Substance Flow Analysis
S_i	Sensitivity index
SMX	Sulfamethoxazole
SRT	Sludge Retention Time
SS	Suspended Solids
TCC	Triclocarban
TCS	Triclosan
v_p	Vapour pressure
WWTP	Wastewater Treatment Plant

List of figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

List of tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

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- C.G.J -

Chapter 1

GENERAL INTRODUCTION

Table of contents

1.1. Objectives of the thesis

In the last decades, the rise of the global population and the steady growth of the healthcare sector have led to a considerable increase in pharmaceutical consumption worldwide (Van Boeckel et al., 2014). On the one hand, this means that modern medicine has developed significantly, but on the other, this brought to the possibility that residues of these compounds reach surface water, soil and plants through several routes during their manufacture, use, and disposal, resulting in potential environmental risks.
In this context, many reports about the occurrence of pharmaceutical active compounds (PhACs) and their transformation products in the environment can be found in the existing scientific literature. As an example, Galindo-Miranda et al., 2019 provided a summary on the occurrence of emerging contaminants (among them PhACs and pesticides) in different types of surface water, including rivers, lakes, creeks, and wetlands all around the globe; Al-Farsi et al., 2017 reviewed the recent research about plant uptake, focusing on the occurrence of pharmaceuticals and personal care products (PPCPs) in crops irrigated with reclaimed water and Carter et al., 2019 presented an in-depth analysis to establish the potential pathways the PhACs may take in the agricultural environment and discussing how they can impact on human and agroecosystem health, to name just a few.
These findings have been made possible also by the advancement of laboratory instrumentation and analytical methods, which enabled the detention of a wide range of different substances in different environmental matrices. And it's expected that the occurrence in the environment of even more compounds and their corresponding transformation products may be precisely monitored in the upcoming future (Daverey et al., 2019).

Among the different PhAC emission pathways, the wastewater treatment plant (WWTP) effluent and combined sewer overflows (CSOs) are worth mentioning (Verlicchi et al., 2017), and therefore many recent studies tried to evaluate their contribution to surface water pollution (among them Baranauskaite-Fedorova et al., 2016 and Chèvre et al., 2013). But at the same time, considering a peri-urban or a rural area, PhACs, and other emerging contaminants may reach the receiving water bodies also through other ways, such as surface runoff, tile drainage, and percolation from manure or sewage sludge-amended arable lands (Figure 1.1). Here, differently from WWTP effluents and CSOs, these last emission pathways are non-point sources. Thus, their contribution to the PhAC occurrence in surface waters can be only indirectly estimated through a comprehensive sampling campaign in which samples are collected in strategic locations, such as different points of a stream which passes through arable lands and also receive WWTP effluents and CSOs (Hanamoto et al., 2018).
This thesis takes place in this background trying to, first of all, characterize the PhAC content in different sources (such as different manures from different animals and treated sewage sludge, also called biosolid) and emission pathways (such as surface runoff, tile drainage, and percolation from manure and sewage sludge-amended soils) by means of an exhaustive analysis of the recent and past literature. This part was carried

out during the first year of this PhD (see Figure 1.2) to have an overall view of the literature state-of-the-art, and this led to the publication of two review articles: the first one related to the occurrence of veterinary PhACs and microorganisms in different zootechnical wastes and the corresponding environmental risk due to their application onto arable land, and the second one regarding the occurrence of PhACs and microorganisms in surface runoff and tile drainage from soils that received biosolid.

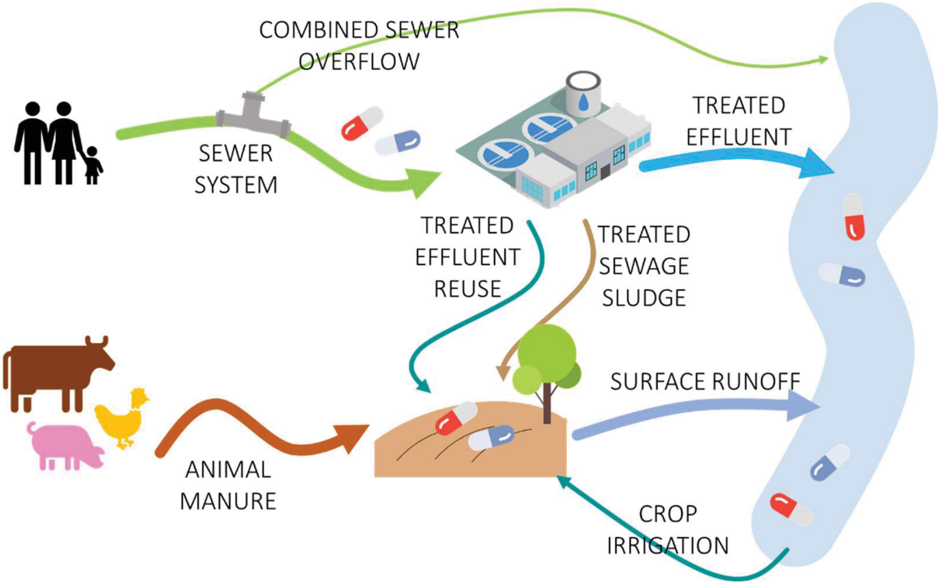

Figure 1.1. Most relevant sources and emission pathways of PPCPs in surface water bodies on a catchment scale.

After that, surface water sampling practices were analysed, in order to discuss how different sampling modes and frequencies may influence the campaign measurements. This insight has not only led to the publication of a related research article (Figure 1.2) but also helped to characterize better the literature findings of PhAC occurrence in surface runoff and tile drainage. At the same time, it was a valuable input toward understating the data uncertainty, which has been useful in the second and main part of this PhD.

In this regard, all the elements were now on the table to try to evaluate the contribution of three different emission pathways (namely WWTP effluent, CSOs, and runoff and tile drainage from manure and biosolid-amended arable lands) to the occurrence of selected PhACs into the receiving water body, considering a defined catchment area. Of course, this task required a comprehensive state-of-the-art about the pathways a PhACs may take from its human and animal consumption to the receiving catchment river and a consistent amount of data and information related to the catchment under study. Thus, in this regard, the collaboration with Dr Zoboli and Professor Zessner of the Institute for Water Quality and Resource Management of the TU Wien – carried out from the second year of this PhD (Figure 1.2) – has been an invaluable help which takes this work further.

They provided not only a precious scientific experience on the topic, but also an exhaustive dataset about annual river water flows, WWTP effluent and CSO discharges, arable land dimension and characteristics, types of animals and manure produced, and so forth, regarding a specific Austrian catchment. The article resulted from this collaboration represented the main destination of this PhD work because it outlined a method to assess the occurrence of selected micropollutants on a catchment scale in different environmental compartments, also estimating the corresponding PhAC emission pathways contributions.

Finally, another question came up as a consequence of the previous work, outlining an issue which could be taken as the completion of this PhD thesis: what kind of effects on crops may result when plants are irrigated with surface water containing PhAC residuals? This subject has been studied during the research visit at the University of York (Figure 1.2). Thanks to the precious and advanced experience of Dr Sallach, Dr Wilkinson, and Professor Boxall, from the Department of Environment and Geography, it was possible to carry out a dedicated laboratory experiment, in which plants were watered with mixtures of selected PhACs in concentrations equal to those found in some past monitoring campaign on surface water all around the world.

The results of this work not only helped to supplement the current thesis with a different point of view but also have led to the writing of a corresponding research article.

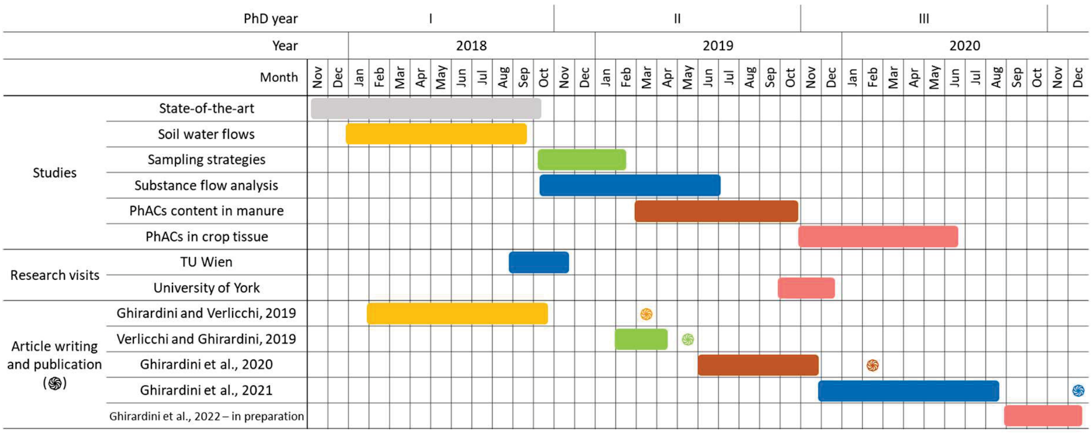

Figure 1.2. Gantt chart on PhD topic evolution and development.

In the conclusion of this PhD thesis, the opportunity has been taken to try providing the whole picture about the pathways a PhACs may take from its consumption to its release in surface waters and then its return into the water cycle *via* crop irrigation. The topic has been there discussed focusing on the advances made in these years and considering the difficulties found on the way of this work, concluding with the main future research perspectives.

1.2. Main characteristics of pharmaceuticals and personal care products in the environment

Due to the worldwide people aging, the constant healthcare development, and the consistent use of growth promoters and treatment therapies in animal husbandry, large quantities of pharmaceuticals, hormones, and personal care products may occur in surface waters all around the globe (Tijani et al., 2016).

As reported in Table 1.1, different types of micropollutants can be measured in human and livestock wastes. In this context, household wastewaters may contain a broad spectrum of pharmaceuticals active compounds (PhACs), such as analgesic and anti-inflammatories, antibiotics, antidiabetics, antihistamines, antineoplastics, antiseptics, beta-blockers, lipid regulators, and psychiatric drugs, to name just a few. Not only, but also hormones, stimulants (such as caffeine and nicotine), and personal care products (such as UV filters, insect repellents synthetic musks), and microplastics may occur (Alvarino et al., 2018).

Some of these compounds persist in the sewer network and enter in wastewater treatment plant (WWTP) or are directly released in water streams through combined sewer overflows (CSOs) during high-intensity rainfall events (Launay et al., 2016). Once in the WWTP, they may undergo different processes, which will be further discussed later, been discharged in the receiving water bodies through the treated effluent, or been absorbed into sludge, which may be applied onto arable land as amendment and fertiliser (Verlicchi and Zambello, 2015).

The compounds commonly found in zootechnical wastes mainly belong to few PhAC classes (antibiotics, antifungals, and anti-inflammatories, in most cases) and hormones, due to the different treatments the animals are subjected to, typically regarding disease prevention and medication, and growth promotion (Boxall et al., 2004). Notwithstanding this, antibiotics and hormones in raw manure may occur at a high concentration level ($\mu g\ g^{-1}$ to $mg\ g^{-1}$) (Arikan et al., 2007). Thus, when zootechnical wastes are applied onto arable land, microcontaminants may be potentially remobilised in the water phase through surface runoff, tile drainage, and percolation, primarily when rainfall occurs within few days from manure amendment. Not only that, once pharmaceuticals and hormones are applied onto the soil, they can be available for plant roots uptake and then cause phytotoxic effects or enter the food chain, or remain sequestered in the solid particles, potentially posing an environmental risk for the soil microbial community.

That said, as schematically represented in Figure 1.1, the pathways pharmaceuticals and personal care products may take, from their consumption to the receiving water bodies, are complex and may differ depending on each specific case and will be anyway thoroughly examined in chapter 6.

It is important to highlight that these micropollutants are characterised by various structures and physicochemical properties, which make them exhibit very different behaviours in the environment. It is thus necessary, for the aim of this thesis, to have a quick overview of the essential characteristics that define pharmaceuticals and personal care products (PPCPs) and that distinguishes them and their potential fate in WWTPs, soil flows, and surface waters.

Table 1.1. Micropollutants that could be found in sewage sludge, animal manure, and WWTP effluent.

Micropollutant family	Therapeutical class	Presence in sewage sludge[a]	Presence in animal manure[b]	Presence in WWTP effluent[c]
Personal care products		✓		✓
Hormones		✓	✓	✓
Pharmaceuticals	Analgesics and anti-inflammatories	✓	✓	✓
	Anticonvulsants	✓		✓
	Anthelmintics	✓	✓	✓
	Antibiotics	✓	✓	✓
	Beta-blockers	✓	✓	✓
	Diuretics	✓		✓
	Lipid regulators	✓	✓	✓
	Psychiatric drugs	✓		✓
	Receptor antagonists	✓		✓
	Stimulants	✓		✓

[a]Verlicchi and Zambello, 2015
[b]Boxall et al., 2004
[c]Verlicchi et al., 2012

1.2.1. Molecular weight (M_W)

The molecular weight is evaluated as the sum of the atomic weights of the atoms making up the substance's molecular formula. It is used to determine stoichiometry in chemical reactions and equations, and it can be expressed in atomic mass units or be unitless.

1.2.2. Solubility (S)

The solubility is defined as the ability of a chemical substance (here referred to as the *solute*) to dissolve in a *solvent* at a specific temperature (typically 20°C or 25°C). In this thesis, solubility will be generally used to measure the quantity of a particular compound that can be dissolved in water (*water solubility*). It will be expressed in mg/L or ppm.

1.2.3. Vapour pressure (v_p)

The vapour pressure (v_p) is the maximum pressure a compound can produce at a given temperature (25°C). It is used to measure the volatility of that compound, and it is typically expressed in mmHg.

1.2.4. *Octanol-water partition coefficient (K_{OW})*

K_{OW} is a measure of the tendency of a (not ionised) compound to be more soluble in a fat-like solvent – as the octanol used in the measurement – or water. It is evaluated with the following equation:

$$K_{OW} = \frac{[compound]_{octanol}}{[compound]_{water}} \qquad \text{(eq. 1)}$$

In which $[solute]_{octanol}$ is the concentration of the solute in octanol, and $[solute]_{water}$ is the solute concentration in water. Here, if K_{OW} is greater than 1, the compound under study exhibits a hydrophobic character, so it shows the tendency to be sequestered in a solid phase. On the contrary, if the value of K_{OW} is less than 1, the compound is considered hydrophile, also suggesting a polar character (Cairns, 2012).

The high extension of the potential values the octanol-water partition coefficient may take is often expressed in a base-10 logarithmic scale, allowing one to compare its result with specifically dedicated tables. In this regard, as a rule of thumb, if $logK_{OW}$ is greater than 4, it is expected that the compound is highly adsorbed into a solid phase, while if $logK_{OW}$ is less than 2.5, the compound likely tends to be more soluble in water (Rogers, 1996).

It should be noted that the octanol-water partition coefficient does not consider the potential ionisation of a substance. Thus, according to Cunningham, 2008, the evaluation of the octanol-water *distribution* coefficient (D_{OW}) could be more appropriate for assessing the partition behaviour of acidic and basic compounds.

Therefore, defining an acid as compounds that ionise to release hydrogen ions, or protons, to their surroundings, and a base as a compound that can accept hydrogen ions (Cairns, 2012), the octanol-water distribution coefficient can be calculated, according to Schwarzenbach et al., 2003 based on the *log Kow* with the following equations:

$$\log D_{OW} = \log K_{OW} + \log \frac{1}{1 + 10^{pH - pK_a}} \qquad \text{(in case of acidic compounds)} \qquad \text{(eq. 2)}$$

$$\log D_{OW} = \log K_{OW} + \log \frac{1}{1 + 10^{pK_a - pH}} \qquad \text{(in case of basic compounds)} \qquad \text{(eq. 3)}$$

$$\log D_{OW} = \log K_{OW} \qquad \text{(in case of neutral moieties)} \qquad \text{(eq. 4)}$$

In which pK_a is the dissociation constant (defined below), and pH is evaluated at the solution equilibrium.

Here, the rule of thumb is to consider the compound as adsorbed in the solid phase if its $logD_{OW}$ is greater than 3, and more soluble in water if its $log\ D_{OW}$ is less than 1 (Cunningham, 2008).

1.2.5. *Dissociation constant (pKa)*

Defining an acid as a substance HA which can dissociate in aqueous solution into A^- (the conjugate base of the acid) and H^+ (a hydrogen ion), its equilibrium constant can be written as:

$$K_a = \frac{[A^-][H^+]}{HA} \qquad \text{(eq. 5)}$$

Or alternatively:

$$pK_a = -\log_{10} K_a = \log_{10} \frac{[HA]}{[A^-][H^+]} \qquad \text{(eq. 6)}$$

Where *[HA]*, *[A⁻]*, and *[H⁺]* are the concentrations of *HA*, A^- and H^+ in the solution, respectively.

The dissociation constant is thus a particular example of an equilibrium constant, in which the substance under study is an acid. With this definition, pK_a may be considered as a quantitative measure of the tendency an acid has to dissociate in an aqueous solution, or in other words, its strength (Cairns, 2012).

1.3. Fate of micropollutants in wastewater treatment plants

Conventional WWTPs are currently designed to remove suspended solids, dissolved organic matter, and nutrients, such as nitrogen and phosphorus (Metcalfe and Eddy, 2014). Thus, micropollutant removal is not ensured, even if they may be affected by the complex processes occurring in a reactor. In this context, the removal mechanisms the PPCPs may undergo in a WWTP mainly belong to sorption onto particulate matter (sludge), volatilisation, biological transformation, and abiotic degradation (Margot et al., 2015).

In the first two processes, the micropollutants are not mineralised but only partitioned between two phases (water-solid or water-gas). Thus, the removal of a compound, evaluated with the conventional formula (eq. 7), is greater than zero but the compounds are still persisting in the environment (for example, as sequestered in the sewage sludge, which may be applied on arable land). On the contrary, biological transformation and abiotic degradation directly lead to the transformation of micropollutants, or, in the best cases, even their complete mineralisation, defined as the production of H_2O, CO_2, and minerals from the initial compound's structure (Knapp and Bromley-Challoner, 2003).

In any case, assuming a constant flow, the removal of a compound can be calculated with the following equation:

$$\%_{removal} = \frac{\dot{m}_{PPCP,in} - \dot{m}_{PPCP,eff}}{\dot{m}_{PPCP,in}} \times 100 \qquad \text{(eq. 7)}$$

In which $\dot{m}_{PPCP,in}$ and $\dot{m}_{PPCP,eff}$ are the load of a PPCP in WWTP influent and effluent, respectively.

It should be noted here that equation 7 is commonly evaluated considering the concentrations of a micropollutant, instead of the influent and effluent load. As a result, the obtained removal may be subjected to uncertainties related to the fact that part of the mass of a compound entered in the reactor is sequestered in the sludge. Therefore, an overall mass balance of the substance can be evaluated only taking into account also the SRT of the plant, and consequently, the mass came in and came out, in a specific period of time.

1.3.1. Sorption onto particulate matter

Sorption onto activated sludge may be ascribed to two different interactions:

According to Ternes et al., 2004, when aliphatic and aromatic groups of a compound interact with the microorganisms' lipophilic cell membrane or the lipid fraction of sludge, the mechanism is defined *absorption*, referring specifically to the hydrophobic behaviour of a contaminant.

Differently, when a positively charged group of a compound comes into contact with the negatively charged surface of microorganisms in the sludge, the mechanism is defined *adsorption*, indicating an electrostatic interaction of the contaminant.

In this context, the sorption tendency of a compound can differ from what is estimated based on its *LogK*$_{OW}$ or *LogD*$_{OW}$, as these values are calculated considering a lipophilic interaction only (with octanol, as already remarked in Section 1.1.4). If a PPCP exhibit a *LogK*$_{OW}$ greater than 4, it is expected its aliphatic and aromatic groups interact with the lipophilic cell membrane of microorganisms, colloidal particles, and other suspended solids, which may be present in a biological reactor. In this case, even if colloidal particles occur in a relatively small fraction of the total particle mass in an activated sludge reactor, they provide a large surface area that can enable covalent, electrostatic, and hydrophobic binding of micropollutants (Das et al., 2017). In addition, if a compound contains functional groups which can be protonated and de-protonated, also *absorption* may play an important role in the sorption process.

For example, some pharmaceuticals, such as fluoroquinolones, exhibit a strong tendency to interact with the solid sludge phase, even if their *LogK*$_{OW}$ is extremely low (*e.g.*, norfloxacin with a *LogK*$_{OW}$ = -1) (Golet et al., 2003).

For this reason, the whole sorption mechanism occurring in a WWTP is often described using Equation 8 (Ternes et al., 2004). This equation gives a linear relationship between the PPCP concentration sorbed onto particulate $[PPCP]_{ads}$, the concentration of suspended solids in the reactor *SS* and the PPCP concentration dissolved in the water phase $[PPCP]_{dis}$ (Das et al., 2017):

$$[PPCP]_{ads} = K_d \times SS \times [PPCP]_{dis} \qquad \text{(eq. 8)}$$

In which, K_d is the *partition coefficient*, expressed in L/gSS, which is a compound-related indicator that measures its affinity to a defined solid phase (Ternes et al., 2004):

$$K_d = \frac{[compound]_{solid}}{[compound]_{water}} \qquad \text{(eq. 9)}$$

Where $[compound]_{solid}$ is the compound solid-sorbed concentration (*e.g.,* mol/gSS) and $[compound]_{water}$ is the dissolved aqueous compound concentration (*e.g.,* mol/L).

This coefficient can be directly correlated to K_{OW} by means of many empirical formulas and models that will be further discussed in Chapter 6.

Sorption into solid-phase results in the occurrence of PPCPs in raw and treated sewage sludge, which, due to the potential risks posed by sludge application on arable land, will be considered in more detail in Chapter 3.

1.3.2. Volatilisation

In a WWTP, micropollutant transfer from the water to air is mainly due to stripping during aeration. Thus, it depends on compound characteristics (especially on Henry's law constant K_H) and reactor operating conditions, such as type of aeration, agitation, and temperature (Margot et al., 2015).

This mechanism leads only to a negligible degradation for the majority of PPCPs, except for musk fragrance, which can be volatile (Fernandez-Fontaina et al., 2014). In any case, stripping should not be considered for micropollutant removal, as the reactor's gas flow are not commonly treated afterwards.

1.3.3. *Biological transformation*

This mechanism occurs when organic micropollutants are metabolised or react with the bacteria cells of the reactor. In the first case (microorganism metabolism), the compounds are used by bacteria as a source of energy and carbon (catabolism and anabolism), and thus directly supporting cell maintenance and growth.

In the second case (co-metabolic reaction), micropollutants are biologically transformed by side reactions occurring in the bacteria cell and do not serve for cell development (Margot et al., 2015).

In any case, due to the strict dependence of this process by bacteria colonies in the reactor, all the parameters which drive microbial growth can play an important role. For example, it was observed that increasing the sludge retention time (SRT) and the hydraulic retention time (HTR) may entail a pronounced biological transformation of micropollutants by co-metabolic reactions (Clara et al., 2005), mainly due to nitrifying bacteria which increased the more the aeration in the reactor is extended (Metcalfe and Eddy, 2014).

Furthermore, the temperature may significantly influence microbial growth and activity and solubility, and other physicochemical characteristics of the compounds, resulting in a higher biological transformation in summer than winter (Vieno et al., 2005).

Thus, it is difficult to describe these phenomena fully, and only empirical formulas may be derived. In this context, the biotransformation (first-order) kinetic is commonly expressed with the following equation (Pomiès et al., 2013):

$$biotrans_{rate} = k_{biol} \times SS \times [PPCP]_{dis} \qquad \text{(eq. 10)}$$

In which k_{biol} is the biodegradation rate constant of the selected PPCP (commonly expressed in L gSS^{-1} h^{-1}), SS is the concentration of suspended solids in the reactor, and $[PPCP]_{dis}$ are the PPCP concentration dissolved in the water phase.

Of course, the equation represents a simplification of the complex phenomena. As well as the previously mentioned models, it could be further discussed and amplified in many different, more exhaustive, and comprehensive ways (Pomiès et al., 2013), going far beyond the limited framework of this thesis.

It is interesting here to highlight that k_{biol} can be used to preliminary classify the micropollutants for their tendency to be biodegraded in a reactor. For instance, Joss et al., 2006 observed that the pharmaceuticals that exhibit a k_{biol} less than 0.1 (*e.g.*, carbamazepine, diazepam, diclofenac, and tonalide) generally persist in conventional activated sludge treatment. On the contrary, those with a k_{biol} higher than 10 (*e.g.*, estradiol, ibuprofen, and paracetamol) are easily biodegraded.

1.3.4. *Abiotic degradation*

As the name suggests, abiotic degradation does not involve microorganisms or any *biota*. On the contrary, the compound is transformed by different reactions, such as photolysis or hydrolysis. The first one is uncommon in a biological reactor due to the turbidity of wastewater, making it difficult for the photons of sunlight to reach the compounds and then cleave the chemical bonds. On the contrary, this mechanism can occur in the plant's tertiary treatment if wastewater disinfection is carried out with UV lamps (Rodríguez-Chueca et al., 2018).

Hydrolysis consists of the cleavage of micropollutant's chemical bonds and additional substitution of an atom (or a group of atoms) of the compound with water molecules or hydroxide ion (OH^-). This mechanism has been shown to occur in the removal of macrolide and tetracycline antibiotics and a few other compounds, while it can be considered negligible for others (Schwarzenbach et al., 2003).

Finally, micropollutants may also be affected by the solution pH, which, in the range of 6-8 that could be commonly found in wastewaters (Metcalfe and Eddy, 2014), may be able to ionise those compounds containing at least one functional group with pK_a value in the range of 5-10 (Das et al., 2017).

1.4. Legal framework about micropollutants in the water environment

Nowadays, no legal limits regarding pharmaceuticals, hormones, and personal care products concentration in WWTP effluent are defined.

Nevertheless, in Europe, water resources and aquatic environments are protected by the Water Framework Directive (2000/60/EC), adopted from October 23rd, 2000. This directive aims to achieve a *good ecological and chemical status* in the ground and surface waters all around the EU territory by 2015, which can be postponed with a maximum deadline of 2027 (EC, 2019). Here, the ecological and chemical status can be considered a qualification of the functionality of the ecological services and is assessed according to the evaluation of defined criteria. Among these are worth mentioning the biological quality (e.g., aquatic flora), chemical quality (in terms of pollutants concentration), physicochemical quality (e.g., temperature, oxygenation, and nutrient conditions), and hydro-morphological quality (e.g., river continuity) (Zacharias et al., 2020).

Therefore, identifying a list of substances that can pose an environmental risk in the water environment is necessary to follow the objectives of the Water Framework Directive (WFD).

In this context, in its latest version, the WFD established a list of pollutants which need further monitoring in order to collect information regarding their concentrations in the water environment (EC, 2013). The list included traditional pesticides (*e.g.*, atrazine, diuron, simazine, DDT, and cyclodienes), polycyclic aromatic hydrocarbons, brominated and chlorinated compounds, dioxins, and heavy metals, and poses the bases for the creation of an appropriate Watch list in which contaminants of emerging concern (*e.g.*, pharmaceuticals and hormones) can be included (EC, 2013).

In this regard, the European Commission published in March 2015 the so-called Watch list (EC, 2015), including 17 compounds from different classes (among them the antibiotics azithromycin, clarithromycin, and erythromycin, the hormones estrone and estradiol, and also pesticides and herbicides such as acetamiprid and triallate). This Commission Implementing Decision made it mandatory for the EU member states to monitor each substance of the list in at least one monitoring station over at least 12 months to report the campaign results to the Commission and take further decisions in the upcoming future.

The Watch list was updated in June 2018 (EC, 2018), adding three compounds (the antibiotics amoxicillin and ciprofloxacin, and the insecticide metaflumizone) and removing five compounds from the previous version (namely, the anti-inflammatory diclofenac, the UV filter EHMC, the antioxidant BHT and the herbicides oxadiazon and triallate). The last update of the list occurred in August 2020 (EC, 2020). Here, the added compounds are the antibiotics sulfamethoxazole and trimethoprim, the antidepressant venlafaxine and its metabolite O-desmethylvenlafaxine, a group of three azole pharmaceuticals (clotrimazole, fluconazole and miconazole), seven azole pesticides (imazalil, ipconazole, metconazole, penconazole, prochloraz, tebuconazole, tetraconazole), the fungicides famoxadone and dimoxystrobin.

A list of the compounds included in the three versions of the Watch list is reported in Table 1.2. It will be further mentioned in this PhD thesis, as the listed micropollutants frequently occur in treated effluent, combined sewer overflow, sewage sludge, animal manure, and thus soil vadose zone water flows in arable lands amended with sludge and manure.

Table 1.2. Resume of the compounds included in the first (2015) and second (2018) version of the Water Framework Directive Watch list. Italic was used for compounds included in one Watch list version and not in the previous one.

Watch list version	**Class (number of compounds included)**	**Compounds included**	**Total number**
1st – **2015**/495	Antibiotics (3)	Azithromycin, clarithromycin, erythromycin;	17
	Anti-inflammatories (1)	Diclofenac;	
	Antioxidants (1)	Butylated hydroxytoluene (BHT)	
	Hormones (3)	Estrone (E1), estradiol (E2), ethinylestradiol (EE2);	
	Pesticides (6)	Acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam, methiocarb;	
	Herbicides (2)	Oxadiazon, triallate;	
	UV filters (1)	Ethylhexyl methoxycinnamate (EHMC).	
2nd – **2018**/840	Antibiotics (5)	*Amoxicillin*, azithromycin, *ciprofloxacin*, clarithromycin, erythromycin;	15
	Hormones (3)	Estrone (E1), estradiol (E2), ethinylestradiol (EE2);	
	Insecticides (1)	Metaflumizone;	
	Pesticides (6)	Acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam, methiocarb.	
3rd – **2020**/1161	Antibiotics (4)	Amoxicillin, ciprofloxacin, *sulfamethoxazole*, *trimethoprim*;	19
	Antidepressants (2)	*Venlafaxine*, *O-desmethylvenlafaxine*;	
	Fungicides (2)	*Dimoxystrobin*, f*amoxadone*;	
	Insecticides (1)	Metalflumizole;	
	Pesticides (10)	*Clotrimazole*, *fluconazole*, *imazalil*, *ipconazole*, *metconazole*, *miconazole*, *penconazole*, *prochloraz*, *tebuconazole*, *tetraconazole*.	

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Chapter 2

INSIGHTS ABOUT SURFACE WATER SAMPLING

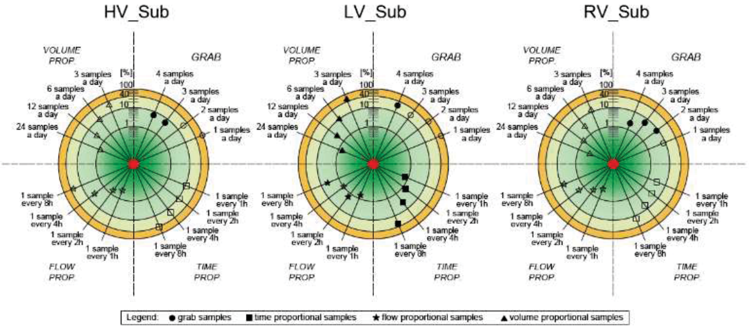
INFLUENCE OF THE ADOPTED SAMPLING METHOD ON SURFACE WATER QUALITY EVALUATION

Table of contents

Aims of the chapter, in a nutshell

- *The main different approaches adopted for water flow sampling were described.*
- *The average concentration in water flow measured with four different sampling strategies was compared with its ideal theoretical value.*
- *Three representative substances with different patterns of concentration versus time were considered for the numerical example.*
- *The results of this chapter were published in 2019 in Water (MDPI), 11, 1152 with the title: "Occurrence of Micropollutants in Wastewater and Evaluation of their Removal Efficiency in Treatment Trains: The Influence of the Adopted Sampling Mode" (Verlicchi and Ghirardini, 2019).*

Graphical Abstract



2.1. Introduction

In monitoring surface waters, WWTP influent and effluent, soil water flows (such as surface runoff, and tile drainage), it is essential to plan a sampling campaign which can be able to provide samples that can be considered *representative* of the phenomenon under study.

Water samples can be grab (instantaneous) or composite (showing thus the result of a defined time window), and in the last case, they may be evaluated as time, flow, or volume proportional. Also, in any case, the frequency of sampling is a crucial parameter to pinpoint the different behaviours in the occurrence of the pollutants monitored.

The issue requires even more attention if the compounds under study occur, as in the context of this PhD thesis, at ng L^{-1} or μg L^{-1}, being thus considered micropollutants.

For example, consider those PhACs which are specifically administered in hospitals (among them the diagnostic agents gadolinium and iopamidol, or the cytostatic 5-fluorouracil) in a defined time window (*e.g.*, in the morning). These chemicals reach the sewer system and then the WWTP within a specific period, and their concentration may be very high in an hour rather than another.

A monitoring campaign of these substances should be planned in order to detect all the potential concentration ranging values, but this could be challenging in case of PhACs with such various pattern. In addition, pinpointing a concentration peak (or in contrary, a local minimum) may lead to an overestimation (or an underestimation) of the real occurrence of a chemical, entailing an inaccurate environmental risk assessment.

The same issue can be easily observed in a larger scale for those compounds which pattern of concentration vary on a weekly basis, such as fluorouracil, diatrizoate, iomeprol and iohexol (Weissbrodt et al., 2009), or monthly basis, such as cefazolin or carbamazepine (Verlicchi, 2018), or even on an annual basis, as for antibiotics.

For this reason, the influence of the sampling method and frequency adopted has been studied by many researchers in the last ten years.

Some of them (for example Ort et al., 2010a, 2010b, 2010c; Ort and Gujer, 2006; Verlicchi, 2018; Weissbrodt et al., 2009) addressed this issue with great detail, considering different sampling modes in a sophisticated way. Some others did not focus specifically on this subject but pointed the attention on it (Verlicchi and Zambello, 2014). Particularly worth of mention are the investigations of Ort and colleagues which provided – following an in-depth mathematical analysis – interesting suggestions for monitoring campaigns of micropollutants, taking into account many parameters, as the number of toilets flushing in the catchment under study (Ort et al., 2010a, 2010b, 2010c; Ort and Gujer, 2006).

In this background, to provide a general overview regarding the uncertainty connected to water flow sampling – which represents an essential step for the core of this PhD thesis, as it will be further discussed below in Chapter 6 – this chapter aims to present a brief description and discussion of the most adopted sampling modes.

Also, the results of these different sampling strategies are here compared in terms of their percentage deviations from the ideal solution, which theoretically represents the most representative of the case under study.

To address this issue, three *ad hoc* curves of concentration *versus* time were defined, each one representative of a group of compounds which can be found in the effluent of a WWTP.

The first curve corresponds to those substances of which the concentration in the water flows present few but evident variations overt the day. This is the case, for example, of the cytostatic 5-fluorouracil (Weissbrodt et al., 2009) or the diuretic furosemide (Nelson et al., 2011). This group of compounds will be from now on referred as *high variability substances* (HV_Sub).

The second curve was defined emulating the profile of the concentration of substances that present a moderate variation over the whole day. This almost constant behaviour can be observed, for example, for the antiinflammatory ketoprofen (Khan and Ongerth, 2005), the antiseptic triclosan and the anticonvulsant phenytoin (Nelson et al., 2011). This profile is called from now on to a *low variability substance* (LV_Sub).

The third curve regards to those compounds which present a pattern of concentration *versus* time in the water flow that randomly varies over the day. This is the case, for example, of the antibiotics ciprofloxacin (Duong et al., 2008) and lincomycin (Hong et al., 2015), or the antiinflammatory diclofenac (Nelson et al., 2011). This group of compounds take here the name of *random variability substances* (RV_Sub), and its profile pattern is not easily predictable.

The values of concentration *versus* time used to outline these curves were taken from the literature cited above regarding gadolinium, ketoprofen and, ciprofloxacin for HV_Sub, LV_Sub, and RV_Sub, respectively, and are reported Table 2.1.

Table 2.1. Concentrations of the three representative compounds and values of flow rates used for defining the corresponding profile of concentrations and flow rate over the day.

Time [h]	**HV_Sub** [ng L^{-1}]	**LV_Sub** [ng L^{-1}]	**RV_Sub** [ng L^{-1}]	**Flow rate** [m^3 h^{-1}]
12:00 AM	1000	1500	14,000	16
1:00 AM	1000	1100	13,500	18
2:00 AM	1000	850	15,500	20
3:00 AM	1000	550	19,000	21
4:00 AM	1000	280	22,500	19
5:00 AM	1000	100	26,000	18
6:00 AM	1000	100	29,000	19
7:00 AM	1000	100	31,000	24
8:00 AM	1000	100	32,000	30
9:00 AM	5500	300	32,000	36
10:00 AM	17,000	550	31,500	39
11:00 AM	30,000	750	31,000	39
12:00 PM	40,000	850	30,000	37
1:00 PM	46,000	900	29,000	35
2:00 PM	48,000	900	29,000	34
3:00 PM	46,000	800	29,500	33
4:00 PM	40,000	650	30,500	31
5:00 PM	37,000	450	32,000	28
6:00 PM	34,500	300	34,000	24
7:00 PM	35,000	200	36,500	21
8:00 PM	37,000	250	38,000	22

Time	HV_Sub	LV_Sub	RV_Sub	Flow rate
9:00 PM	38,500	450	38,000	27
10:00 PM	35,000	850	35,500	28
11:00 PM	22,000	1300	29,000	17
12:00 AM	1000	1500	14,000	16

It should be noted that the selected values of concentration *versus* time may be different and case-specific. Nevertheless, the nature of the comparison does not depend on the case. On the contrary, the pattern of concentration *versus* time is here meaningful, and others concentration data can be used instead of those employed in here.

To create the curves, the concentrations reported in Table 2.1 were fitted with a nonlinear curve using the software MATLAB. In this way, the concentration *c versus* time *t* was set as a continuous curve.

The resulting patterns of concentration for the three representative substances are represented in Figure 2.1.

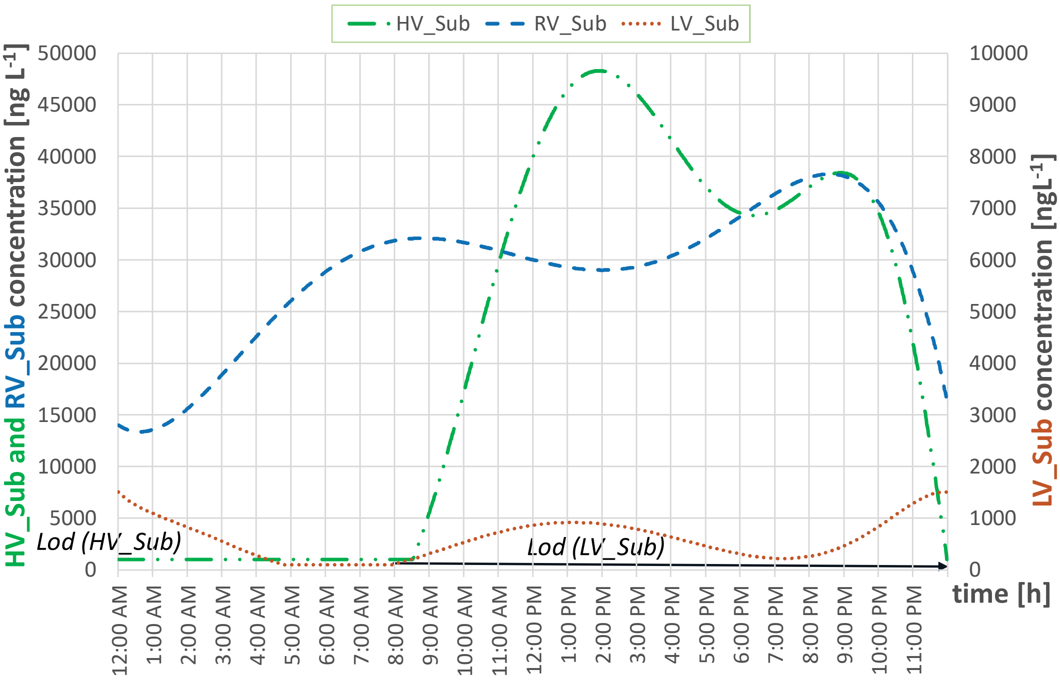


Figure 2.1. Concentrations *versus* time for the three key compounds considered in the studied example. Note that Y-axis for LV_Sub is on the right and Y-axis for HV_Sub and RV_Sub is on the left.

In addition, their mathematical formulas, excluded here for the sake of brevity, are provided in the main work this chapter is referring to, published in volume 11 of Water (MDPI) on May 31st, 2019 (Verlicchi and Ghirardini, 2019).

A similar procedure was involved in designing the water flow curve, essential here to evaluate the load of the substances, considering which the comparison of the reliability between the different sampling modes will be tested.

This was done assuming the wastewater generated by a small catchment area (around 3,500 inhabitants), with individual water consumption of 200 L *per* inhabitant *per* day.

The daily flow (thus equal to 634.5 m3 d^{-1}) was divided in the 24 hours based on wastewater flow curves found in the literature (Duong et al., 2008; Verlicchi et al., 2013, 2010).

The 24 values of flow rate, reported in Table 2.1, were processed in MATLAB as for the curves described above, and the resulting curve is represented in Figure 2.2.

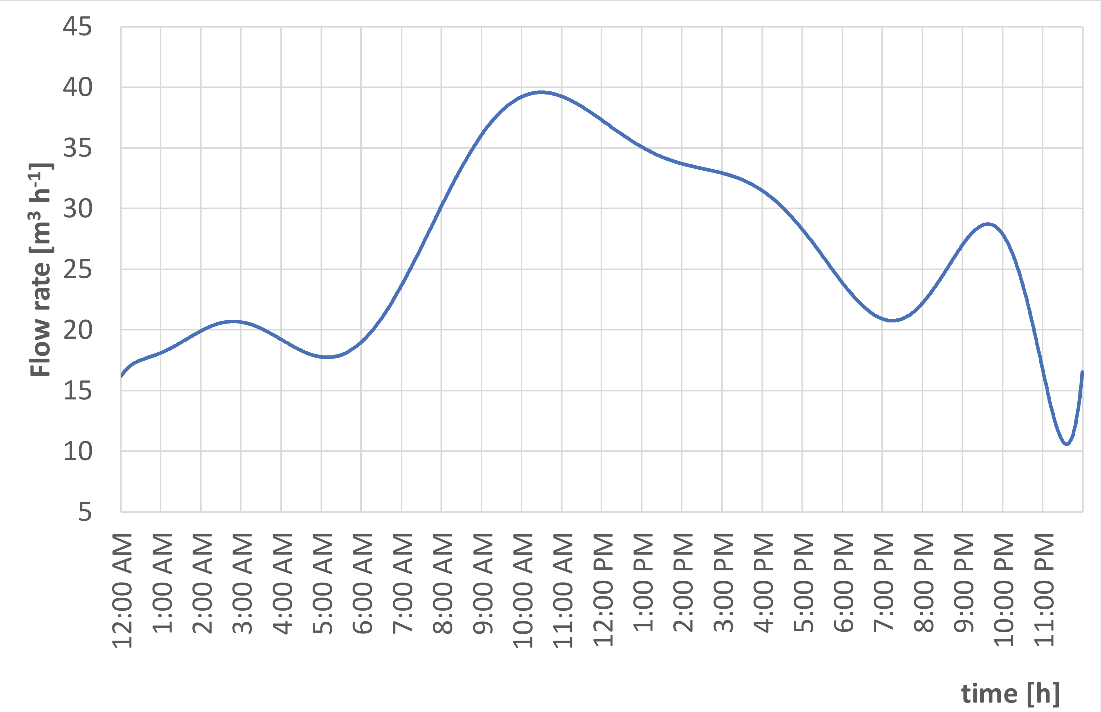


Figure 2.2. Flow rate *versus* time for the case study considered.

These theoretical curves were then used to calculate the occurrence (in terms of daily average concentration) of the three representative substances evaluated with the different sampling methods.

Finally, the resulting values of concentration were compared with the real value (theoretical) and between each other, in order to discuss what of the sampling modes could present the lower level of uncertainty for a specific group of compounds.

2.2. Insights on the sampling strategies commonly adopted in wastewater and surface water monitoring

Four sampling strategies are commonly adopted in collecting water samples.
The discussion herein reported considers average concentrations on a daily basis, but similar findings can be obtained in shorter or larger scales (*e.g.*, hourly, or monthly).
Here, consider that the *ideal* obtainable daily average concentration (ng L^{-1}) for each key compound can be evaluated with the following equation:

$$c_{ideal} = \frac{\sum_{i=1}^{1440} c_i \, Q_i}{\sum_{i=1}^{1440} Q_i} \qquad \text{(eq. 1)}$$

where c_i is the concentration (ng L^{-1}) at the minute i (in total 60x 24 min = 1440 minutes) and Q_i is the flow rate (L min^{-1}) at the same minute i.
This ideal concentration will be then compared with the results of the emulated sampling campaigns carried out with different strategies. Among them, the first one to mention, and the easiest one, is the **grab sampling**.
In this case, the daily average concentration of a compound (ng L^{-1}) is based on the number of water withdrawals n, which, in this thesis, was assumed to be 1,2,3, or 4 in a day:

$$\bar{c}_{grab} = \frac{\sum_{i=1}^{n} c_i}{n}, \; n = 1, 2, 3, 4 \qquad \text{(eq. 2)}$$

where c_i is the concentration of the compound (ng L^{-1}) in the i-th sample.
In grab sampling, the frequency of the withdrawals is defined by the monitoring protocol, for example randomly in a day, or at equally distanced time windows (*e.g.*, every 6 hours, if the objective is to collect 4 samples in a day). In this discussion, this second option was followed. An example of the withdrawal results (in terms of volume collection and sampling frequency) in grab sampling is provided in Figure 2.3.

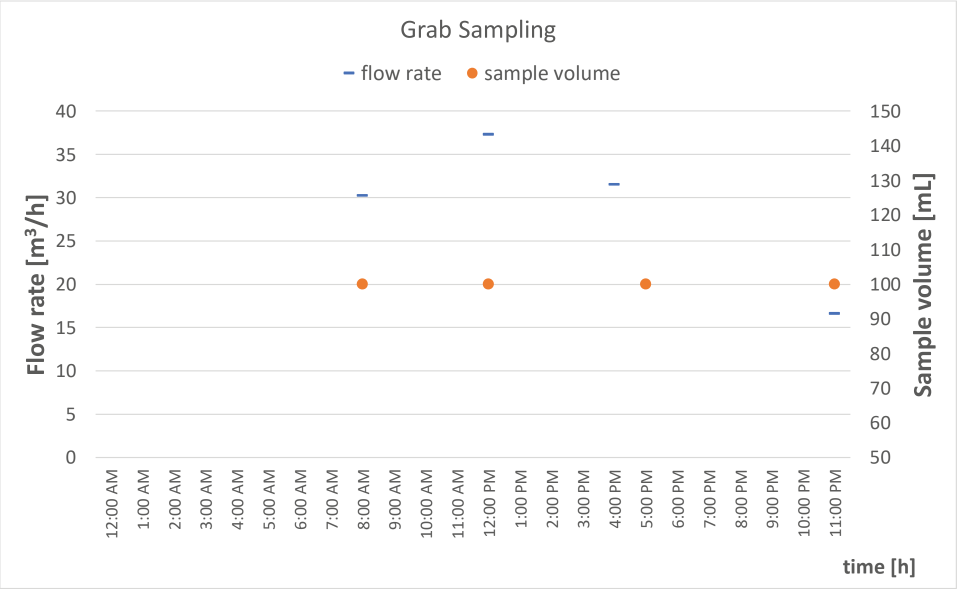


Figure 2.3. Flow rate profile (dashes) and withdrawn volume (full circles) for each grab sample. Note that the volume is always the same at the defined instants of time in case of four grab samples (i.e. 8:00 am; 12:00 pm; 5:00 pm and 11:00 pm).

In the case of composite sampling, three different procedures may be adopted to obtain a 24-h representative sample.

Firstly, suppose the daily average concentration is evaluated performing withdrawals of a defined volume (e.g., 1 L) at specific time intervals (e.g., every 1, 2, or 3 hours). In that case, the obtained result refers to a 24-h **time proportional composite sample**.

This method also called constant time-constant volume (CTCV) is the most commonly adopted sampling approach. The daily average concentration of a compound is calculated with the following equation:

$$\bar{c}_{time\ prop} = \frac{\sum_{i=1}^{k} c_i V_{sample}}{k\, V_{sample}} = V_{sample} \frac{\sum_{i=1}^{k} c_i}{k\, V_{sample}} = \frac{\sum_{i=1}^{k} c_i}{k}, \qquad k = 24, 12, 6, 3 \qquad \text{(eq. 3)}$$

where c_i is the concentration (ng L^{-1}) of the key compound in sample i, V_{sample} is the wastewater volume sampled (mL) at each withdrawal (always the same), and k is the number of samples taken according to the defined monitoring protocol. In this thesis example, it was assumed to collect samples every hour, every 2 hours, every 4 hours, or every 8 hours. Therefore, k was 24, 12, 6, or 3. An example of the withdrawal results (in terms of volume collection and sampling frequency) in time proportional composite sampling is provided in Figure 2.4.

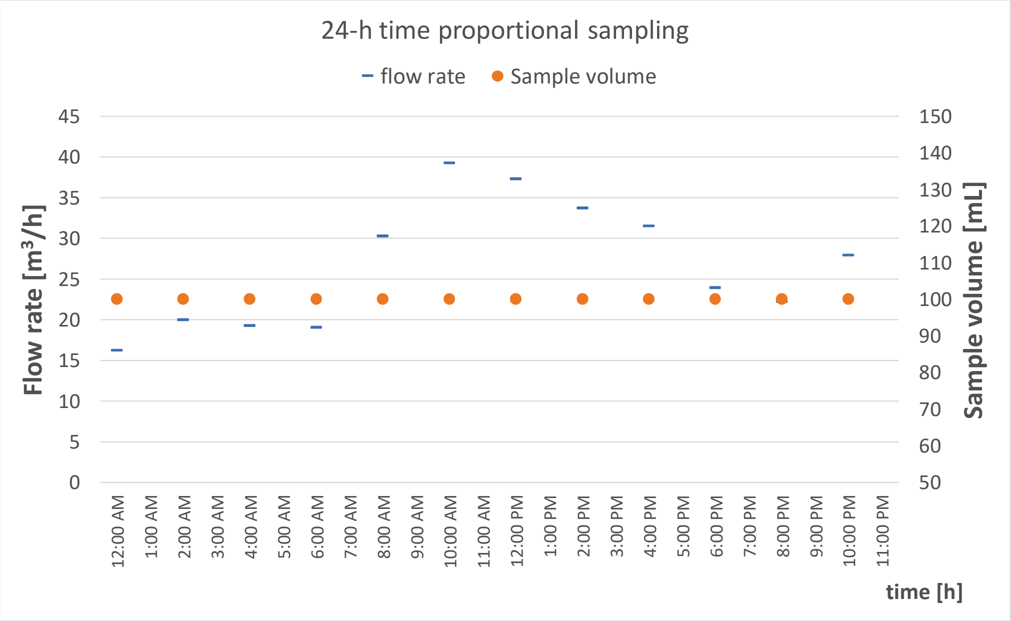


Figure 2.4. Flow rate profile (dashes) and the withdrawn volume (full circles) for the 12 water samples. Also, in this case, the sample volume is constant. Samples are taken every 2 hours.

A second commonly used composite sampling strategy consists of collecting a volume of water that is proportional to the flow rate flowing at the same time as the withdrawal. In this case, the result is considered representative of a 24-h **flow proportional composite sample**. The volume is calculated by means of a linear interpolation curve defined between the minimum and the maximum water flow observable during the day. This method is also called constant time-variable volume (CTVV).
The daily average concentration of the tested substance (ng L^{-1}) is evaluated according to the following equation:

$$\bar{c}_{flow\ prop} = \frac{\sum_{i=1}^{k} c_i\ \alpha\ Q_i}{\sum_{i=1}^{k} \alpha\ Q_i}\ ,\quad k = 24, 12, 6, 3 \qquad \text{(eq. 4)}$$

where c_i is the concentration of the key compound in sample i, in ng L^{-1}, αQ_i is the withdrawn wastewater volume (mL) being α the coefficient of direct proportionality (in this thesis example equal to 2) between the flow rate Q_i flowing at the sampling point at that instant and the volume to be sampled. The sampling protocol used in this chapter case study, as already mentioned before, set k equal to 24, 12, 6, or 3. An example of the withdrawal results (in terms of volume collection and sampling frequency) in flow proportional composite sampling is provided in Figure 2.5.

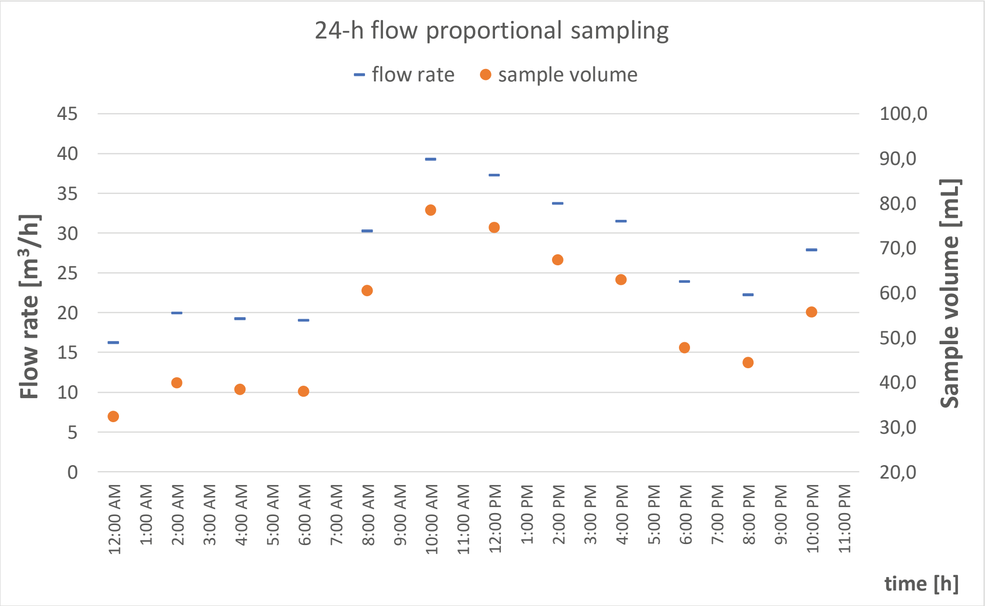


Figure 2.5. Flow rate profile (dashes) and the withdrawn volume (full circles) for the 12 water samples. The volume taken for the different samples is proportional to the flow rate at the sampling time. Samples are taken every 2 hours.

Finally, a third strategy may be adopted in water. In this last case, the withdrawal of a defined volume (*e.g.*, 1 L) only occur when a defined volume of water has passed the sampling point. This last volume can be assumed, for example, as the total daily volume passing the sampling point divided by the number of withdrawals one requires. The approach aims to collect a 24-h **volume proportional sample**, and thus is also called constant volume-variable time. Here, the average daily concentration is calculated with the following equation:

$$\bar{c}_{volume\ prop} = \frac{\sum_{i=1}^{k} c_i\, V_{sample}}{k\, V_{sample}} = V_{sample}\frac{\sum_{i=1}^{k} c_i}{k\, V_{sample}} = \frac{\sum_{i=1}^{k} c_i}{k}, \quad k = 24, 12, 6, 3 \qquad \text{(eq. 5)}$$

An example of the withdrawal results (in terms of volume collection and sampling frequency) in volume proportional composite sampling is provided in Figure 2.6.

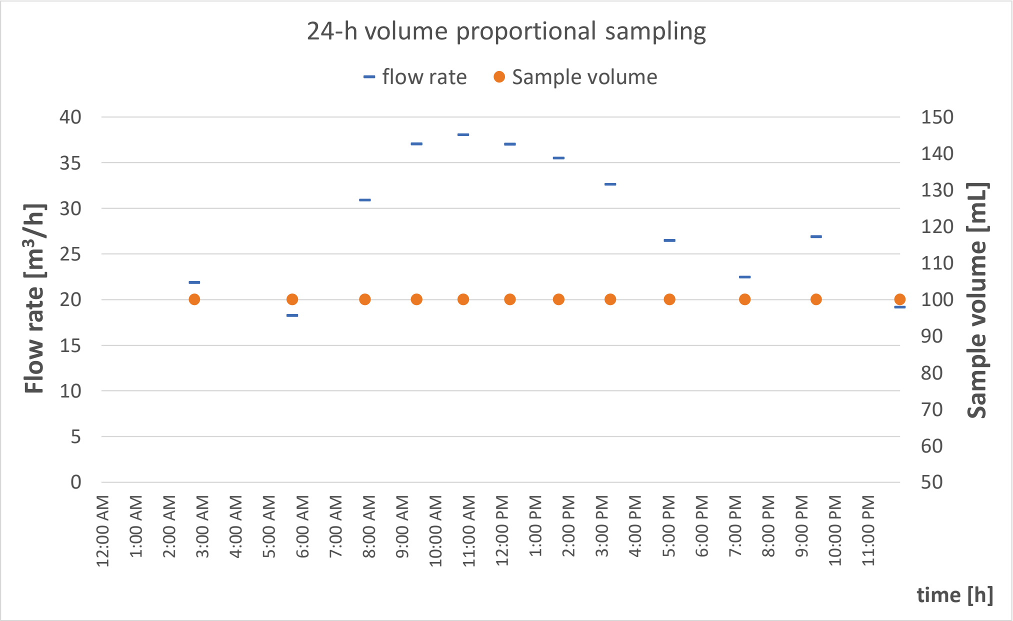


Figure 2.6. Flow rate (dashes) profile and the withdrawn volume (full circles) for the 12 water samples. The volume taken for the different samples is constant. Samples are taken when $\frac{1}{12}Q_{daily}$ is passed at the sampling point.

A brief resume of the main characteristics of the sampling strategies mentioned above, and the monitoring protocols considered for the specific example here carried out, is provided in Table 2.2.

Table 2.2. Description of the sampling modes adopted and compared in the example provided in this chapter, for the average concentration of the different compound.

Sampling	**Description**	**Water volume sampled**	**Sampling time (number of samples)**
Grab	The sampling consists of instantaneous (grab) wastewater withdrawal(s). The monitoring may include only one grab sample or a number of grab samples. The sampling time is defined by the investigation (monitoring protocol).	The requested wastewater volume for analysis	8 am, (1) 8 am+5 pm, (2) 8 am+12 pm+5 pm, (3) 8 am+12 pm+4 pm+11 pm (4)
24-h time proportional composite	The sampling is performed at constant time intervals. It is the most common sampling mode. It is also called Constant Time, Constant Volume (CTCV)	A constant volume V_{sample} took at each sampling instant	Every hour, (24) Every 2 hours (12) Every 4 hours (6) Every 8 hours (3)

Sampling	Description	Water volume sampled	Sampling time (number of samples)
24-h flow proportional composite	The sampling is performed at constant time intervals. The volume of wastewater taken is proportional to the flow rate flowing at each instant of the sampling. It is also called Constant Time, Variable Volume (CTVV)	A linear interpolation curve is defined between the minimum and maximum wastewater flow and wastewater sampled over the whole observed range of variability of the wastewater flow	Every hour (24) Every 2 hours (12) Every 4 hours (6) Every 8 hours (3)
24-h volume proportional composite	The sampling takes the same wastewater volume at variable time intervals after a defined volume of wastewater has passed the sampling point. It is also called Constant Volume, Variable Time (CVVT)	A constant volume V_{sample} is taken at each defined sampling time	Frequency: Three times a day (3) Six times a day (6) Twelve times a day (12) Twenty-four times a day (24)

In the context of sampling of micropollutants in surface water, the passive sampling devices (PSDs) should be mentioned. Passive sampling technique is based on the retaining of micropollutants in a receiving phase (e.g., solvent, solid sorbent) that receive the flow of the environmental medium (Godlewska et al., 2020).

In this context, due to the relatively low uptake of micropollutants in the receiving phase, PSDs generally need a long exposure time to work, allowing the measurement of the time-weighted average concentration of the target compounds and consecutively reflecting their long-term behaviour (Wang et al., 2020).

Among the most used and known PSDs, are worthy of mention the SPMD (Semi-Permeable Membrane Device), Chemcatcher, PISCES (Passive in Situ Concentration-Extraction Sampler), MESCO (Membrane Enclosed Sorptive Coating), and the polar organic chemical integrative samplers (POCIS). Here, SPMD, MESCO, and PISCES passive dosimeters are most often used for the collection of hydrophobic analytes, whereas, POCIS dosimeter is specifically designed to polar analytes monitoring (Godlewska et al., 2020).

Nowadays, PSDs showed to be a promising alternative to conventional water sampling for the monitoring of pharmaceuticals and other micropollutants, but on the other hand, due to their uptake principle, they may be susceptible to hydrodynamic conditions, temperature, and water quality parameters (e.g., pH, dissolved organic matter, ion strength). For this reason, these approaches require a proper in situ calibration to reduce the uncertainty related to the estimation of the aqueous concentrations of the micropollutants under study (Vrana et al., 2021).

However, an in-depth description of PSDs is out of the scope of this thesis, and for further information, the reader is referred to Gallé et al., 2019; Godlewska et al., 2020; Vrana et al., 2021; and Wang et al., 2020.

2.3. Influence of sampling mode and frequency on the evaluation of the load of micropollutants in water flows

The ideal concentration for the three key substances evaluated with Equation 1 was 24,561 ng L^{-1} for HV_Sub, 586 ng L^{-1} for LV_Sub, and 29,609 ng L^{-1} for RV_Sub.

These were taken as the theoretical values to which compare the results of the simulated sampling campaigns.

In particular, daily average concentrations evaluated with the grab strategy are reported in Table 2.3. It emerged here that for all the three key compounds, the average concentrations presented the widest range of variability, among all the sampling approaches adopted.

Also, the grab sampling may lead to a significant underestimation or overestimation, especially for those compounds which the concentration *versus* time profile is represented by the HV_Sub and RV_Sub curves.

Table 2.3. Average concentrations (in ng L^{-1}) of the three substances in case of grab sample (with the different number of samples collected).

Number (#) of grab samples	HV_Sub	LV_Sub	RV_Sub
1	1000	112	31,852
2	19,041	287	31,954
3	26,014	478	31,301
4	26,117	724	30,263

It should be noted that the grab sampling strategy was the only one which does not ensure a lower deviation (as the difference between measured and ideal concentration) when the number of sampling rises. In this context, the three target diagrams in Figure 2.7 provide the percentage deviation ($= \frac{c - c_{ideal}}{c_{ideal}} \times 100$) between the ideal daily average concentration and the measured daily average concentrations obtained with the four different sampling approaches.

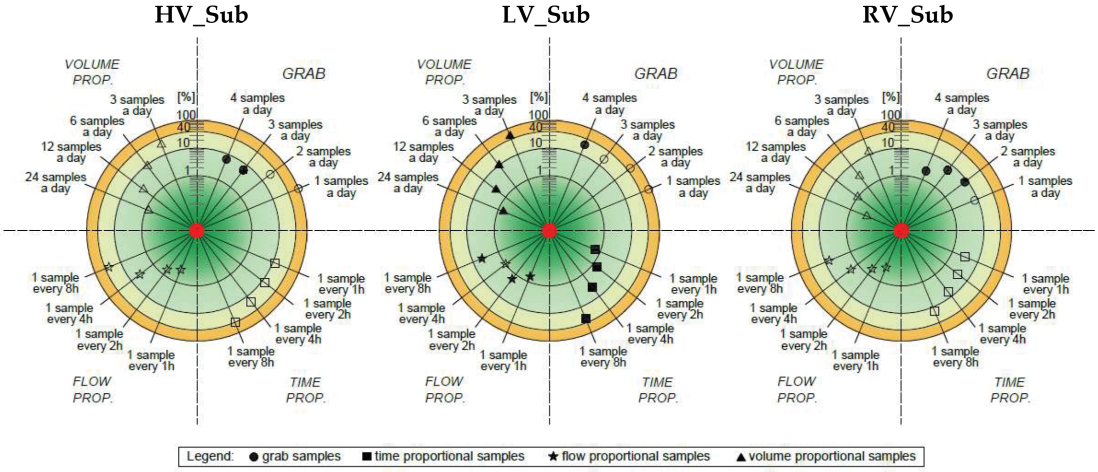


Figure 2.7. Percentage deviations between the ideal concentration of each substance (red dot) and the measured average concentrations found following the different sampling modes, defined in Table 2.1. Circumferences in the three graphs refer to different values of percentage deviation on a log scale. Full symbols correspond to an overestimation and empty symbols to an underestimation.

As for 24-h time proportional sampling, the daily average concentrations resulted for the three key substances are reported in Table 2.4. Here, it is possible to note that, overall, the discrepancy between these concentrations and c_{ideal} are lower than those provided with grab sampling. The highest deviation found in case of time proportional composite samples was found for HV_Sub when collecting 1 sample every 8 hours, equal to -41%.
For the other sampling frequencies and substances, the deviation was always lower than 35%.

Table 2.4. Average concentrations (in ng L^{-1}) of the three substances in case of time proportional sampling (with the different number of samples collected).

Interval [h], (#of samples)	HV_Sub	LV_Sub	RV_Sub
1 (24)	21,751	590	28,664
2 (12)	21,518	595	28,472
4 (6)	20,270	608	27,799
8 (3)	14,535	750	25,409

The best results in terms of the difference between measured and ideal concentrations were always found in the case of flow proportional composites (Table 2.5). This sampling strategy provided results which show the smallest range of variability, among all the sampling modes, and for all the three key substances.
As represented in Figure 2.7, the concentrations monitored with flow proportional sampling were always (with the only exception of HV_Sub when the withdrawal occurred once every 8 hours) within the 40% of deviation from the ideal. Furthermore,

in the best-case scenarios – corresponding to 1 sample every hour – the discrepancies were almost negligible for all the three compounds (<1% deviation).

Table 2.5. Average concentrations (in ng L^{-1}) of the three substances in case of flow proportional sampling (with the different number of samples collected).

Interval [h], (#of samples)	HV_Sub	LV_Sub	RV_Sub
1 (24)	24,477	590	29,525
2 (12)	24,412	596	29,443
4 (6)	23,550	581	29,000
8 (3)	17,406	612	27,543

Good results were also found for 24-h volume proportional composite sampling.
In this case, the best single result was found concerning RV_Sub sampled every hour (-0.2% discrepancy from the ideal concentration). Despite this, the range of variability of the measured volumes was higher than those found with 24-h flow proportional composite sampling and similar to those found in 24-h time proportional composite samples (Table 2.6).

Table 2.6. Average concentrations (in ng L^{-1}) of the three substances in case of volume proportional sampling.

Frequency (#/d)	HV_Sub	LV_Sub	RV_Sub
3	18,848	888	25,948
6	22,359	644	28,702
12	23,867	602	29,314
24	24,365	590	29,541

Concluding, these results are case-specific and may differ for other substance-curves and other water flows. Nevertheless, it emerged that the daily average concentration evaluated with the selected sampling strategies lead to an uncertainty varying in the range between <1% and 30% for 24-h flow proportional composite sampling, between <1% and 40% for 24-h time proportional composite sampling, between z1% and 51% for 24-h volume proportional composite sampling, and even up to 95% in case of one grab sample in a day.
These values confirm the findings of the previous studies, for which flow proportional sampling entailed a deviation of around 10% (Ort et al., 2010a), and time proportional also showed higher discrepancies, from 25% to 100% (Verlicchi et al., 2014).
These considerations remark the importance to define a sampling mode well to provide data of occurrence and of removal of micropollutants from wastewater with a high level of reliability.

Main conclusions

- *The most adopted approaches for water flow sampling are grab sampling, time-proportional composite sampling, flow-proportional composite sampling, and volume-proportional composite sampling.*

- *The sampling strategy that led to the lower discrepancy from the ideal concentration was flow-proportional composite sampling, followed by time-proportional and volume proportional. Grab sampling showed the higher uncertainty related to the campaign results.*

- *Grab sampling strategy may be adopted anyway, without resulting in significant measurement errors, in case of substances which pattern of concentration versus time tends to be constant (e.g., the anti-inflammatory ketoprofen and the antiseptic triclosan). On the contrary, it may entail to an under- or over-estimation of about 100%.*

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Chapter 3

OCCURRENCE OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN RAW AND TREATED SEWAGE SLUDGE

Table of contents

Aims of the chapter, in a nutshell

- *The different types of raw and treated sludge produced by a wastewater treatment plant were described.*
- *The occurrence of pharmaceutical and personal care products (PPCPs) in different raw and treated sewage sludge has been reviewed.*
- *The legal requirements for sludge reuse as amendment were presented, with regard to some countries of Europe, USA, Australia, and Canada.*
- *Further details about the occurrence of PPCPs in untreated and treated sludge, and the environmental risk in the case of application on soil, can be retrieved in Verlicchi and Zambello, 2015, to which this chapter refers.*

3.1. Introduction

Based on Eurostat statistics, more than 10 million tons (in dry matter) of sewage sludge are produced in one year by WWTPs within EU-28 countries (Eurostat, 2020).
This number is even expected to increase due to requirements regarding the effluent quality of WWTPs, which are becoming more stringent in recent years (Collivignarelli et al., 2019b). Consequently, the institutions and technicians are forced to take action for proper sewage sludge management.
To this end, and to face the growing attention on the issue by citizens who perceive sewage sludge as dangerous for the environment (Collivignarelli et al., 2019a), the European Commission provided the Directive 2018/851/EC (EC, 2018a).
The directive introduces a waste hierarchy that aims to promote legislative actions on waste prevention and management, encouraging to reduce the sewage sludge production, energy recovery (*e.g.*, from sludge treatment), and treated sludge reuse.
Nowadays, the main disposal routes include incineration, landfilling, and application to arable land as amendant or fertilizer, with percentages varying from country to country (Malmborg and Magnér, 2015). And, in the context of Directive 2018/851/EC, this last sludge reuse method is expected to gain special attention.
Here, if on one hand sludge soil-amendment may improve nutrient content, water holding capacity, and overall physicochemical characteristics of the soil (Clarke and Smith, 2011), on the other, it may expose the soil and water environment to the potential risk due to contaminant of emerging concern sequestered in the sludge.
For example, it has been recognized that the accumulation of pharmaceutical and personal care products (PPCPs) in the soil matrix may represent a threat to the soil living organisms (Camotti Bastos et al., 2020), and furthermore, the occurrence of antibiotics may entail resistance in pathogens (Aryal et al., 2020).
These aspects encouraged the scientific community to investigate the occurrence of PPCPs in raw and treated sewage sludge in order to evaluate the potential environmental risk posed by this agricultural practice.

This chapter thus aims to briefly present the literature results on this subject, which represent an essential step regarding the core of this thesis: the evaluation of the behaviour of micropollutants, from their consumption to their release to the receiving water body, on a catchment scale.
Here, the discussion will follow the findings of Verlicchi and Zambello, 2015, which reviewed the occurrence of PPCPs in different types of raw and treated sludge, also providing a comparison between the main aspects (among them, sludge characteristics, wastewater treatment type, compound physicochemical properties, and so forth) influencing their concentration level.
The study reviewed 59 papers published between 2002 and 2015 regarding the occurrence of 169 compounds (152 pharmaceuticals and 17 personal care products).
The list of compounds included together with their characteristics – which will be frequently involved in discussions of this thesis – is reported in Appendix 1.

Regarding the terminology adopted, the term sewage sludge will be from now on use to identify a mixture of WWTP residues exhibiting a solid fraction varying between 0.25 and 12% by weight, depending on the processes involved in a WWTP (Metcalfe and Eddy, 2014).

The term *biosolid* – which was introduced by the US Water Environment Federation in 1991 indicating the sewage sludge which has been undergone treatment and fulfils the legal requirements (in terms of nutrients and pathogens concentration) for reuse in the land amendment – is also used as a synonym of treated sewage sludge.

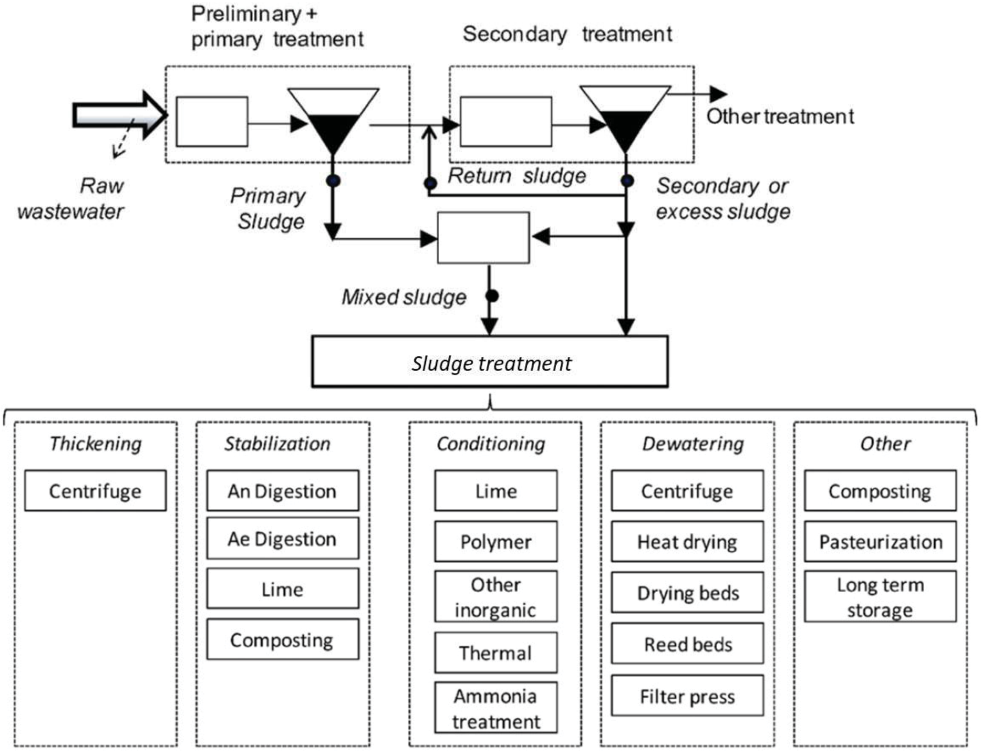

Figure 3.1. Schematic representation of the types of raw and treated sludge that can be produced in a treatment plant. Figure retrieved in Verlicchi and Zambello, 2015.

The following section will resume and compare the occurrence of PPCPs in raw and treated sludge, considering the sludge types outlined in Figure 3.1 and briefly described below.

Anyway, the discussion here provided only summarizes some of the results of Verlicchi and Zambello, 2015. For further details, which were not included here to focus on the aim of this thesis strictly, the reader is referred to the cited study.

3.2. Micropollutants occurrence in different types of raw and treated sewage sludge

3.2.1. Micropollutants occurrence in raw sewage sludge

Among the raw sewage sludge types schematically represented in Figure 3.1, the first one which can be collected is the primary sludge. This raw sludge derives from the primary clarifier of a WWTP and contains about 2-8% of total dry solids (Metcalfe and Eddy, 2014). It generally has a larger particle size compared to the secondary sludge, and it may also be chemically enhanced (for example, with $FeCl_3$ addition).

A consistent concentration of fragrances was found in primary sludge by Ternes et al., 2004, which measured a maximum concentration of galaxolide and tonalite of 187 µg g^{-1} DM and 183 µg g^{-1} DM, respectively. High concentration fragrances were also found by McAvoy et al., 2002 and Khan and Ongerth, 2002, which measured triclosan and salicylic acid occurring at 14.7 µg g^{-1} DM and 13.8 µg g^{-1} DM, respectively.

Overall, the most investigated compound in primary sludge were antibiotics, analgesics and anti-inflammatories, antifungals, hormones, and psychiatric drugs. Most of the concentration data were available for the antibiotics ciprofloxacin and norfloxacin, the anti-inflammatory ibuprofen, and the hormones estradiol and ethinylestradiol, showing a range of variability of 2 order of magnitude (Verlicchi and Zambello, 2015).

More various are the findings regarding secondary sludge, which derives from the secondary biological treatment, such as conventional activated sludge (CAS) or membrane biological reactor (MBR). In this case, probably due to the different possible biological reactor configurations, the ranges of concentration observed were about 3-4 orders of magnitude for many compounds (Verlicchi and Zambello, 2015). Among these worth mentioning are the antibiotics azithromycin, ofloxacin, and sulfamethoxazole, and the fragrance tonalide, which were found to occur at high concentration (64 µg g^{-1} DM, 21 µg g^{-1} DM, 68 µg g^{-1} DM, and 10 µg g^{-1} DM, respectively) by Göbel et al., 2005. Relevant concentrations were also reported in Göbel et al., 2005 for clarithromycin (67 µg g^{-1} DM) and trimethoprim (41 µg g^{-1} DM), in Heidler and Halden, 2009 for triclosan and triclocarban, and in Tavazzi et al., 2013 for Galaxolide (131 µg g^{-1} DM).

Overall, most of the data collected in Verlicchi and Zambello, 2015 refers to antibiotics (135 data regarding 29 compounds), analgesics and anti-inflammatories (36 data regarding 7 compounds), and hormones (49 data regarding 4 compounds).

It is here interesting to mention that a seasonal variation of PPCPs concentration was observed. For example, Gao et al., 2012 and Martín et al., 2012, measured a consistent variability of antibiotic occurrence in secondary sludge, with the highest peaks in winter and lowest peaks in autumn. This is probably due to the higher human consumption of these PhACs, and mutatis mutandis the higher concentration of the compounds and their metabolites in the sewer network. This suggests that human consumption is the main source of PhACs in a WWTP influent.

PhACs concentration data in mixed sludge (as the sum of primary and secondary sludge) are provided by Jones et al., 2014 which monitored the occurrence of 7 compounds in sludge samples collected in different WWTPs in the UK. The authors reported high

concentrations regarding the antibiotic oxytetracycline (7.6 µg g^{-1} DM), the antiseptic triclosan (4.9 µg g^{-1} DM), and the non-ionic surfactant nonylphenol tri ethoxylate (176 µg g^{-1} DM), nonylphenol mono ethoxylate (5 µg g^{-1} DM), and nonylphenol di ethoxylate (1 µg g^{-1} DM).
Relevant occurrence data were also found for diclofenac, ibuprofen, propranolol, erythromycin, ofloxacin, and fluoxetine, with maximum concentrations ranging between 60 and 270 ng g^{-1} DM.

A comparison between the studies' findings revealed that differences between PPCPs concentration among the different sludge types might depend on various factors:
Firstly, as mentioned in Chapter 1, the sorption of micropollutants onto sewage sludge can be ascribed to two mechanisms: adsorption and absorption, related to the lipophilicity and the electrostatic interaction of a compound, respectively. Thus, if a chemical exhibits low lipophilicity ($LogK_{OW} < 2$), it may anyway occur at a high concentration in the sludge that better facilitate electrostatic bindings. For instance, some authors (among them Martín et al., 2015 and Stasinakis et al., 2013) measured higher concentrations of diclofenac, ibuprofen, caffeine, and nonylphenol, in primary sludge instead of secondary sludge and attributed this difference to the protonation at lower pH values of the two environments. In fact, the pH of primary and secondary sludge is different (e.g., around 6.5 and 7.5, respectively, Verlicchi and Zambello, 2015), and thus the dissociation of a compound (as mentioned in Chapter 1 regarding pK_a) may drive its behaviour in the reactor.
Secondly, the sorption of a chemical onto sewage sludge may depend on the biological reactor type. In this regard, it is well-known that the typical higher biomass concentration in MBRs rather than CAS may result in enhanced biodegradation of PPCPs, and thus influencing the compound behaviour in sewage sludge (Fernandez-Fontaina et al., 2013). Despite this, Jones et al., 2014 did not observe significant differences, in terms of PPCPs concentration in sewage sludge, among various secondary treatments (CAS, MBR, biological nutrient reactors (BNR), and biological filtration). Thus, the authors' findings suggest that the type of reactor influences only secondarily the behaviour of a chemical.
Instead of reactor type, the reactor characteristics may play an important role in PPCPs sorption onto sludge. In this context, Li, 2014 showed that a longer SRT entails higher sorption of quinolones (among them ciprofloxacin and norfloxacin). Differently, Muller et al., 2010 found that a longer SRT promotes an enhanced degradation of different hormones, and, consecutively, a lower occurrence in sewage sludge.
Overall, the results mentioned so far highlighted that the behaviour of micropollutants in WWTP and their sorption onto sewage sludge is strongly compound specific. The physicochemical properties of a substance define if it may be more subjected to sorption, transformation, or biodegradation, thus showing to be more influenced by environment pH, sludge type, reactor type, SRT, and so forth.

3.2.2. Micropollutants occurrence in treated sewage sludge

One of the most common treatments for sewage sludge consists of keeping the sludge in particular tanks (digester) in which sludge and environmental characteristics are controlled (Metcalfe and Eddy, 2014). Digestion aims to reduce sludge organic content, pathogens, and odours, obtaining the so-called sludge stabilization. To do this, in a digester, different parameters can be varied (such as sludge pH, sludge solid content, residence time), and different conditions can be achieved. For instance, sludge can be stabilized in aerobic or anaerobic conditions, and in mesophilic (at around 35°C) or thermophilic (at around 55°C) conditions, promoting the growth of different bacteria populations, which will drive the stabilization process. The waste resulting from this process is from now on referred to as digested sludge.

Regarding PPCPs occurrence in digested sludge, the most investigated compounds belong to psychiatric drugs and antibiotics. In this context, many data are reported in the literature about carbamazepine (41 values), ibuprofen (27 values), estradiol (26 values), diclofenac (22 values), estrone (21 values), and ciprofloxacin (20 values), showing ranges of concentration variability of 2-3 magnitude order (Verlicchi and Zambello, 2015).

High concentrations of micropollutants in digested sludge were reported by Stevens et al., 2003 for galaxolide (81 μg g^{-1} DM) and traesolide (16 μg g^{-1} DM), by Heidler and Halden, 2009 for triclocarban (63 μg g^{-1} DM) and triclosan (46 μg g^{-1} DM), and by Malmborg and Magnér, 2015 for estrone (22.5 μg g^{-1} DM).

Seasonal variation for some antibiotics and anti-inflammatories concentration was also noted in the case of digested sludge by Nieto et al., 2010, confirming that the human consumption of PhACs greatly influences their occurrence in the sewer system and thus in the WWTP.

Other than aerobic digestion, sludge stabilization in aerobic conditions can also be achieved through composting and lagooning. In the first case, sewage sludge is piled in specific heaps that are periodically turned and watered to ensure good aeration and particular water content. Within a heap, many different microorganisms can proliferate and coexist due to the various environmental conditions – mesophilic and thermophilic phases in different moments and in different layers of the heap – which take place. Although under ideal conditions the composting process proceeds through specific stages and steps, in practice, the heap is subjected to several variables, especially when maintained in open-air conditions.

Regarding lagooning, sludge stabilization is carried out by the accumulation of liquid sludge in specific ponds for up to 20 years (Hamilton et al., 2006). In a lagoon, the sludge settles from the surface layer (which can be assumed in aerobic conditions) to the bottom (in anaerobic conditions). The water phase of a lagoon (also called lagoon effluent) is often reused for agricultural purposes, such as crop irrigation, and has a residence time of 3-6 months (Bodman, 1996). An in-depth description of sludge treatments is out of the scope of this thesis. Anyway, for further information, the reader is referred to Metcalfe and Eddy, 2014.

The studies concerning PPCPs occurrence in compost revealed that the composting practice generally entails a consistent degradation of many compounds. This may be due to the high microbial diversity, abundant substrates, and various pH and redox conditions, which facilitate the transformation of a broad spectrum of chemicals with different characteristics (Xia et al., 2005). Despite this, some PPCPs showed recalcitrant behaviour in composting. This is the case of the personal care products triclosan, galaxolide, and tonalide, which were measured at a concentration up to 4-5 µg g^{-1} DM by Kinney et al., 2006, Peysson and Vulliet, 2013 and Tavazzi et al., 2013, and ibuprofen, found at 1 µg g^{-1} DM by Martín et al., 2012.

Regarding lagoon sludge, Martín et al., 2015 monitored the occurrence of 42 PhACs in a wastewater stabilization pond, observing wide ranges of concentration for many compounds.

Among the PhACs found with the highest concentration higher than 100 ng g^{-1} DM, the authors reported acetaminophen, salicylic acid, ciprofloxacin, gemfibrozil, and caffeine.

Overall, sludge stabilization showed to be effective in reducing the concentration of many studied compounds. This is the case of ibuprofen, salicylic acid, caffeine, and gemfibrozil, for example, which concentrations consistently decreased during both digestion and composting, as reported by Martín et al., 2012. In this regard, even more comprehensive are the results of Martín et al., 2015. Here, the authors studied the occurrence of 42 PhACs belonging to eight pharmaceutical classes in different sludge (namely, primary, secondary, mixed, anaerobically digested, aerobically digested, composted, and settled in a lagoon), highlighting that PhACs concentration was reduced from untreated to treated sludge. In both studies, the authors speculated that the concentration of PPCP was attenuated since during the treatment the chemicals tend to desorb, becoming exposed to biodegradation reactions.

Different results were found for hormones by Andersen et al., 2005, which measured an incremented estradiol concentration from untreated to treated sludge (via anaerobic digestion). As suggested by Khan and Ongerth, 2002, this may be due to the cleavage of conjugated steroid estrogens during treatment. In this regard, estrone is reduced in estradiol during anaerobic digestion, and the mesophilic conditions of the digester require high sludge retention times to significantly reduce bot estrone and estradiol (Paterakis et al., 2012).

Conflicting results were found for the antiseptics triclosan and triclocarban, for which some authors reported a sufficient removal during digestion (among them McAvoy et al., 2002), and others even found an accumulation (and thus a rising in concentration) of the compound in the sludge maintained in the digestor (Heidler and Halden, 2009).

In any case, also for treated sludge, the occurrence of PPCPs strongly depends on the specific physicochemical characteristics of a compound, which drives their behaviour during the treatment.

3.3. Legal requirements for sewage sludge application to soil

As briefly mentioned in the introduction of this chapter, the European directives have aimed to improve the protection of the aquatic environment by promoting the upgrade of the existing WWTPs (Directive 91/271/EC - (EC, 1991), and Directive 2000/60/EC - (EC, 2000)), and encouraging proper management of wastes (Directive 2018/851/EC (EC, 2018a)).

In terms of sewage sludge application on arable land, the European Community – through Directive 86/278/EEC – has introduced limits regarding the content of heavy metals that can be added onto the soil with sludge (EC, 1986). These regulations allow the member states to choose to pose maximum concentration of metals in sludge or the receiving soil.

Nowadays, each European country has applied the provisions of Directive 86/278/EEC, implementing them, in some cases, defining cap values also for nutrients and pathogens (Collivignarelli et al., 2019a). This is the case of France, Italy, Austria, and Bulgaria, to name just a few.

In this context, it is important to mention that the current legislation present different restriction also regarding tilling procedures, tilling periods within the year (*e.g.*, summer months), maximum slope values, distances from the waterways (such as irrigation channels, or groundwater), and soil characteristics (pH, CEC, and so forth).

In this regard, Table 3.1 and Table 3.2 briefly report a description of the legal requirements and limits set on the pathogen content of biosolid for some representative countries (among them, some EU member states, USA, Australia, and Canada).

Table 3.1. Comparison of the main issues addressed in regulations in different countries.

Country	HM	Pathogens	N/P	Organic micropol.	Other restrictions			
					Max rate of application	Slope limits	soil pH limits	Others
EU	•							
Ireland	•		•		In function of nutrients (N and P) and metals applicated to soil or 2 tons/ha year			Untreated sludge may be used in agriculture provided that it is previously injected into the land;
Italy	•	•	•		15 t/ha (3 years)	<15%	>5,0	Cation exchange capacity of the soil have to be greater than 8 meq/100 gr; Solid content of the sludge has to be greater than 20%;

Country	**HM**	**Pathogens**	**N/P**	**Organic micropol.**	**Other restrictions**			
					Max rate of application	**Slope limits**	**soil pH limits**	**Others**
New South Wales (Australia), Restricted use 1	•	•	•	•	In function of nutrients (N and P) and metals applicated to soil	<10%	>5,5	minimum distances to the aquifer, surface waters, and other sensitive areas; sludge should be incorporated into the soil within 36 h of spreading;
New South Wales (Australia), Restricted use 2	•		•	•	In function of nutrients (N and P) and metals applicated to soil	<10%	>5,5	minimum distances to the aquifer, surface waters, and other sensitive areas; sludge should be incorporated into the soil within 36 h of spreading;
Ontario (Canada)	•	•			function of contaminants concentration	<12%		minimum distances to surface water of 20m; sludge should be incorporated into the soil within 24 h of spreading; max content of plastic in sludge (0,5% of dry weight)
USA class A	•	•			In function of nitrogen and metals applicated to soil			minimum distances to surface water of 10m;
USA class B	•	•			In function of nitrogen and metals applicated to soil			minimum distances to surface water of 10m;

Policy details, **for EU**: (EC, 1986); **for Ireland**: (MSDEHLG, 2010; MSDELG, 2001, 1998); **for Italy**: (DL, 1992); **for New South Wales (Australia)**: (Ang and Sparkes, 1997); **for Ontario (Canada)**: (GO, 2002); **for USA**: (Lu et al., 2012); **for other EU nations**: (Mininni et al., 2015).

Table 3.2. Limits set in different countries for microorganisms occurrence in sludge to be applied onto the soil.

	Cryptosporidium	Enteric Bacteria	Enteric Virus	E. coli	Faecal Coliforms	Faecal Streptococcus	Giardia	Helminth eggs	Salmonella
Denmark	-	-	-	-	-	<100/g DM	-	-	ND
EU	-	-	-	-	-	-	-	-	-
Finland	ND	-	-	1,000 CFU/ g DM	-	-	-	-	-
France	-	-	3 MPN/10 g DM	-	-	-	-	3/ 10 g DM	8 MPN/10g DM
Ireland	-	-	-	-	-	-	-	-	-
Italy	-	-	-	-	-	-	-	-	1,000 MPN/g DM
Luxemburg	-	100/g	-	-	-	-	-	-	
New South Wales (Australia) RU1	-	-	-	100 MPN/g DM	1,000 MPN/g DM	-	-	-	ND
New South Wales (Australia) RU2	-	-	-	-	-	-	-	-	-
Ontario (Canada)	ND	-	-	1,000 CFU/g DM	-	-	ND	-	3 MPN/ 4g DM
Poland	-		-	-	-	-	-	-	ND
USA Class A	-	-	1 PFU/ 4 g DM	-	-	-	-	1/4 g DM	3 MPN / g DM
USA Class B	-	-	-	-	2,000,000 CFU/g DM	-	-	-	-

Policy details, **for EU**: (EC, 1986); **for Ireland**: (MSDEHLG, 2010; MSDELG, 2001, 1998); **for Italy**: (DL, 1992); **for New South Wales (Australia)**: (Ang and Sparkes, 1997); **for Ontario (Canada)**: (GO, 2002); **for USA**: (Lu et al., 2012); **for other EU nations**: (Mininni et al., 2015).

These tables are far from being exhaustive, as they were prepared as a complement for a specific review work, which will be better discussed later in Chapter 5 (Ghirardini and Verlicchi, 2019).
Anyway, it is possible to note that limits regard heavy metals, pathogens, nutrients, and organic compounds (such as adsorbable organic halides AOX, polychlorinated biphenyl PCB, and dioxins and furans PCDD/F). There is no mention regarding the contaminants of emerging concern, such as pharmaceuticals, hormones, and fragrances.

That said, the debate is still open. In this regard, the growing attention on the EU Watch list (EC, 2018b) and the introduction of Directive 2018/851/EC (EC, 2018a) are expected to entail a *minimization* of the quantity of sludge produced and to encourage adequate sludge *reuse* (Collivignarelli et al., 2019a). The latter may results, in addition to the above-mentioned benefits, also in facing the phosphorus needing of agricultural soils (Grames et al., 2019).

Main conclusions

- *Pharmaceutical and personal care products may be found in raw and treated sewage sludge at ng g^{-1} / μg g^{-1} concentration levels.*

- *The treatment (e.g., aerobic or anaerobic digestion, composting, etc.) may consistently contribute to the degradation of many studied micropollutants sequestered in sewage sludge.*

- *The in-force regulations do not set any limit on the occurrence of PPCPs in sewage sludge before its application onto arable land.*

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Chapter 4

OCCURRENCE OF PHARMACEUTICALS AND HORMONES IN ANIMAL MANURE

Table of contents

Aim of the chapter, in a nutshell

- *Different types of raw and treated zootechnical wastes from various animals (cattle, swine, and poultry) were characterized.*

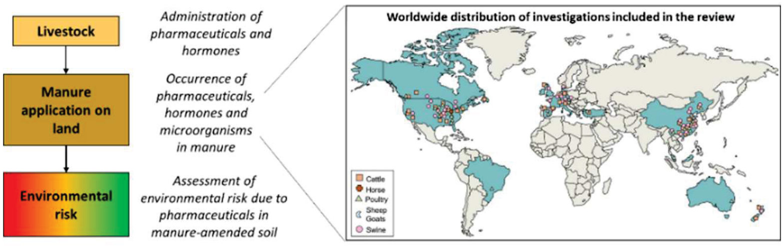
- *Concentration of pharmaceuticals and hormones in different types of raw and treated zootechnical wastes from various animals (cattle, swine, poultry) has been reviewed.*

- *Concentration of microorganisms in different types of raw and treated zootechnical wastes from various animals (cattle, swine, poultry) has been reviewed.*

- *The legal requirements for manure reuse as amendment were briefly presented, with regard to Europe countries.*

- *The results of this chapter were published in 2020 in Science of the Total Environment, 707, 136118 with the title: "A review of the occurrence of selected micropollutants and microorganisms in different raw and treated manure – Environmental risk due to antibiotics after application to soil" (Ghirardini et al., 2020).*

Graphical Abstract



4.1. Introduction

With an estimated population of around 1.4 billion cattle, 1.9 billion sheep and goats, 1 billion pigs, and 19.6 billion chickens, livestock is one of the main economic activities all around the globe, ensuring the livelihoods and food security of almost a billion people and affects the diet and health of many more (Robinson et al., 2014).

Against this background, and assuming that the 1000 heads manure production ranges between 17,400 and 26,100 kg/d for cattle, from 21,000 to 25,000 kg/d for swine, from 1000 to 1800 kg/d for sheep and goats, and from 45 to 60 kg/d for poultry (Sims and Maguire, 2004), it is possible to have an overall view on the issue related to zootechnical wastes management.

A valid option for reusing animal manure is represented by its application onto arable land as soil amendant and fertiliser due to its relevant nutrients content in terms of C, N, and K (Combalbert et al., 2012).

As an example, in Table 4.1 are reported the characteristics and nutrients concentration of the most common types of manure (among them cattle bedding manure, swine slurry, and poultry litter) collected from the existing scientific literature.

On one hand, the manure soils-amendment can play an important role in facing the agricultural needs, but on the other, this practice, that is commonly followed in many countries, may represent a potential route for micropollutants entering the environment (Bloem et al., 2017).

In this regard, in the last decades and increasing attention has been paid to the occurrence of contaminants of emerging concern in animal manure by means of dedicated sampling campaigns monitoring pharmaceuticals and hormones concentration in different types of zootechnical wastes, before, during, and after the treatment, as well as in manure amended soil (Bartelt-Hunt et al., 2012; Gros et al., 2019; Wallace et al., 2018).

These studies revealed that PPCPs frequently occur in manure also at relevant concentrations, and furthermore, some of that persist even after the treatment (typically composting, stirring, or anaerobic digestion) and once applied onto the soil (Spielmeyer, 2018).

This is not surprising, as many different classes of PhACs are commonly administered to farm animals for treatment and prevention of diseases, as well as for the control of the hormone activity and growth promotion (Boxall et al., 2004).

As reported in a recent European Commission report (Tavazzi et al., 2018), the main groups of veterinary medicines administered in the EU belong to antimicrobials, endectocides, antifungals, hormones, growth promoters, anaesthetics, tranquilisers, nonsteroidal antiinflammatories agents, and euthanasia products. In this context, antibiotics are the main animal consumed group of PhACs, as they are commonly administered not only for disease control (therapeutic use) but also for prevention and growth promotion (sub-therapeutic use), reaching the level of 11 million kg of antibiotics sold in the US every year for veterinary purposes (USFDA, 2018).

Thus, even if many countries have banned the use of antibiotics for growth promotion – for example, Sweden and Switzerland in 1986 and 1999 respectively (Haller et al., 2002) and the European Commission in January 2006 (EC, 2003) – many different types of antibiotics are commonly detected in zootechnical wastes (Bloem et al., 2017) suggesting that this issue needs to be addressed in order to properly assess to what extent the manure-soil amendment practice could represent a threat or a benefit.

Here, before going into the details of the occurrence of PhACs and hormones in animal manure, it could be useful to briefly define what is meant for zootechnical wastes and what type of data on micropollutants occurrence in them one can expect to find:

The term animal manure can be used for many different types of zootechnical wastes with various characteristics. Of course, it is impossible to strictly compile a list of manure types, but at the same time, a classification is needed to compare and draw conclusions.

Table 4.1. Definition of the different types of raw and treated manure considered in this chapter, their typical content of macronutrients, and the corresponding references.

Manure categories	**Manure types**	**Description** **[unit of measurement for micropollutant concentrations]**	**Dry matter [%]**	**C_{tot} [%]** **N_{tot} [%]** **P_{tot} [%]** **K_{tot} [%]**	**References**
		Raw manure			
Bedding manure	Cattle, horse, sheep, or pig bedding manure	Mixture of faeces, urine, bedding material (including straw, wood shavings, and sawdust) and other dry adsorbents, low-cost material. [ng g^{-1} DM]	20.9–69.9	11.8–12.9 0.4–2.2 0.2–4.0 0.9–4.0	Arikan et al., 2009; Derby et al., 2011; Hutchison et al., 2004; Patten et al., 1980
	Poultry litter	Mixture of faeces, urine, spilt feed, animal waste (feathers, blood, etc.), and bedding material. Generally deriving from indoor ground breeding of broiler chickens. [ng g^{-1} DM]	33.3–78.5	12.6–50.4 1.1–5.9 1.1–3.2 2.0–3.3	Arikan et al., 2016; Aznar et al., 2018; Dutta et al., 2010; Jenkins et al., 2006; Leal et al., 2012; Nichols et al., 1997
Solid manure	Cattle and horse solid manure	Manure with medium-high dry matter content that could be scraped from stalls (mostly faeces, but may contain urine), or solid fraction of slurry obtained with separation processes. [ng g^{-1} DM]	24.4–65.0	10.4–48.1 0.6–4.6 0.1–2.5 0.1–3.2	Amarakoon et al., 2014; Arikan et al., 2016; Aust et al., 2008; Karci and Balcioğlu, 2009; Ray et al., 2017; Wallace and Aga, 2016; Wallace et al., 2018
	Pig solid manure		28.0–29.0	35.3–41.0 1.3–2.7 1.5–3.2 0.7	Bao et al., 2009; Gros et al., 2019; Zhang et al., 2019
	Poultry manure	Mixture of faeces, urine, and, to a lesser extent, animal waste (feathers, blood, etc.). Bedding material is absent. Generally obtained from the shallow scrape of an alley in an egg production facility (e.g., from laying hens in battery cages). [micropollutants in ng g^{-1} DM]	33.0–79.4	24.9–46.2 1.7–7.1 0.7–6.7 1.9–5.0	Bao et al., 2009; Conde-Cid et al., 2018; Delgado et al., 2018; Dutta et al., 2010; Ho et al., 2014; Karci and Balcioğlu, 2009

Manure categories	Manure types	Description [unit of measurement for micropollutant concentrations]	Dry matter [%]	C_{tot} [%] N_{tot} [%] P_{tot} [%] K_{tot} [%]	References
Semiliquid manure	Cattle slurry	Faeces and urine (often accumulated from the slatted floor) collected in the slurry pit. [ng g^{-1} DM and ng L^{-1}]	0.5–8.3	17.5–36.5 0.2–2.8 0.04–0.1 0.4–0.5	Conde-Cid et al., 2018; Khan and Lee, 2012; Peyton et al., 2016; Wallace et al., 2018
	Pig slurry		0.3–8.3	16.3–41.4 0.1–3.4 0.01–3.1 0.1–2.5	Blackwell et al., 2009; Conde-Cid et al., 2018; Gros et al., 2019; Hutchison et al., 2004; Jacobsen and Halling-Sørensen, 2006; Joy et al., 2014; Kjær et al., 2007; Lamshöft et al., 2010
	Cattle and horse liquid (fraction) manure	Liquid fraction of manure obtained through percolation, centrifugation, or other separation practices. [micropollutants in ng g^{-1} DM and ng L^{-1}]	4.9	NA NA 0.05 0.2	Wallace and Aga, 2016; Wallace et al., 2018
	Pig liquid (fraction) manure		<1–1.6	NA 0.1 1.0 NA	Combalbert et al., 2012; Gros et al., 2019
Liquid manure	Cattle, horse, and pig urine	Liquid waste generated by any animal species. [ng L^{-1}]	NA	NA 0.1–1.7 NA NA	Hoogendoorn et al., 2010
	Cattle shed flushing material	Dirty water composed of faeces, urine, wash water from stalls, and, if collecting tank is outdoors, rainwater. [ng g^{-1} DM and ng L^{-1}]	<2	NA NA NA NA	Hutchison et al., 2004
	Pig house flushing material		<2	NA 0.6^{1} 0.1^{1} 0.4^{1}	Edwards and Daniel, 1994; Hutchison et al., 2004
	Poultry house flushing material		<2	NA NA NA NA	Hutchison et al., 2004
Treated manure					
Lagooning sludge		Sludge accumulated in 1–5 m deep open-air or covered ponds. Generally removed from 5–20 years and applied on the soil as amendment (Hamilton et al.,2006). [ng g^{-1} DM and ng L^{-1}]	3.2–25	NA 0.5 0.06 0.4	Frey et al., 2013; Kuchta and Cessna, 2009; Wallace et al., 2018
Lagoon effluent		Water collected from the upper part of lagoon receiving manure (water phase). Residence time generally varies from 2–6 months. Often used for irrigation purposes (Bodman 1996). [ng L^{-1}]		NA 0.04–0.15 0.03–0.14 0.02–0.04	Khan and Lee, 2012

Manure categories	Manure types	Description [unit of measurement for micropollutant concentrations]	Dry matter [%]	C_{tot} [%] N_{tot} [%] P_{tot} [%] K_{tot} [%]	References
Compost		Mixture of manure and organic material (e.g. hay, straw, or decomposed leaves) that results from aerobic composting process favoured by regular turning and controlling of moisture and temperature. [micropollutants in ng g^{-1} DM]	33.5–79.0	10.1–48.8 0.8–3.6 0.2–3.7 1.4–3.2	Aznar et al., 2018; Biswas et al., 2017; Cessna et al., 2011; Derby et al., 2011; Larney et al., 2003; Liu et al., 2015; Ray et al., 2017
Digested manure		Mixture of manure and organic material (e.g. hay, straw, or decomposed leaves) that results from anaerobic digestion process generally occurring at least at 40 °C for up to 6 months. [ng g^{-1} DM and ng L^{-1}]	4.3	NA 0.3 0.02 0.1	Wallace et al., 2018
Pellet manure		Extremely dense and low moisture content manure granules made by compression of dung at high temperature (at last 100 °C). [ng g^{-1} DM]	78–94	NA 2–4.5 1.6–1.8 NA	Dutta et al., 2010; Haggard et al., 2005; McMullen et al., 2005
Alum treated manure		Manure in which $Al_2(SO_4)_3$ is added to reduce water-extractable constituents between flocks. [ng g^{-1} DM]	75.0–78.5	NA 5.2 1.3 NA	Haggard et al., 2005; Nichols et al., 1997

[1] Estimated assuming a bulk density of 1000 kg m^{-3}.

To this end Table 4.1 was drawn up following a scientific literature review concerning the analysis of different animal manures. Here, it is possible to see that manure can be firstly classified based on its dry matter, and then on the producer animal.

As to raw manure, four categories has been distinguished:

- *Bedding manure*, in which faeces and bedding material (such as straw, wood shavings, sawdust and other dry adsorbents and low-cost material) are collected together. This zootechnical waste presents a high dry matter content (20-80% DM), and it is typical of dairy cattle and horses stalls, or poultry houses, especially from indoor ground breeding or broiler chickens. The concentration of micropollutants in bedding manure is typically reported in ng g^{-1} DM.
- *Solid manure*, which embraces different types of zootechnical wastes which are commonly linked by their high dry matter content (20-80% DM) but without bedding material. Here, three main types can be distinguished: firstly, is considered solid manure the mixture of faeces and urine (with a majority of faeces) that can be scraped from, as an example, cattle stalls, in which the animals are raised in intensive rearing.

  Secondly, it can be considered solid manure also the solid fraction obtained with separation processes (*e.g.,* percolation or centrifugation) of slurry (that will be defined later).

And finally, it can be considered solid manure that mixture of faeces, urine, and animal wastes (blood, nails, feathers, etc.) that is generally obtained from the shallow scrape of an alley in egg production facilities (*e.g.* from laying hens in battery cages).
Similarly to bedding manure, the micropollutants concentration contained in solid manure is typically expressed in ng g^{-1} DM.

- *Semiliquid manure*, which can refer to 2 distinguished wastes that present a low dry matter content (0.5-8% DM):
  The first one, generally referred as *slurry*, can be defined as a mixture of faeces and urine collected without other specific accumulation, or ageing, of material. It is often conveyed in specific tanks (slurry pits) after the accumulation from the so-called slattered floor of some stalls, and it can be spread onto the soil as liquid waste.
  The second meaning of semiliquid manure may concern that liquid fraction obtained with separation processes of other different manures. This meaning is only rarely used, and it is included in the semiliquid category for its agricultural use, which, similarly to slurry, can be applied directly onto the soil as liquid waste, and for this reason, the micropollutant concentration in slurry and manure liquid fraction is generally reported in ng L^{-1}. Notwithstanding this, some study expressed micropollutants concentration in semiliquid manure also in ng g^{-1} DM, if measured after a proper dewatering process.
- *Liquid manure*, which is defined based on its very low or almost absent dry matter. In this category can be found urine and/or dirty water collected after the cleaning of stalls, and thus containing residues of faeces, urine, and others. The latter is generally referred also as *flushing material*. The occurrence of micropollutants in this type of zootechnical waste is, by its nature, typically expressed in ng L^{-1}.

Once excreted, manure is generally stored in heaps, pits, or lagoons – depending on their characteristics or the type of animal and stall – or it can be treated. In this regard, the wastes resulting from prolonged storage or the most common treatments can be summarised as follows:

- *Lagooning sludge* may be defined as the sludge sedimented and accumulated in slurry lagoons, which are 1-5 m deep open-air or covered ponds in which slurry, flushing material and other zootechnical effluents are conveyed. This waste is generally removed from the bottom of the lagoon after 5-20 years of accumulation (Hamilton et al., 2006). It is semi-solid (3-25% dry matter content), and it could thus be applied as a soil amendment. Micropollutants concentration in lagooning sludge can be expressed both in ng g^{-1} DM or ng L^{-1}.
- *Lagoon effluent* is thus the water collected from the shallow part of the lagoon (water phase). It has a very low or absent dry matter, as, assuming a residence time that generally varies from 2 to 6 months, suspended solid may have already settled down forming the above described lagooning sludge. It is typically used for irrigation purposes (Bodman, 1996). Micropollutants concentration in lagoon effluent is generally reported in ng L^{-1}.

- *Compost* is the result of the composting process, which consists in the regular turning and moisture and temperature control of manure heaps (in which are commonly added hay, straw or decomposed leaves) in order to favour aerobic conditions. It presents a high dry matter content (33-80% DM). Thus micropollutants concentration in it is typically expressed in ng g^{-1} DM.
- *Digested manure* (also called *digestate*) is the result of the aerobic or anaerobic digestion process, typically occurring (in this specific context) at least at 40°C for up to 6 months. It can be considered as a semiliquid material, thus micropollutants concentration in digestate is often expressed in ng L^{-1}, but it could also be reported in ng g^{-1} DM.
- *Pellet manure* is extremely dense and low moisture content granules made by mechanical compression of manure at high temperature (at least 100°C). The pelletisation process is frequently carried out with poultry dung (but other solid manures can also be adopted). It produces a high dry matter content waste (80-95% DM) with high fertilising potential (Valentinuzzi et al., 2020). Micropollutant concentration in pellet manure is expressed in ng g^{-1} DM.
- *Alum treated manure* is manure in which $Al_2(SO_4)_3$ has been added to reduce water-extractable constituents between flocks. The main objective of the alum treatment is to reduce the bacteria load in the raw manure, and the resulting waste presents a high dry matter content (75-80% DM). Its reuse in agriculture is uncommon but possible if its nutrient content (and thus, its application rate) is properly evaluated in advance (Guo and Song, 2009). Micropollutant concentration in alum treated manure is expressed in ng g^{-1} DM.

A detailed resume of these manure categories, their definition, the terminology commonly adopted, and their typical characteristics (in terms of dry matter and nutrient content), is reported in Table 4.1, together with the references of the extreme values indicated.

4.2. Occurrence of micropollutants in raw and treated manure

As mentioned above, pharmaceuticals and hormones occurrence in different types of manure from different animals has been investigated in many studies in the last decades. To have an overall view of the concentration level of micropollutants that can be found in manure, and then have the possibility to estimate the corresponding soil and water pollution due to manure soil-amendment, as it will be carried out in Chapter 6, a large number of studies (a total of 104) published between 1980 and 2019 providing data on PPCPs occurrence in manure were reviewed.

The results of this review process were published on March 10th, 2020 in Science of the Total Environment (Ghirardini et al., 2020), and are here resumed.

All the studies considered here monitored the occurrence of the compounds in manure from animals that received therapeutic or sub-therapeutic treatments, and in which the resulting PPCPs concentrations can be considered as a *real* case scenario. On the contrary, all the studies in which micropollutants were spiked in manure, or in which their concentration was monitored before the excretion (e.g., intestinal manure grab), were excluded.

A large number of studies did not strictly focus on monitoring compounds occurrence in zootechnical wastes, but in other related issues, such as micropollutants occurrence in soil or water after manure application, or in manure before and after a defined treatment, but they were included in this chapter discussion as they anyway provided information regarding pharmaceuticals and hormone concentration in raw and treated manure.

As shown in Figure 4.1, the 104 studies refer to samples of manure collected all around the globe. Many of them were conducted in US, China, Canada, Spain, and Germany, concerning manure produced (in descending order) by pigs, cattle, poultry, and, to a lesser extent, sheep and horses.

The great majority of data reported referring to manure produced by cattle (37%), swine (34%) and poultry (27%). Information about micropollutants in horses, sheep and goats' manure are scarce (less than 1% of the collected data) and have been thus excluded in this chapter. Nevertheless, some horse manure PhAC concentrations can be found in Busheé et al., 1998, and, regarding sheep and goats manure, they can be retrieved from Sarmah et al., 2006.

The data reported in the articles can refer to grab or composite samples of manure. In the first case, every single value of the sampling campaign (corresponding to each grab sample collected) was elaborated in then reported in this chapter graphs. In the second case, the concentration of the resulting composite sample, made by uniform mixing of more than one grab samples in a different point of a manure pile or slurry pit, has been considered. In some cases, the studies only reported a single average of more grab or composite samples results. Thus, that value has been reported here as a single value, losing other information about ranges of concentration in that specific case study.

It should be mentioned that compounds concentration may be reduced in manure even without specific treatment. This could be due to many different chemical and biological processes which may occur both in aerobic (e.g. within the first metre in the depth of lagoons) and anaerobic (e.g. in manure heaps) conditions (Hafner et al., 2017; Sura et al., 2014).

Pharmaceuticals and hormones concentration reduction during manure stock is not discussed here. Still, it will be detailed separately in Chapter 6 to give its estimation for the modelling of PPCPs fate in the environment.

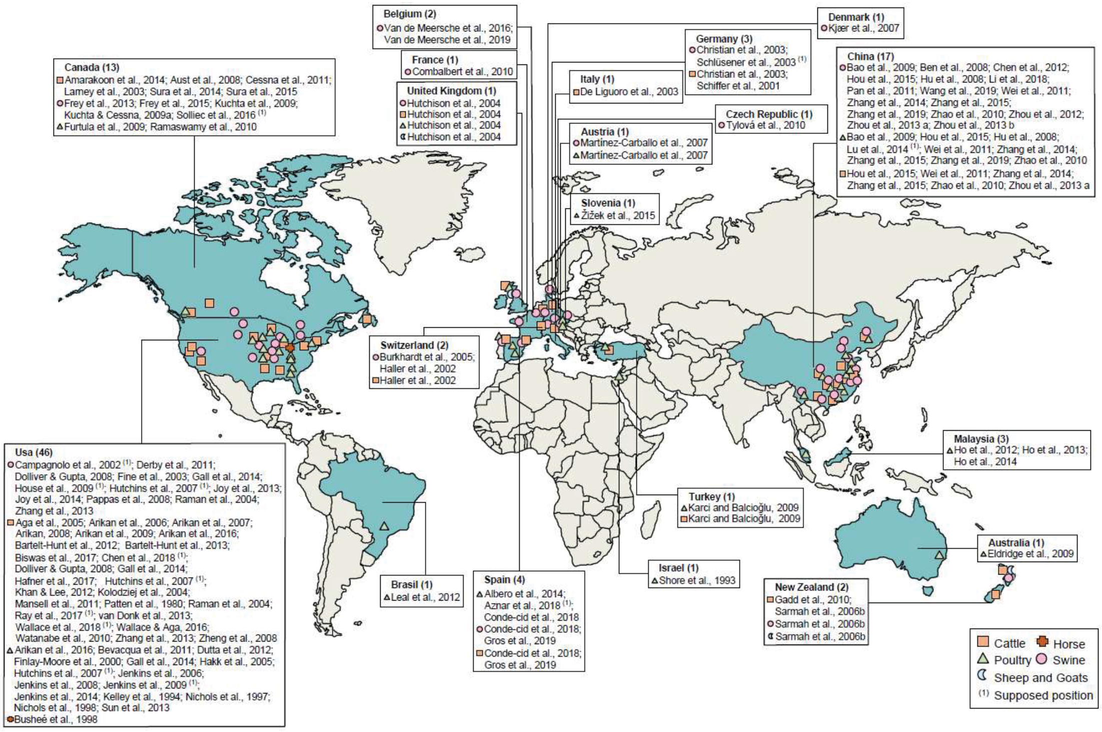


Figure 4.1. Location (clearly reported or assumed) of the manure sampling campaign with the corresponding animal producer and study reference. In each box, the numbers in brackets after the country name refer to the number of studies carried out in the specific country.

In this chapter context, concentrations of in zootechnical wastes were found for a total of 145 compounds mainly including antimicrobials (85) and hormones (39) (for this reason, micropollutants will be herein referred in the text as pharmaceuticals and hormones, excluding personal care products, which very rarely occur in animal manure), and considering parent compounds, metabolites and transformation products. Table 4.2 summarises the micropollutants included in this analysis, and Appendix 1 provide further information concerning the physicochemical characteristics of each chemical.

Table 4.2. Micropollutants included in this analysis, grouped according to their therapeutic class. The number in brackets corresponds to the number of compounds included in the group.

Class	Compounds included
Analgesics and anti-inflammatories (8)	Acetaminophen; fenoprofen; flunixin; ibuprofen; ketoprofen; mefenamic acid; naproxen and salicylic acid
Anticonvulsants (1)	Carbamazepine
Antihelminthics (1)	Flubendazole
Antimicrobials (85)	Amoxicillin; amprolium; anhydrochlortetracycline; anhydrotetracycline; atrazine; azithromycin; bacitracin A; benzylpenicillin (or Penicillin G); carbadox; ceftiofur; chloramphenicol; chlortetracycline; ciprofloxacin; clarithromycin; cloxacillin; colistin A; colistin B; cyromazine; danofloxacin; demeclocycline; difloxacin; doxycycline; enrofloxacin; epi-anhydro-tetracycline; epi-chlortetracycline; epi-iso-chlortetracycline; epi-oxytetracycline; epi-tetracycline; erythromycin; erythromycin H_2O; fleroxacin; florfenicol; flumequine; furazolidone; iso-chlortetracycline; lasalocid; leucomycin A5; lincomycin; lomefloxacin; marbofloxacin; metacycline; minocycline; monensin; n^4-acetyl-sulfamethazine; narasin; nicarbazin; norfloxacin; novobiocin; ofloxacin; oleandomycin; ormetoprim; oxytetracycline; pefloxacin; pirlimycin; pristinamycin (or virginiamycin); roxithromycin; salinomycin; sarafloxacin; sulfacetamide; sulfachlorpyridazine; sulfadiazine; sulfadimethoxine; sulfadoxine; sulfaguanidine; sulfamerazine; sulfameter; sulfamethazine; sulfamethizole; sulfamethoxazole; sulfamethoxypyridazine; sulfamonomethoxine; sulfanilamide; sulfapyridine; sulfaquinoxaline; sulfathiazole; sulfisoxazole; tetracycline; thiamphenicol; tiamulin; tilmicosin; toltrazuril; trimethoprim; tylosin; α-apo-oxytetracycline; β-apo-oxytetracycline
Antiseptics (3)	Methyl triclosan; ortho-phenylphenol; triclosan
Beta-blockers (1)	Metoprolol
Hormones (39)	11-ketotestosterone; 17α-estradiol (E2α or alfatradiol); 17α-estradiol-3-sulfate; 17α-ethynylestradiol (EE2 or ethinyl estradiol); 17α-hydroxyprogesterone; 17α-trenbolone; 17β-estradiol (E2β or estradiol); 17β-estradiol-3, 17-diglucuronide; 17β-estradiol-3, 17-disulfate; 17β-estradiol-3-glucuronide; 17β-estradiol-3-glucuronide-17-sulfate; 17β-estradiol-3-sulfate; 17β-

Class	Compounds included
	estradiol-3-sulfate-17-glucuronide; 17β-estradiol-17-sulfate; 17β-estradiol-17-glucuronide; 17β-trenbolone; androstadienedione; androstenedione; androsterone; dienestrol; diethylstilbestrol; epiandrosterone (or trans-androsterone); estriol (E3); estriol-3-glucuronide; estriol-3-sulfate; estrone (E1); estrone-3-glucuronide; estrone-3-sulfate; hexestrol; medroxyprogesterone; melengestrol acetate; mestranol; progesterone; testosterone; trendione; α-zearalanol; α-zearalanol; β-zearalanol; β-zearalanol
Inhibitors (xanthine oxidase) (1)	Allopurinol
Lipid regulators (3)	Clofibric acid; fenofibrate; gemfibrozil
Parabens (2)	Methylparaben; propylparaben
Plasticiser (1)	Bisphenol A

For the sake of clarity, the occurrence of micropollutants in different types of manure from different animals are in this chapter discussed and graphically presented divided between raw and treated manure, then based on the animal producer and finally on the unite of measurement provided by the studies reviewed.

It should be noted that compound concentration in semiliquid manure (such as slurry or flushing material) can be expressed both in ng g^{-1} DM or in ng L^{-1}. This manure type can be thus reported in both the discussion and the graphical representation regarding the two units of measurement.

Finally, regarding the elaboration of the concentration values collected in this literature review, if a value was reported below its limit of detection (LOD) or its reporting limit (RL) it was assumed to equal to the LOD or the RL and if it was reported below its limit of quantification (LOQ), it was assumed to be half its LOQ value, as suggested by Armbruster and Pry, 2008. In the same way, in the following text, a study result expressing a non-detection of a compound will not be reported as 0, but with its LOD value. This may be in some case confusing, for example, discussing a range of concentrations found in a specific manure type, two different compounds can be found <LOD and with two different LOD values, and thus in this chapter, the lowest detected concentration will be assumed for the compound measured with the lowest LOD. This was an unavoidable choice done considering that, in any case, it is not possible to infer that a compound does not occur just because it was not detected (Armbruster and Pry, 2008) and thus, the value characterised by a more precise (and then lower) LOD was here assumed to be the lowest value of concentration effectively detectable.

4.2.1. Micropollutants occurrence in raw manure

The results reported in the scientific literature reviewed regarding micropollutants concentration in raw manure are here summarised.

Before going into the details, it is worth noting that some compounds are commonly detected in each manure type and from each animal. The antibiotics chlortetracycline and oxytetracycline were the most investigated compounds in the studies considered, and they also commonly presented the highest average concentrations.

In any case, some pharmaceutical remains strictly animal correlated. It is the case, for example, of monensin, which is mainly detected in cattle manure and rarely in others. This can be explained by the fact that this pharmaceutical is typically administered to cattle, as it promotes ruminants' food metabolism (Łowicki and Huczyński, 2013).

It is worth noting that a significant amount of the plasticiser Bisphenol-A was monitored in various zootechnical wastes from different animals. This may be ascribed to the fact that Bisphenol-A can be released from materials lining the inner surface of food containers – and thus reach the animal's intestines – or directly from manure storage tanks (Zhang et al., 2014).

Finally, in the data discussed below, no considerations are reported about seasonal variation in antibiotic concentrations, even if it may be expected. It should be mentioned that seasonal fluctuations in antibiotic occurrence can be due both for differences in their administered amount and climatic reasons. The first case regards, for example, tetracyclines, which are mainly administered in winter to prevent flu and other respiratory illness (Ben et al., 2013), or, on the contrary, some sulfonamides, which are used to treat a variety of bacterial and protozoal infections which occur more frequently in the hot seasons (Pan et al., 2011). Otherwise, in the second case (climatic reason), the presence of PhACs may be affected by the enhanced degradation which occurs at high temperatures, such as in summer (Raman et al., 2004).

4.2.1.1. Micropollutants occurrence in raw cattle manure

Concerning raw cattle manure measured as ng g^{-1} DM, collected data refer to 98 compounds, in which the most reported were sulfamethazine and tylosin (with 69 data for each of them), followed by chlortetracycline (68), oxytetracycline (62), tetracycline (56), sulfadimethoxine (42) and epi-tetracycline (35). As expected, all of them are antibiotics, and other groups' compounds (e.g., analgesics/antiinflammatories, anticonvulsants, etc.) were detected at a lesser extent.

Overall, compounds concentration ranges between 0.02 ng g^{-1} DM (<LOD) for the hormone trendione in cattle slurry and 225,000 ng g^{-1} DM for the antibiotic oxytetracycline in bedding manure.

In this regard, the widest variability range was found for oxytetracycline (6 order of magnitude: from 0.1 to 225,000 ng g^{-1} DM), followed by chlortetracycline, enrofloxacin and sulfamethoxine (5 order of magnitude).

Among all the detected compounds and limiting the attention to those in which more than five values were collected, the highest average concentration (± standard deviation) was found once again for oxytetracycline (5,815 ± 29,452 ng g^{-1} DM), followed by monensin (2,434 ± 2,272 ng g^{-1} DM) and enrofloxacin (2,318 ± 10,176 ng g^{-1} DM). Here, it is noteworthy that the 75th percentile of many compounds' concentration values (27 out of 98) does not reflect the high standard deviation, and are lower compare to the average, suggesting that extreme values (max and min) are outliers. For example, 75th percentile of oxytetracycline and enrofloxacin occurrence values resulted equal to 166 and 33 ng g^{-1} DM respectively, which is one order of magnitude lower than the average, and two orders of magnitude lower than the calculated standard deviation. Further statistical information and details (namely, number of data, min, max, average, standard deviation and 75th percentile) are summarised in Table M1 of Appendix 2 for all the compounds monitored.

Figure 4.2 shows all the occurrence data found in the literature review for antibiotics, while Figure 4.3 shows the values regarding all the other classes of compounds found in cattle manure and measured in ng g^{-1} DM (namely analgesics and antiinflammatories, anticonvulsants, anthelmintics, hormones and plasticisers).

A rapid glance on these figures reveals that the maximum concentration values mainly occurred in solid manure (57%) followed by slurry (20%) and then bedding manure (22%).

Regarding liquid/semiliquid cattle manure, in which micropollutants concentrations are measured in ng L^{-1}, it emerges that the collected occurrence data refer to 80 compounds. Among them, 62 are antimicrobials, 15 are hormones, and the others refer to analgesic and antiinflammatories, anticonvulsants and plasticisers). Their concentrations varied between 0.5 ng L^{-1} (<LOD) for some sulfonamides (among them sulfachlorpyridazine, sulfadimethoxine and sulfamethoxazole) and 5.86×10^6 ng L^{-1}. Differently from cattle manure measured in ng g^{-1} DM, the most monitored compounds were the hormones 17β-estradiol and estrone (with 34 collected data for each one), followed by the antibiotics oxytetracycline, sulfamethazine and tetracycline and the hormone 17α-estradiol (with 32 values collected for each of them).

The widest variability ranges were found for chlortetracycline and epi-chlortetracycline (6 order of magnitude), followed by iso-chlortetracycline, sulfamethoxazole, and sulfamethazine (5 orders of magnitude).

Epi-chlortetracycline, chlortetracycline and iso-chlortetracycline exhibited a high average concentration (± standard deviation), resulted equal to $766{,}691 \pm 1.65 \times 10^6$ ng L^{-1}, $281{,}281 \pm 1.24 \times 10^6$ ng L^{-1}, and $280{,}274 \pm 687{,}556$ ng L^{-1} respectively. These three compounds also showed the highest difference between 75th percentile and average, suggesting, as already mentioned above, a potential driving of the calculation by outliers.

Apart from epi-chlortetracycline, chlortetracycline, and iso-chlortetracycline, also other 22 compounds (thus a total of 25 out of 80) had a 75th percentile lower than their corresponding average value.

As represented in Figure 4.4 and Figure 4.5, and reported in detail in Table M2 of Appendix 2, most of the maximum concentration values were found in flushing material (75%), followed by slurry (11%), urine (10%) and then the liquid fraction of manure (4%).

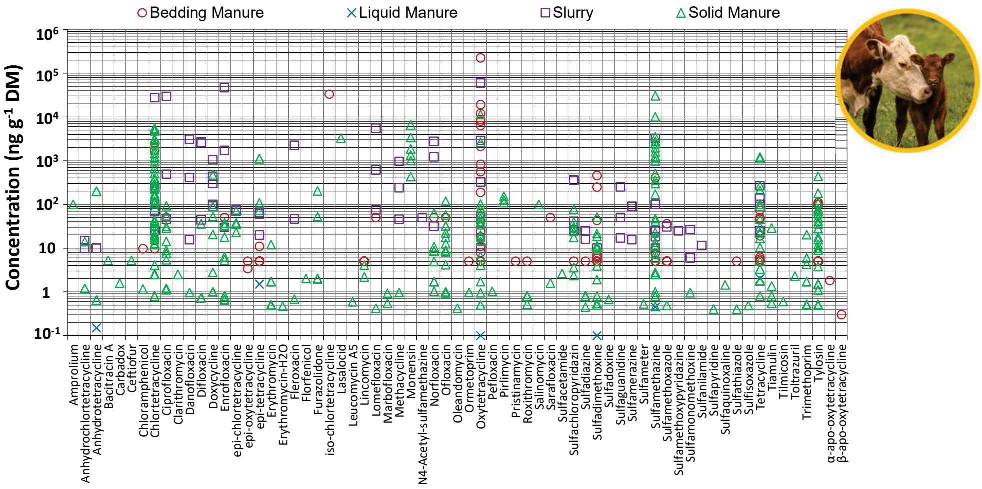


Figure 4.2. Occurrence of antibiotics in different types of raw cattle manure. Data from Aga et al., 2005; Amarakoon et al., 2014; Arikan et al., 2006, 2007, 2009, 2016; Aust et al., 2008; Cessna et al., 2011; Chen et al., 2018; Christian et al., 2003; Conde-Cid et al., 2018; De Liguoro et al., 2003; Dolliver and Gupta, 2008; Gros et al., 2019; Hafner et al., 2017; Haller et al., 2002; Hou et al., 2015; Karci and Balcioğlu, 2009; Patten et al., 1980; Ray et al., 2017; Sura et al., 2014, 2015; Wallace et al., 2018; Wallace and Aga, 2016; Watanabe et al., 2010; Zhang et al., 2015; Zhao et al., 2010; Zhou et al., 2013.

Figure 4.3. Occurrence of other micropollutants, belonging to classes A (analgesics and antiinflammatories), B (anticonvulsants), C (anthelmintics), G (hormones) and K (plasticisers) in different types of raw cattle manure. Data from Bartelt-Hunt et al., 2012, 2013; Biswas et al., 2017; Gall et al., 2014; Gros et al., 2019; Mansell et al., 2011; Raman et al., 2004; Schiffer et al., 2001; Van Donk et al., 2013; Watanabe et al., 2010; Zhang et al., 2014; Zheng et al., 2008.

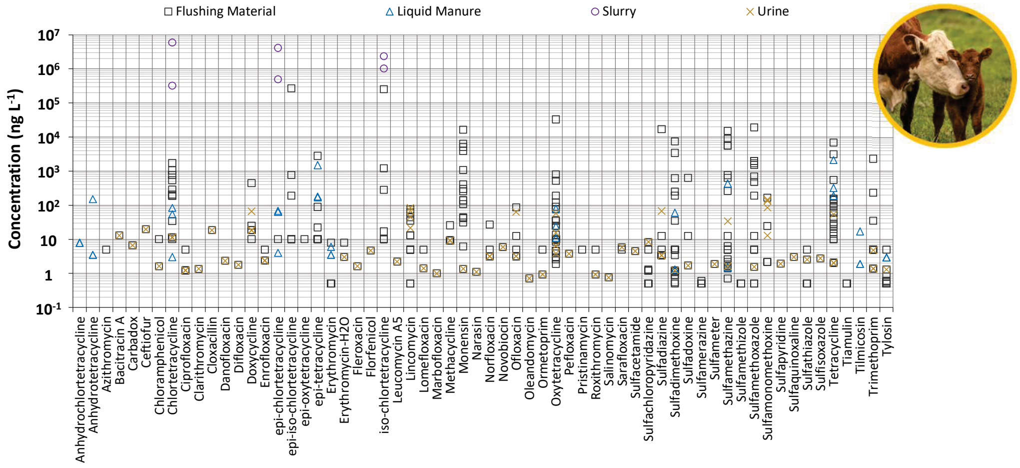


Figure 4.4. Occurrence of antibiotics in different types of liquid/semiliquid raw cattle manure. Data from Arikan, 2008; Hafner et al., 2017; Wallace and Aga, 2016; Watanabe et al., 2010; Wei et al., 2011; Zhang et al., 2013; Zhou et al., 2013.

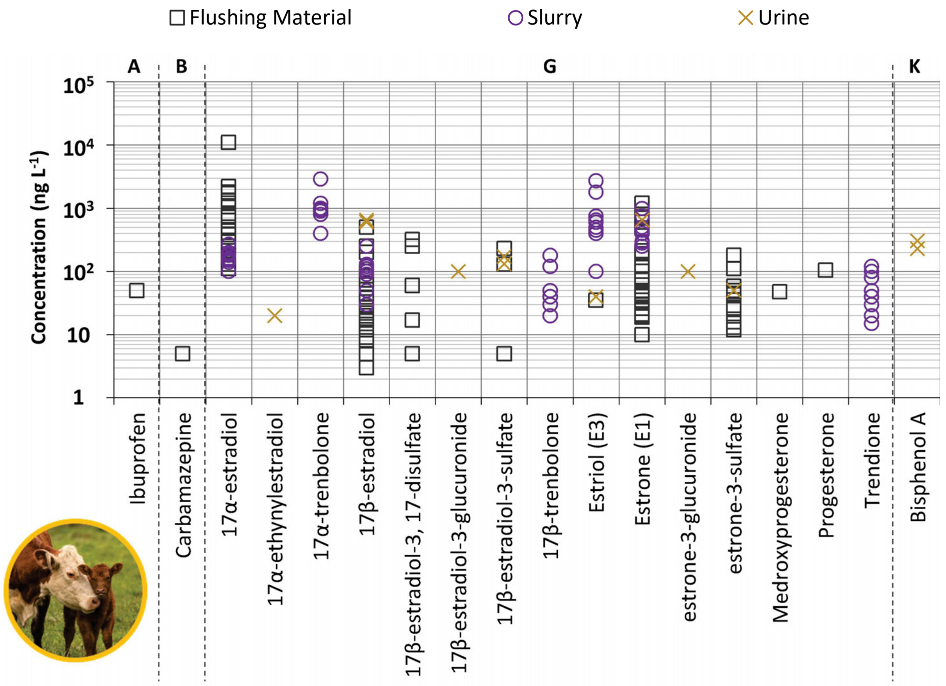


Figure 4.5. Occurrence of other micropollutants, belonging to classes A (analgesics and antiinflammatories), B (anticonvulsants), G (hormones) and K (plasticisers) in different types of liquid/semiliquid raw cattle manure. Data from Gadd et al., 2010; Khan and Lee, 2012; Watanabe et al., 2010; Zhang et al., 2014; Zheng et al., 2008.

4.2.1.2. Micropollutants occurrence in raw swine manure

Regarding swine manure, measured in ng g^{-1} DM, occurrence data refer to 77 compounds (62 of them antimicrobials and 11 hormones). Here, doxycycline was the most reported one (100 values), followed by sulfadiazine (93 values), oxytetracycline (72 values), chlortetracycline (65 values), tetracycline and sulfamethazine (59 values), tylosin (44 values) and ciprofloxacin (41), confirming that antibiotics are the most studied compounds even in this zootechnical waste.

The highest concentration was found for chlortetracycline in solid (fraction) manure (879,600 ng g^{-1} DM), which also exhibited the highest average (± standard deviation) concentration value (76,667 ± 176,264 ng g^{-1} DM), followed by bacitracin A (28,133 ± 85,165 ng g^{-1} DM), chloramphenicol (11,693 ± 28,761 ng g^{-1} DM) and oxytetracycline (11,180 ± 43,662 ng g^{-1} DM).

Moreover, the compounds cited above also showed the widest variability ranges, found for oxytetracycline and chlortetracycline (6 order of magnitude), followed by bacitracin A, doxycycline, tetracycline, lomefloxacin, enrofloxacin, tylosin, sulfamethazine, sulfamonomethoxine, lincomycin and sulfathiazole (5 order of magnitude).

Similarly to the results discussed for cattle manure, the 75th percentile is lower than the average value for 36 out of 77 compounds, that can be explained looking at the extraordinary high maximum values for the corresponding PhACs (see Table M3 of Appendix 2 for other information).

A rapid glance to Figure 4.6 and Figure 4.7 regarding occurrence in the manure of antibiotics and other micropollutants respectively, reveals that the maximum values of concentration were found in manure solid fraction (62%) followed by slurry (32%) and then (6%).

As to liquid and semiliquid manure, in which micropollutants concentrations are measured in ng L^{-1}, data were collected for 72 compounds, even if studies reported only a limited number of values: the most investigated compound was chlortetracycline (19 values), followed by oxytetracycline and sulfamethazine (18 values each one) and then ciprofloxacin and tylosin (15 values each one).

Overall, the collected concentrations vary between 0.1 ng L^{-1} for sulfamonomethoxine in flushing material, and 1.1×10^8 ng L^{-1} for chlortetracycline in the slurry, found by Hoese et al., 2009.

Here chlortetracycline concentration variability range (9 orders of magnitude) is wider than the previous findings reported for cattle manure and swine manure measured in ng g^{-1} DM, which presented a maximum variability range of 6 order of magnitude. And in any case, in raw swine liquid and semiliquid manure, higher variability range were observed, such as for sulfamethazine (8 orders of magnitude) and sulfamonomethoxine and oxytetracycline (7 orders of magnitude).

Chlortetracycline also showed the highest average (± standard deviation) concentration, with 5.78×10^6 ng L^{-1} ± 2.5×10^7 ng L^{-1}, followed by lincomycin ($2.9 \times 10^6 \pm 7.7 \times 10^6$ ng L^{-1}), and 11 compounds out of 72 exhibited a 75th percentile lower than the average. Further information can be found in Table M4 of Appendix 2.

Finally, as can be seen in Figure 4.8 and Figure 4.9, the maximum concentration values occurred mainly in slurry (45%), flushing material (34%), urine (11%) and the liquid (fraction) manure (10%)

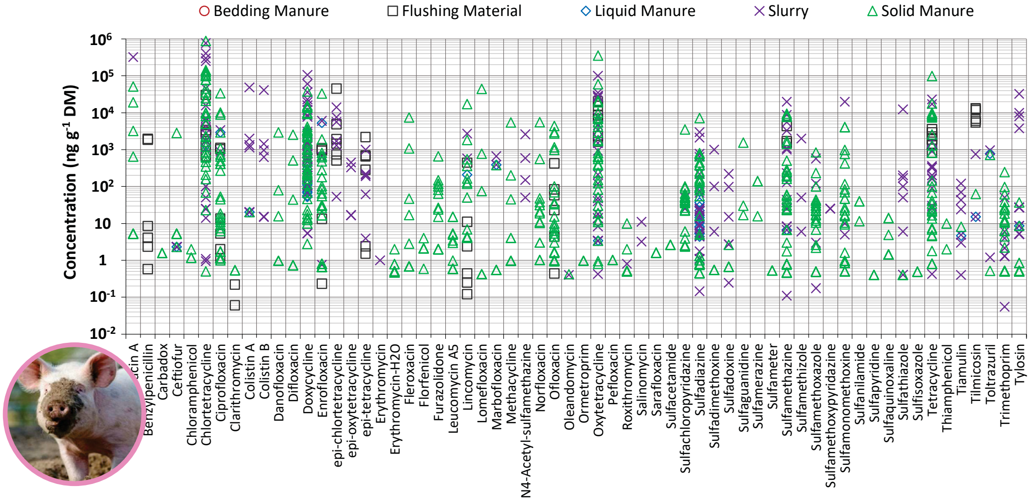


Figure 4.6. Occurrence of antibiotics in different types of raw swine manure. Data from Bao et al., 2009; Chen et al., 2012; Christian et al., 2003; Conde-Cid et al., 2018; Gros et al., 2019; Haller et al., 2002; Hou et al., 2015; Hu et al., 2008; Jacobsen and Halling-Sørensen, 2006; Joy et al., 2013, 2014; Martínez-Carballo et al., 2007; Pan et al., 2011; Schlüsener et al., 2003; Tylová et al., 2010; Van den Meersche et al., 2016, 2019; Wang et al., 2019; Zhang et al., 2019; Zhao et al., 2010; Zhou et al., 2012; Zhou et al., 2013a; Zhou et al., 2013b.

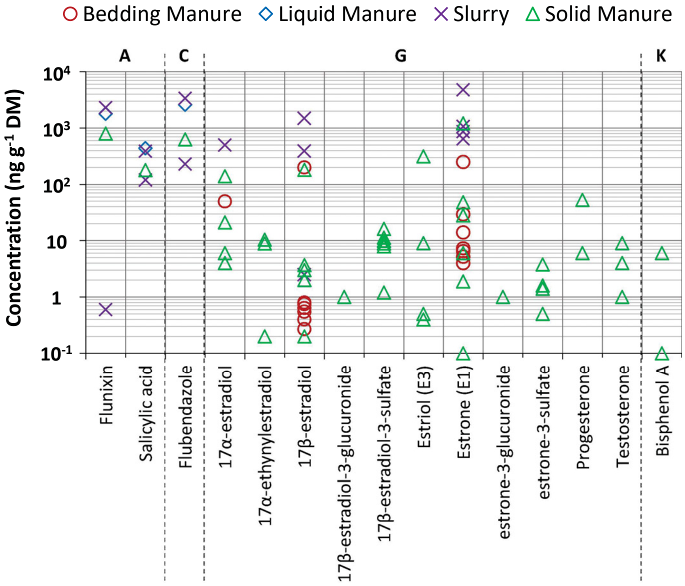


Figure 4.7. Occurrence of other micropollutants, belonging to classes A (analgesics and antiinflammatories), C (anthelmintics), G (hormones) and K (plasticisers) in raw swine manure. Data from Combalbert and Hernandez-Raquet, 2010; Derby et al., 2011; Gros et al., 2019; Kjær et al., 2007; Raman et al., 2004; Zhang et al., 2014.

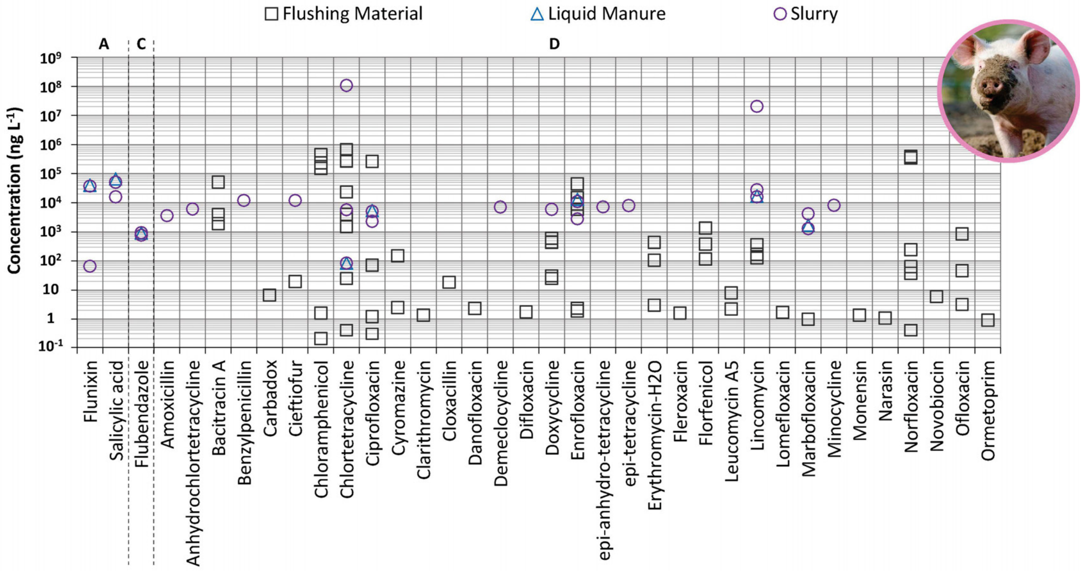


Figure 4.8. Occurrence of analgesics and antiinflammatories (A), anthelmintics (C), antibiotics (D – part 1. The 2nd is represented in Figure 4.9) in raw liquid/semiliquid swine manure. Data from Gros et al., 2019; Hoese et al., 2009; Li et al., 2018; Solliec et al., 2016; Wei et al., 2011; Zhou et al., 2013b.

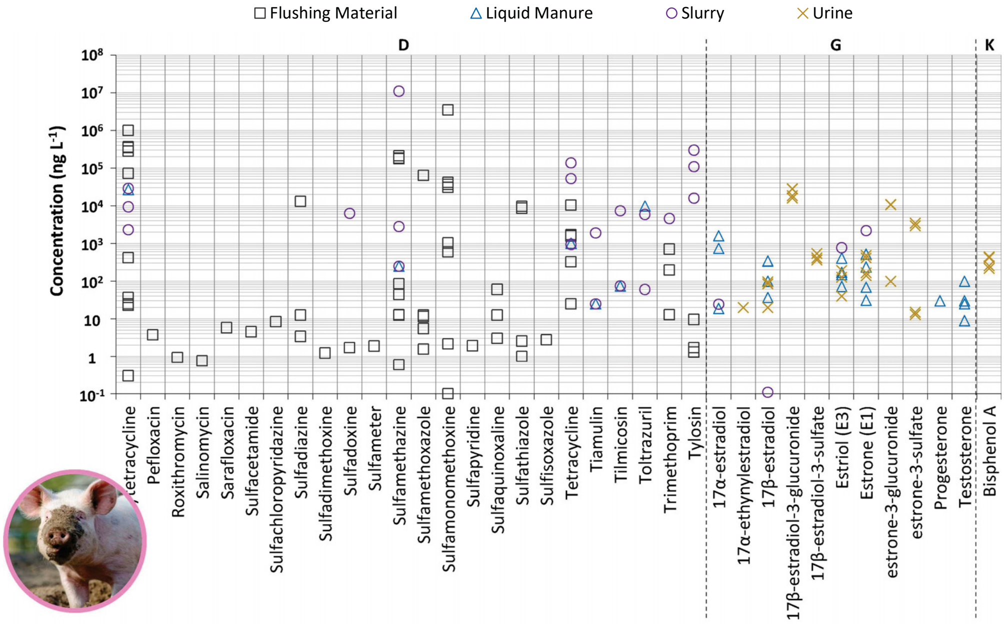


Figure 4.9. Occurrence of analgesics and antibiotics (D – part 1. The 1st is represented in Figure 4.8), hormones (G), and plasticiser (K), in raw liquid/semiliquid swine manure. Data from Burkhardt et al., 2005; Combalbert and Hernandez-Raquet, 2010; Gall et al., 2014; Gros et al., 2019; Hoese et al., 2009; Li et al., 2018; Solliec et al., 2016; Wei et al., 2011; Zhang et al., 2014; Zhou et al., 2013b.

4.2.1.3. Micropollutants occurrence in raw poultry manure

With regard to poultry manure, concentrations are available for 92 micropollutants, in which the antibiotics sulfadiazine, doxycycline and enrofloxacin were the most monitored, with a total of 54, 47, and 44 data respectively.

Overall, the concentrations varied between 0.03 ng g^{-1} DM, for the hormone testosterone in poultry litter, and 1.4×10^6 ng g^{-1} DM, for the antibiotic enrofloxacin in poultry litter. This last antibiotic also showed the widest variability range of concentration (7 orders of magnitude), followed by fleroxacin and oxytetracycline (6 orders of magnitude) and then tylosin, sulfadiazine, salinomycin, trimethoprim, erythromycin and difloxacin (5 orders of magnitude).

Limit the attention to compounds for which more than 5 data has been reported, enrofloxacin exhibited the highest average of concentration (35,774 ± 213,817 ng g^{-1} DM), followed by oxytetracycline (13,769 ± 72,375 ng g^{-1} DM), flumequine (11,833 ± 19,581 ng g^{-1} DM) and then doxycycline (10,935 ± 22,260 ng g^{-1} DM). Here, as better reported in the descriptive statistical analysis of the collected data (Table M5 of Appendix 2), it is interesting to note that for 27 out of 92 compounds the calculated 75th percentile is less than the corresponding average value, indicating the occurrence of extraordinary maximum value. This is the case, for example, of the above-mentioned enrofloxacin and oxytetracycline (of which the 75th percentile were 939 ng g^{-1} DM and 1600 ng g^{-1} DM, respectively).

Figure 4.10 and Figure 4.11 show the collected compounds concentration data for both poultry manure and poultry litter, in which it is possible to see that the maximum values mainly occurred in poultry (solid) manure (61%) followed by poultry litter (39%).

Only a limited number of information has been found regarding poultry house flushing material, and anyway poultry liquid/semiliquid manure (in which compounds concentration were monitored in ng L^{-1}). In this regard, data are available for eight antibiotics (namely chlortetracycline, cyromazine, doxycycline, oxytetracycline, oxytetracycline, sulfadiazine, sulfamethazine, sulfaquinoxaline, and tetracycline), for which the highest concentrations vary between 550 ng L^{-1} (for cyromazine) and 20,700 ng L^{-1} (for oxytetracycline), and average concentrations vary between 90 ng L^{-1} (sulfamethazine) and 950 ng L^{-1} (doxycycline).

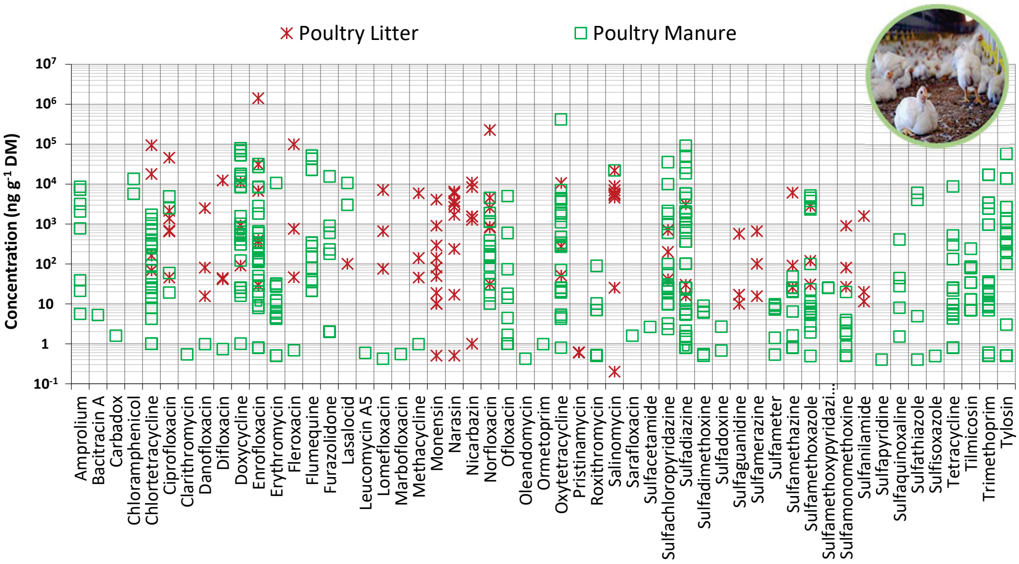


Figure 4.10. Occurrence of antibiotics in different types of raw manure. Data from: Arikan et al., 2016; Bao et al., 2009; Conde-Cid et al., 2018; Furtula et al., 2009; Ho et al., 2012, 2013, 2014; Hou et al., 2015; Hu et al., 2008; Karci and Balcioğlu, 2009; Leal et al., 2012; Martínez-Carballo et al., 2007; Ramaswamy et al., 2010; Sun et al., 2013; Zhang et al., 2015; Zhang et al., 2019; Zhao et al., 2010; Žižek et al., 2015.

Figure 4.11. Occurrence of micropollutants belonging to classes A (analgesics and antiinflammatories), B (anticonvulsants), E (antiseptics), F (beta-blockers), G (hormones), H (inhibitors), I (lipid regulators), J (parabens) and K (plasticisers) in raw poultry manure. Data from: Albero et al., 2014; Aznar et al., 2018; Bevacqua et al., 2011; Dutta et al., 2012; Finlay-Moore et al., 2000; Hakk et al., 2005; Ho et al., 2012, 2013, 2014; Jenkins et al., 2008, 2009, 2006; Lu et al., 2014; Nichols et al., 1997, 1998; Shore et al., 1993; Zhang et al., 2014.

4.2.2. Micropollutants occurrence in treated manure

Some studies (among them Arikan et al., 2009; Ray et al., 2017 and Wallace et al., 2018) reported the concentration of the same compound before and after a specific treatment, allowing to discuss the potential removal efficiency during it.

It emerged that the operational conditions are the main factors affecting the removal of the studied compound. For instance, as reported by Ray et al., 2017 (for cattle solid manure), Arikan et al., 2016 (for both cattle and poultry bedding manure), Liu et al., 2015 (for swine manure) and Zhang et al., 2019 (for swine and poultry manure), thermophilic conditions (45-65°C) during composting allow higher removal efficiency for a broad spectrum of antibiotics.

Bao et al., 2009 and Ho et al., 2013, both studying antibiotics removal during composting of poultry manure, suggested that not only temperature, but also the total organic carbon, total nitrogen, C/N ratio, and metal content (such as copper, as also stated by Liu et al., 2015) may play an important role in micropollutants concentration reduction.

Overall, aerobic conditions (such as those favoured by regular turning of manure piles in which temperature and moisture are systematically controlled, or those occurring within the first metre in the depth of lagoons), have shown to promote degradation of many pharmaceuticals and hormones (Hafner et al., 2017; Zhang et al., 2014). But other compounds, such as chlortetracycline, sulfamethazine, and tylosin, seem to be better degraded under anaerobic conditions, occurring for example in stockpiled manure (Cessna et al., 2011; Sura et al., 2014).

The results reported in the scientific literature reviewed regarding micropollutants concentration in treated manure are here summarised.

4.2.2.1. Micropollutants occurrence in treated cattle manure

Data regarding micropollutants concentration in cattle treated manure (in ng g^{-1} DM) refer to 41 compounds in total, in which the most investigated was chlortetracycline (with 10 data), followed by progesterone, α-zearalanol and estrone (with 9 data each one).

Overall, the most common treated manure investigated was compost, followed by lagooning sludge and then digestate, in which the micropollutants occurred with concentrations varying between 0.06 ng g^{-1} DM (pirlimycin in composted solid manure) and 4,000 ng g^{-1} DM (iso-chlortetracycline in composted bedding manure).

Considering only those compounds with at least five values reported, the highest average concentrations were found for chlortetracycline (179 ± 114 ng g^{-1} DM) and tetracycline (134 ± 148 ng g^{-1} DM). Here, only values of 4 compounds out of 41 presented a 75th percentile lower than the corresponding average value, and this is the case of the hormones 17β-estradiol, estrone, α-zearalanol and β-zearalanol. Further information and descriptive statistical analysis are reported in Table M6 of Appendix 2.

Micropollutants concentrations collected in the considered studies are represented in Figure 4.12, in which it is possible to note that the maximum concentrations mainly occurred in compost (60%) followed by digestate (25%) and lagooning sludge (15%).

About liquid and semiliquid treated cattle manure, in which micropollutants concentrations are reported in ng L^{-1}, data has been collected for 63 compounds, mainly regarding the hormones estrone (42 concentration's values), 17β-estradiol (40 concentration's values) and 17α-estradiol (37 concentration's values). Here, the observed concentrations vary between 0.01 ng L^{-1} for the hormone estriol (in lagoon effluent) and 6.8×10^{6} ng L^{-1} for the antibiotic oxytetracycline (in anaerobically digested bedding manure). The widest variability range was found for oxytetracycline, epi-oxytetracycline, chlortetracycline, and epi- and iso- chlortetracycline (5 order of magnitude).

Considering then the compounds in which more than 5 data are available, the highest average values were found for epi-chlortetracycline ($1.1\times10^{6} \pm 1.3\times10^{6}$ ng L^{-1}), iso-chlortetracycline ($7.3\times10^{5} \pm 1.6\times10^{6}$ ng L^{-1}), epi-oxytetracycline $6.7\times10^{5} \pm 5.4\times10^{5}$ ng L^{-1}) and oxytetracycline ($5.4\times10^{5} \pm 1.7 \times10^{6}$ ng L^{-1}). In this context, as reported in Table M7 of Appendix 2, it emerged that 14 out of 63 compounds exhibited a concentration's 75th percentile lower than the corresponding average value.

Finally, the maximum values occurred mainly in lagoon effluent (88%) and then in anaerobically digested manure (12%), as it can be observed in Figure 4.13 and Figure 4.14.

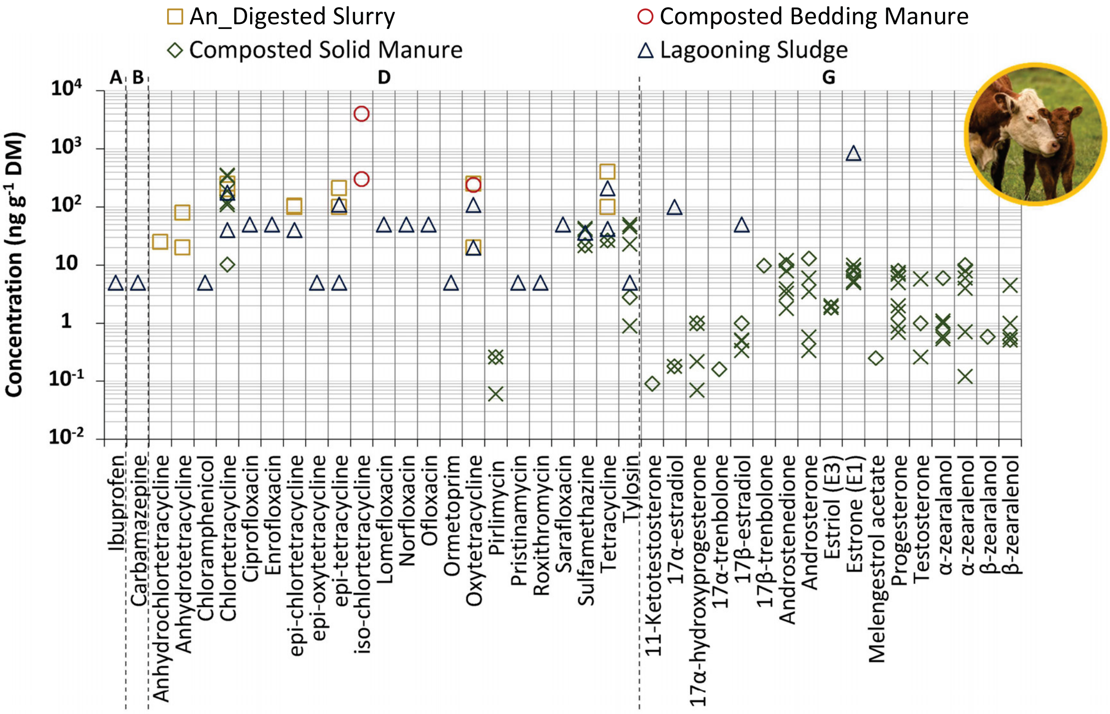


Figure 4.12. Occurrence of micropollutants belonging to classes A (analgesics and antiinflammatories), B (anticonvulsants), D (antimicrobials), and G (hormones) in treated cattle manure. Data from: Arikan et al., 2007, 2009; Bartelt-Hunt et al., 2013; Biswas et al., 2017; Cessna et al., 2011; Chen et al., 2018; Raman et al., 2004; Ray et al., 2017; Van Donk et al., 2013; Wallace et al., 2018; Watanabe et al., 2010.

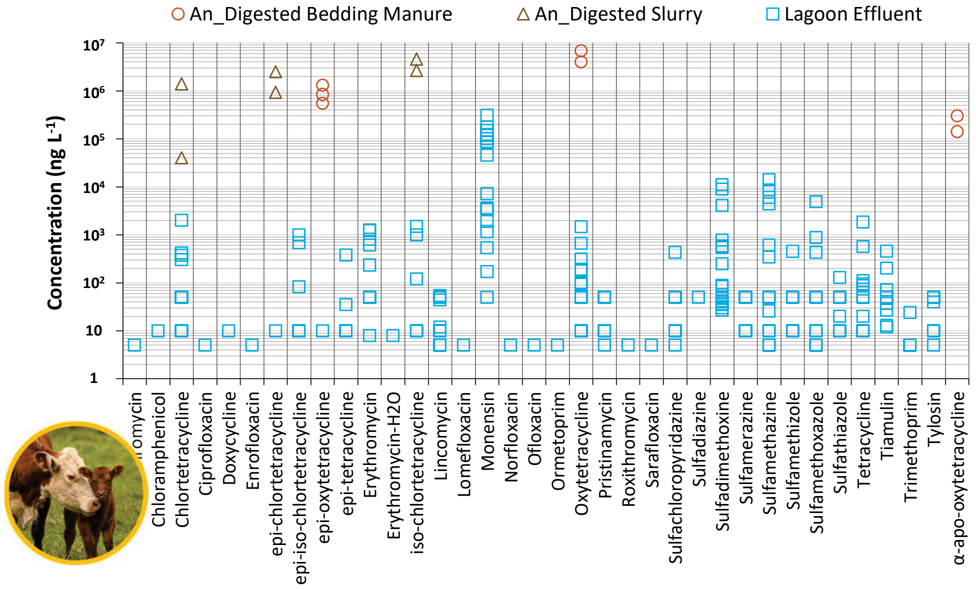


Figure 4.13. Occurrence of antibiotics in different treated liquid/semiliquid cattle manure. Data from: Arikan, 2008; Arikan et al., 2006; Hafner et al., 2017; Watanabe et al., 2010; Zhang et al., 2013.

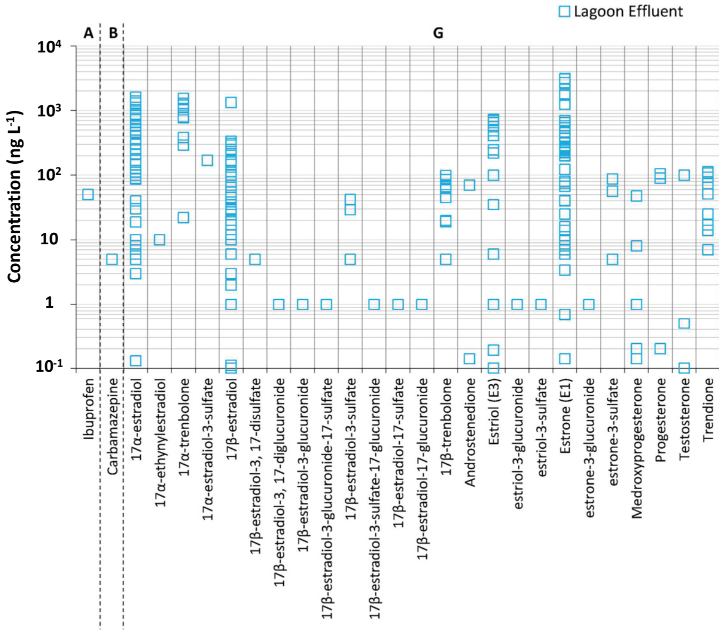


Figure 4.14. Occurrence of other micropollutants belonging to classes A (analgesics and antiinflammatories), B (anticonvulsants), and G (hormones) in the effluent of a lagoon receiving cattle manure. Data from: Gadd et al., 2010; Gall et al., 2014; Hutchins et al., 2007; Khan and Lee, 2012; Kolodziej et al., 2004; Sarmah et al., 2006; Watanabe et al., 2010; Zhang et al., 2014; Zheng et al., 2008.

4.2.2.2. Micropollutants occurrence in treated swine manure

Regarding swine treated manure in which micropollutants were measured in ng g^{-1} DM, data refer to a total of 59 compounds (56 of them are antimicrobials), in which chlortetracycline, doxycycline, oxytetracycline, sulfadiazine and tetracycline were the most studied (21 data for each of them).

Most of the concentrations were monitored in lagooning sludge (427 values of concentrations included), followed by digestate (44 values) and then compost (12 values), confirming the findings of Combalbert et al., 2012 for which the most common treatments for swine slurry and manure are lagooning and anaerobic digestion.

Overall, the observed range of occurrence varies between 0.45 ng g^{-1} DM (danofloxacin in lagooning sludge), and 87,900 ng g^{-1} DM (chlortetracycline in lagooning sludge), and the highest variability range covers four orders of magnitude, in oxytetracycline, tylosin, norfloxacin and sulfamethazine.

The highest average concentrations were due to iso-chlortetracycline (28,200 ± 6,930 ng g^{-1} DM), epi-chlortetracycline (22,800 ± 3,323 ng g^{-1} DM) and chlortetracycline (8,985 ± 21,417 ng g^{-1} DM), with 16 out of 59 compounds presenting a 75th percentile lower than the corresponding average value (Table M8 in Appendix 2).

As represented in Figure 4.15, the distribution of the maximum values reflected the number of data collected, as they were found mainly in lagooning sludge (68%) and then in anaerobically digested flushing material (28%).

As to liquid and semiliquid treated swine manure, the concentration of 74 compounds (56 antimicrobials and 18 hormones) has been reported in the studies considered. Among them, chlortetracycline was the most investigated (29 values), followed by lincomycin (27 values) and tetracycline (26 values).

The lowest and highest concentrations were found for 17β-estradiol (0.1 ng L^{-1} in lagoon effluent) and tylosin (4.9×10^{6} ng L^{-1} in lagooning sludge) respectively, and the widest variability ranges occurred for sulfamethazine, tylosin and lincomycin, covering six orders of magnitude.

The highest average concentration was found for tylosin ($3.9\times10^{5} \pm 1.2\times10^{6}$ ng L^{-1}), followed by chlortetracycline ($1.2\times10^{5} \pm 2.5\times10^{5}$ ng L^{-1}) and lincomycin ($5.4\times10^{4} \pm 7.9\times10^{4}$ ng L^{-1}), and, as reported in Table M9 of Appendix 2, 15 out of 74 compounds exhibited a 75th percentile lower than the average concentration.

Finally, the maximum concentration values were mainly detected in lagoon effluent (88%), and then in lagooning sludge (12%) (Figure 4.16 and Figure 4.17).

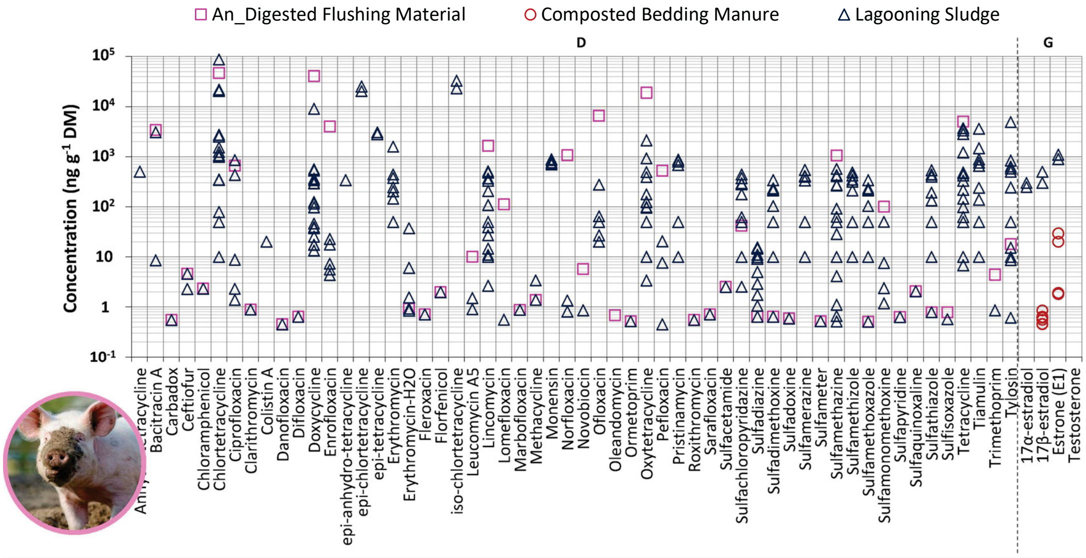


Figure 4.15. Occurrence of micropollutants belonging to classes D (antimicrobials), and G (hormones) in treated swine manure. Data from: Derby et al., 2011; Frey et al., 2015; Raman et al., 2004; Van den Meersche et al., 2019; Zhang et al., 2013; Zhou et al., 2012; Zhou et al., 2013a; Zhou et al., 2013b.

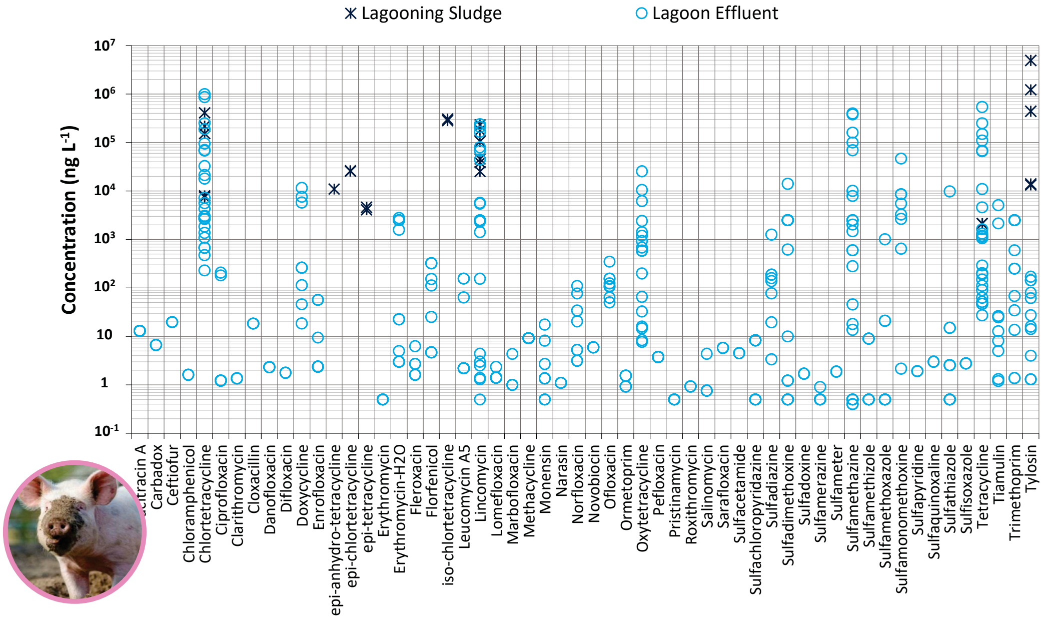


Figure 4.16. Occurrence of selected antibiotics in different treated liquid/semiliquid swine manure. Data from: Ben et al., 2008; Campagnolo et al., 2002; Dolliver and Gupta, 2008; Frey et al., 2015; Kuchta et al., 2009; Kuchta and Cessna, 2009; Zhang et al., 2013; Zhou et al., 2012; Zhou et al., 2013a; Zhou et al., 2013b.

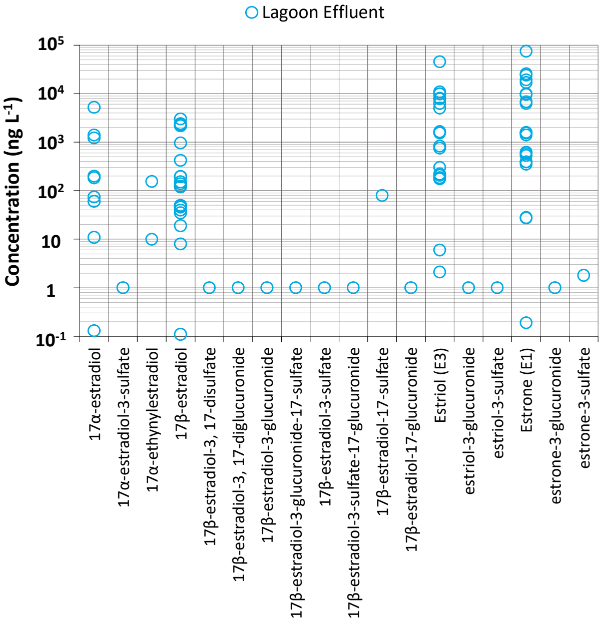


Figure 4.17. Occurrence of selected hormones in the effluent of a lagoon receiving swine manure. Data from: (Fine et al., 2003; Gall et al., 2014; Hutchins et al., 2007; Sarmah et al., 2006; Zhang et al., 2014).

4.2.2.3. Micropollutants occurrence in treated poultry manure

A limited number of data was found regarding treated poultry manure for both micropollutants measured in ng g^{-1} DM and ng L^{-1}.

In the first case, data refer to the antibiotic salinomycin (3 values), and the hormones 17β-estradiol (2 values) and testosterone (3 values).

Thus, some considerations are added here regarding maximum concentrations found, and micropollutants degradation before and after treatment.

For example, during composting, it was found that salinomycin was almost totally reduced (from 22,000 ng g^{-1} DM to 76 ng g^{-1} DM) in the treatment (Ramaswamy et al., 2010), and similarly, but to a smaller extent, happened to 17β-estradiol (reduced from 83 ng g^{-1} DM to 13 ng g^{-1} DM) (Hakk et al., 2005). Contrasting results were found for testosterone, for which Hakk et al., 2005 observed its reduction from 115 ng g^{-1} DM to 11 ng g^{-1} DM, while Shore et al., 1993 found that its concentration increased from 298 ng g^{-1} DM to 525 ng g^{-1} DM, probably due to transformations and reactions of metabolites included in the manure.

Finally, alum treatment could also be effective in reducing the hormone 17β -estradiol, for which Nichols et al., 1997 found that the hormone concentration decreased from 133 ng g^{-1} DM to 101 ng g^{-1} DM when manure is treated.

Same lack of information regards poultry liquid and semiliquid treated manure, for which concentrations of 17 hormones were monitored in the effluent of a lagoon receiving poultry house flushing material by Gall et al., 2014 and Hutchins et al., 2007. It was found that the concentration of the hormones was significantly reduced in the lagoon. For instance, estrone concentration decreased from 2970 ng L^{-1} to 21 ng L^{-1}.

4.3. Occurrence of microorganisms in raw and treated manure

Even if in a limited number of data, also the occurrence of some indicator bacteria (as Heterotrophic bacteria, Total coliforms, Faecal coliforms, *E. coli*, Faecal streptococci) and selected pathogens (namely *Aeromonas hydrophila*, *Campylobacter coli*, *Cryptosporidium parvum*, *Giardia intestinalis*, *Listeria*, *Pseudomonas aeruginosa*, *Salmonella* and *Yersinia enterocolitica*) were monitored in raw and treated manure.
Unfortunately, concentration data were reported in a different unit of measure (*e.g.*, cfu g^{-1}, cells g^{-1}, MPN 100 g^{-1}, and cfu 100 mL^{-1}) avoiding an overall comparison of the results. Thus, the brief discussion made from now on refer to the occurrence data measured in cfu g^{-1}, as they are the most frequently reported. All the data found, and the corresponding references are listed in Table M10 and M11 of Appendix 2.

Regarding raw manure, many concentration data were provided for *E. coli* (54 values), followed by *Campylobacter coli* (32 values), and *Salmonella* (30 values).
In particular, as for indicators, the highest concentrations were found in poultry manure for heterotrophic bacteria (ranging between 10^9-10^{11} cfu g^{-1} DM), followed by total coliforms and *E. coli* (3.8×10^8 cfu g^{-1} DM and 2.6×10^8 cfu g^{-1} DM, respectively). High occurrence values were also measured in cattle manure. For instance, *E. coli*, faecal streptococci and faecal coliforms were reported at a concentration ranging between 3.7×10^7 to 2.3×10^8 cfu g^{-1} DM.
Even in the case of pathogens, the highest concentration was found in poultry manure, regarding Yersinia enterocolitica (2.1×10^6 cfu g^{-1} DM), but in addition, swine and cattle manure exhibited high occurrence values too. For instance, high concentrations were found for *Listeria* and *Giardia intestinalis* in swine manure (9.7×10^5 cfu g^{-1} DM and 1.6×10^5 cfu g^{-1} DM, respectively), and for *Salmonella* and *Campylobacter coli* in cattle manure (5.8×10^5 cfu g^{-1} DM and 1.5×10^5 cfu g^{-1} DM, respectively).
A rapid glance on Figure 4.18 shows that high concentration values were more frequently detected in cattle manure and poultry manure regarding, in both cases, bedding manure. The results reported in Table M10 also highlighted that the difference, in terms of average concentration in cfu g^{-1} DM, from indicators and bacteria, is about 1-5 orders of magnitude.
For example, average concentrations of the indicator bacteria in cattle manure range between 2.1×10^7 cfu g^{-1} DM and 1.3×10^8 cfu g^{-1} DM, while average concentrations for pathogens were measured between 1.9×10^3 cfu g^{-1} DM and 3.1×10^5 cfu g^{-1} DM. Similarly happens in poultry manure, in which average concentrations of indicators range between 1.5×10^5 cfu g^{-1} DM and 2.7×10^{10} cfu g^{-1} DM, and those of pathogens range from 1.1×10^4 cfu g^{-1} DM and 3.4×10^5 cfu g^{-1} DM. Different results were found for swine manure, for which average concentrations of indicators and pathogens are in the same order of magnitude (10^2-10^5 cfu g^{-1} DM), and only slight differences were observed in sheep manure, in which the average concentrations of the indicators were measured with one order of magnitude higher than those of pathogens (10^3-10^4 cfu g^{-1} DM for the first group, and 10^2-10^3 cfu g^{-1} DM for the second).

As to treated manure, a lower amount of data was obtained from past studies, and many of them (50 values out of 64) were collected for microorganisms concentrations in lagooning sludge from lagoons receiving swine or cattle manure. Thus, it was not possible to compare different treatments for the same type of zootechnical waste.

The most studied microorganism was the indicator *E. coli* (20 data) followed by the pathogen Campylobacter coli (13 data).

High values of concentration were measured in cattle lagooning sludge for *Salmonella* (7.2×10^6 cfu g^{-1} DM) and *Listeria* (9.8×10^5 cfu g^{-1} DM), while other microorganisms occurred with concentrations $<2\times10^5$ cfu g^{-1} DM.

Average concentrations of indicator bacteria were observed ranging between 10^3 cfu g^{-1} DM (for *E. coli* in swine lagooning sludge) to 10^5 cfu g^{-1} DM. In comparison, average concentrations of pathogens range between 12 cfu g^{-1} DM (for *Giardia intestinalis* in swine lagooning sludge) to 3.7×10^6 cfu g^{-1} DM (for *Salmonella* in cattle lagooning sludge).

Overall, focusing on the average concentration of each specific microorganisms, it emerges that treatment generally reduces their content.

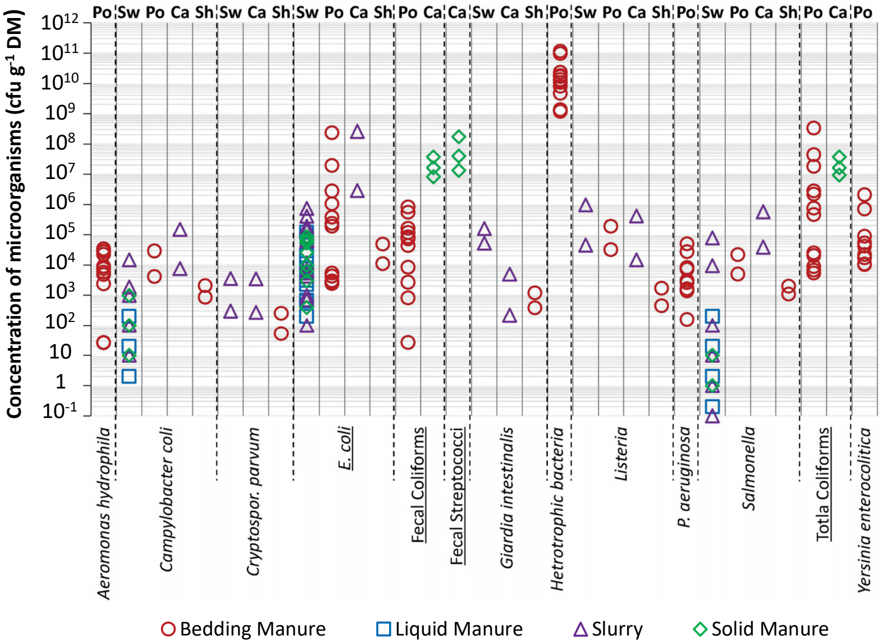


Figure 4.18. Observed concentrations of microorganisms in different types of raw manure generated by different animals (swine, poultry, cattle, sheep). On the x-axis, the underlined names correspond to indicator organisms, those not underlined to pathogens. Data from: Hutchison et al., 2004; Kelley et al., 1994; Patten et al., 1980; Van den Meersche et al., 2019.

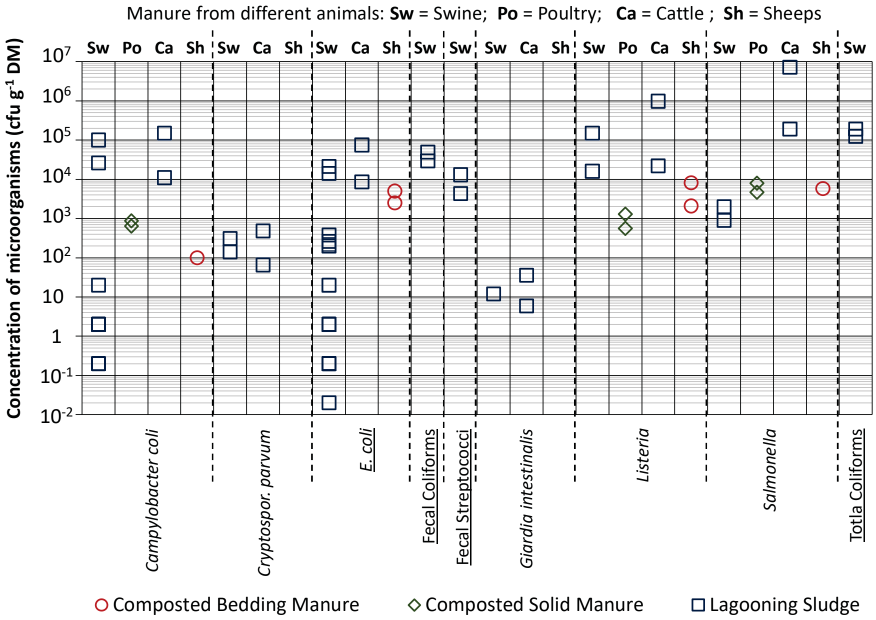


Figure 4.19. Observed concentrations of microorganisms in different types of treated manure generated by different animals (swine, poultry, cattle, sheep). On the x-axis, the underlined names correspond to indicator organisms, those not underlined to pathogens. Data from:(Frey et al., 2013; Hutchison et al., 2004; Van den Meersche et al., 2019.

4.4. Legal requirements for manure application to soil

As already mentioned, manure is commonly applied on arable land to improve soil characteristics (as amendant) and soil nutrients content (as fertiliser). This agricultural practice must fulfil the legal limits set in the various country commonly regarding the maximum quantity of manure to apply, or microorganisms and nutrient content.

An in-depth discussion of the legal requirements set in the different country is beyond the aims of this thesis, but common standards can be outlined, to have a general view of the main parameters monitored before the land application of zootechnical wastes.

Firstly, to avoid the risk of contaminating aquifers due to percolation, a standard parameter which is observed in many policies is organic nitrogen content, based on which the amount of manure to add onto soil is evaluated.

At EU level, the value of 170 kg organic N *per* hectare *per* year is established by the Directive 91/676/EEC (EC, 1991) on regard to areas at risk of nitrate contaminations (vulnerable zones). In some cases, this directive was implemented in the EU countries with further limits. For instance, in Po Valley (Northern Italy), according to the Emilia Romagna regional regulation, limits were also set for areas which are not considered at risk of eutrophication. In this case, a maximum of 340 kg organic N *per* hectare *per* year can be applied with manure amendment.

Precautions to avoid nitrogen pollution have also been taken outside Europe countries, and concern very similar limits. As an example, in Australia, a maximum of 240 kg N year^{-1} hectare^{-1} can be added in soil by manure application (Eldridge et al., 2009).

Main conclusions

- *Pharmaceuticals and hormones occur in various types of zootechnical wastes from different animals (cattle, swine, and poultry) at ng L^{-1} / µg L^{-1} concentration levels.*

- *The treatment (e.g., aerobic or anaerobic digestion, lagooning, composting, etc.) may consistently contribute to the degradation of many studied micropollutants sequestered in sewage sludge.*

- *Compared to sewage sludge, manure exhibited higher concentrations of antibiotics and hormones. On the other hand, sewage sludge generally contains a broader spectrum of compounds, belonging to different families.*

- *The in-force regulations do not set any limit on the occurrence of PhACs in animal manure before its application onto arable land.*

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Chapter 5

FROM ARABLE LAND TO SURFACE WATER: OCCURRENCE OF PPCPs IN SURFACE RUNOFF AND TILE DRAINAGE FROM SEWAGE SLUDGE-AMENDED SOIL

Table of contents

Aims of the chapter, in a nutshell

- *Concentration of pharmaceuticals and personal care products in surface runoff and tile drainage from arable land amended with raw and treated sewage sludge was reviewed.*

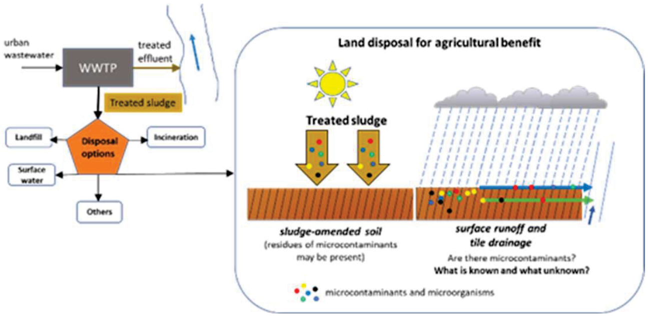
- *Concentration of microorganisms in surface runoff and tile drainage from arable land amended with raw and treated sewage sludge was reviewed.*

- *The factors influencing the transport of micropollutants from sewage sludge-amended soil to surface runoff and tile drainage were discussed.*

- *Lessons learned from the past investigations, and good practices for sludge disposal were briefly reported.*

- *The results of this chapter were published in 2019 in Science of the Total Environment, 655, 939-957, with the title: "A review of selected microcontaminants and microorganisms in land runoff and tile drainage in treated sludge-amended soils" (Ghirardini and Verlicchi, 2019).*

Graphical abstract



5.1. Introduction

In an agricultural land context, during intense rainfall events or due the periodic crop irrigation practices, soil particles are often detached and transported in water streams.
Soil erosion, and the consequent sediment transport, entail the mobilisation of several substances which are adsorbed in the soil matrix, like minerals, metals, and nutrients (Peyton et al., 2016). In this context, considering what has been mentioned so far concerning micropollutants occurrence in sewage sludge and zootechnical wastes, also pharmaceutical compounds, hormones, and other substances may potentially reach the water environment if arable land is amended with these organic fertilisers.
Therefore, in the one hand the application of animal manure and treated sewage sludge may positively contribute to soil properties and fertility (Clarke and Smith, 2011), but on the other, attention must be paid on the adverse effects, in terms of environmental risk due to compound of emerging concern introduction, that this practice may lead to.

From a hydrological point of view, the problem can be schematically represented as in Figure 5.1. Once applied onto the soil (via irrigation, or during rainfall), water starts infiltrating in the unsaturated zone (also referred as vadose zone). Here, it can be intercepted by tile drains, which are specific pipes systems generally placed in agricultural land when soil texture does not provide sufficient water drainage.
The remaining water can continue to percolate, moving to the groundwater, and contributing to soil saturation.
Finally, if the soil surface layer is saturated enough, the exceeding rainwater or irrigation water can no longer infiltrate, generating thus a lateral flowing on the soil surface, the so-called surface runoff.
Further details from a hydrological point of view will be provided below, in Chapter 6, in order to discuss the possible methods to evaluate mathematically the volumes of the vadose zone water flows, and consecutively the PhAC amount they may potentially transport.

Limiting here the attention to micropollutants behaviour in such a complex system, as reported in Figure 5.1, a compound may be subjected to many transformation and degradation processes. Among these, worthy of mention are sorption onto soil particles, photodegradation (occurring in the surface layer of the sludge or manure-amended soil), biodegradation, interactions between chemicals, plant uptake, interactions between pharmaceuticals and plant roots, and finally, once reached the receiving water stream: dilution, further photodegradation, volatilisation, chemical reactions, sorption onto sediments, and further biodegradation.

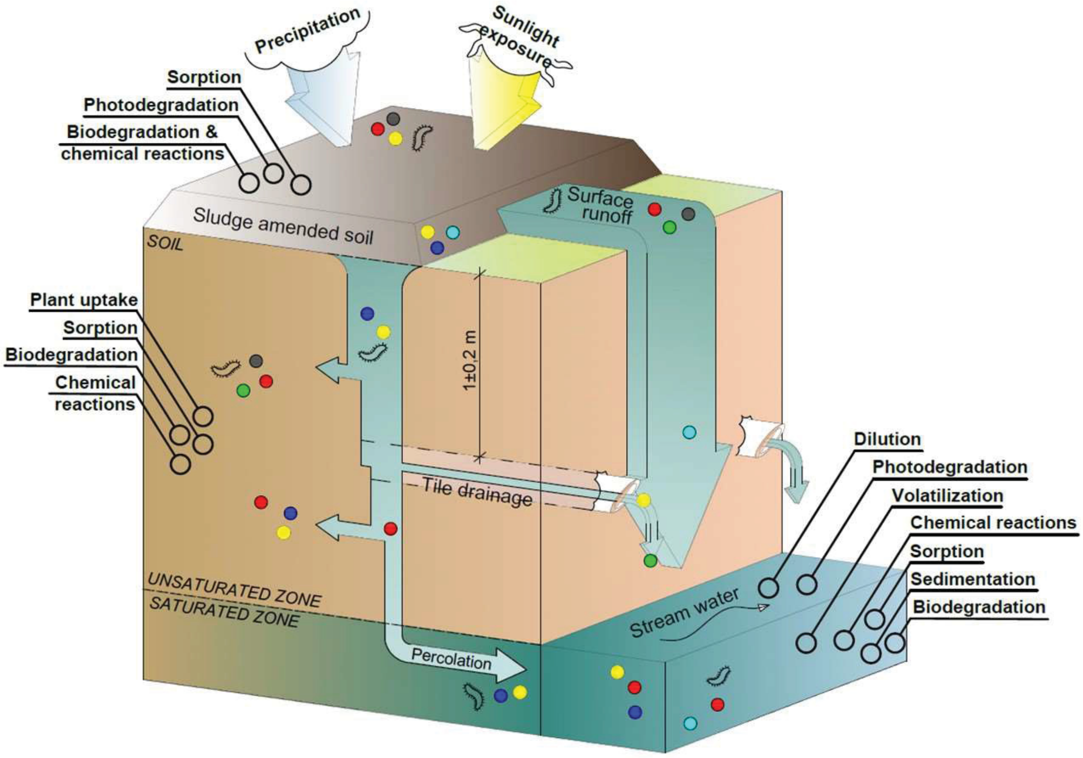


Figure 5.1. Potential soil water flows concerning unsaturated (also referred as vadose zone) and saturated zone, and main removal mechanisms of micropollutants occurring within the soil.

Therefore, to simplify the subject variables, past studies about PPCPs transport in soil water flows mainly focused directly on compound occurrence in surface water after artificial irrigation on specifically constructed soil plots, amended with sewage sludge or manure. The problem was thus generally studied considering the soil as a black box, in which, given an accurate description of the system's variables, PPCPs concentration is measured before the runoff phenomena (in the sludge, or manure, or sludge/manure-amended soil), and after the experiment, in the runoff (or tile drainage) collected.

For example, Biswas et al., 2017 investigated the effects of rainfall timing and tillage practice on hormones transport *via* surface runoff in soil amended with cattle manure; Peyton et al., 2016 compared the runoff quality (in terms of heavy metals, nutrients and microorganisms) from arable land plots that received animal manure and treated sewage sludge; Pan and Chu, 2017 examined the behaviour of veterinary PhACs in percolation (soil column test) through different soil textures, amended with poultry manure; Gottschall et al., 2013 analysed the concentration of selected hormones in tile drainage (1.2 m depth, approximately) coming from an agricultural field (instead of the investigation of the typical plot) amended with treated sewage sludge, to name just a few.

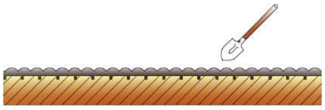
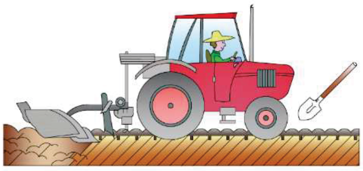
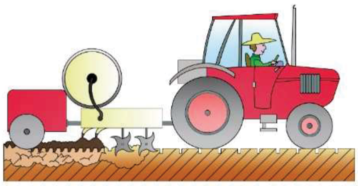
On this background, this chapter aims to provide an overall view on the occurrence of PPCPs in surface runoff and tile drainage from arable land amended with sewage sludge.

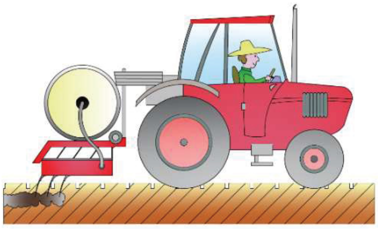
The results here discussed, and further details were published in Volume 655 of Science of the Total Environment (Ghirardini and Verlicchi, 2019).

Here, only plots or fields amended with treated sewage sludge – also referred as *biosolid*, a term introduced in 1991 by the Water Environment Federation, to distinguish raw sludge from treated sewage sludge which could legally be used in agriculture – were considered. On the contrary, experiments regarding manure-amended soils were excluded due to the high variability of their characteristics (as already reported in Table 4.1 of Chapter 4). In this way, it was possible to present the findings of the studies by comparing and discussing the results in terms of compound properties (*e.g.*, $logK_{OW}$), soil characteristics (*e.g.*, texture, organic matter, organic carbon content)., sludge application rate, sludge application rate, and sludge application method and depth.

Regarding this last variable, it should be mentioned that there is not a standardised method to apply sewage sludge in agricultural land. On the contrary, the tilling practice adopted depends on sludge type (mainly in its solid content - liquid or solid sludge), and local habits. A description of the different application methods is provided here in Table 5.1, with typical application depth, a brief description, and literature examples.

Table 5.1. Sewage sludge application methods commonly adopted, with typical application depth and literature references.

Application method	**Graphical description**	**Appl. depth**	**Description**	**References**
Land or Surface spreading		0 cm	With *Land spreading*, biosolids (or manure) are deposited on the land surface without other operations.	Dunigan and Dick, 1980; Eldridge et al., 2009; Healy et al., 2017; Peyton et al., 2016; Wallace et al., 2014
Tilling		5÷20 cm	In the case of *tilling*, sludge is spread on the soil and then it is amended with the first cm of soil within 20-24 h.	Atalay et al., 2007; Edwards et al., 2009; Giudice and Young, 2011; Gottschall et al., 2012, 2013; Gray et al., 2017; Lapen et al., 2008a; Lapen 2008b; Sabourin et al., 2009; Topp et al., 2008; Yang et al., 2012
One-pass Aeration tilling		13÷15 cm	In the case of *one-pass aeration tilling*, just before receiving the sludge, the soil is tilled. It is generally performed by a specific mechanical system that applies the sludge close to the ground, immediately following the passage of rolling tines which affect aerator-type tillage of the soil.	Lapen et al., 2008a; Lapen et al., 2008b

Application method	Graphical description	Appl. depth	Description	References
Subsurface injections		10÷13 cm	*Subsurface injection* consists of an injection of biosolids within 50 cm of the top of the soil surface. Generally, the injection can be used for liquid biosolid application or dewatered that have low solid content and can be easily shovelled.	Edwards et al., 2009; Topp et al., 2008

Overall, this chapter discussion includes the findings of 16 papers, published between 1980 and 2017, referring to 38 investigations regarding the occurrence of PPCPs and some selected microorganism (*E. coli*, faecal coliforms, total coliforms, faecal *streptococcus*, and *clostridium perfringens*) in land runoff and tile drainage from sewage sludge-amended soils.

The term *investigation* is used referring to the focus of a specific study. Selected investigations thus differ in at least one of the following issues: (*i*) soil types, (*ii*) sludge type (liquid or dewatered), (*iii*) sludge application rate, (*iv*) sludge application method, (*v*) flow investigated (runoff or tile drainage), and (*vi*) rainfall frequency and intensity.

Percolation studies were selected only when focusing on tile drainage – instead of groundwater – as tiles are always placed in a well-defined depth range (0.8-1.2 m). A detailed description of the aims and issues addressed by the studies included here is reported in Table 5.2.

Table 5.2. Brief description of the aims of the studies considered, together with experiment characteristics, in terms of soil plot type, rainfall type, rainfall pattern and intensity, sludge type, sludge application rate and type, flow studied and sampling method.

Reference		Main characteristics of the investigations
Atalay et al., 2007	USA	Lab investigations were carried out on the occurrence of 2 microorganisms (*E. coli* and total coliforms), nutrients and heavy metals in the **surface runoff** after treated sludge application. Air-dried sludge was applied at a rate of 2240 kg/ha and mixed with the top 5 cm of the soil bed, on two different soils (a clay loam soil and a sandy loam one called respectively Cullen and Bojac) to compare the influence of soil on the runoff quality. Investigation fields consist of 12 microplots (2 soils x 2 treatments x 3 replicates): tilted aluminium beds (0.8 m x 1.9 m = 1,62 m^2 each) set up in an environmentally controlled greenhouse and used both for treatment and control investigation. Rainfall simulation (deionised water at a rate of 65 mm/h for 45 min) took place immediately before the sludge application (control investigation) and immediately after (treatment investigation), resulting in 2 samples (each with three replicates) per plot available for chemical and microbiological analyses. Number of investigations: 2
Dunigan and Dick, 1980	USA	On-field investigations were carried out on the occurrence of nutrients and Faecal coliforms in **surface runoff** in sludge-amended soil. Treated municipal sludge was applied at different rates (14.8, 16.2, and 28.9 tons/ha) and the concentrations of bacteria were monitored along the following weeks in order to evaluate their temporal variations. Investigation fields consist of triplicate plots and a control one. Rainfall was simulated by applying deionised water at the rate of 1.11 cm/h for 2 hours. Number of investigations: 3

Reference		Main characteristics of the investigations
Edwards et al., 2009	Canada	On-field investigation was carried out on the occurrence (concentrations) and mass loads of 11 PPCPs in agricultural **tile drainage** systems following sludge application. Monitoring lasted approximately 162 d. Dewatered (centrifugated) anaerobically digested sludge was applied to a loam soil at a rate of 8000 kg/ha and mixed with soil bed with two different methods (to compare the results): tilling with the top 10 cm of the soil and direct injections at a depth of 11 cm. The aim is to test the capacity to break apart DMB solid/aggregates and the effect of the atmosphere exposure and the soil environment to the PPCPs. Investigation fields consist of 8 plots (100 m x 15 m= 1500 m^2 each) in a field with tiles posed 0,8 m below the soil surface and spaced 15 m each one by the others; 2 of them were hydraulically isolated and used for control investigation (never received DMB). Sampling occurred after a real rain event (in case of a rainfall depth of 5 mm/h in summer and 7 mm/24 h in fall achieved in a rainwater collection vessel) occurred in the study period with a total depth of 413 mm. Samples were collected with an automatic water sampler when a rainfall depth of 5mm/h (summer) and 7 mm/d (fall) was achieved in a rain collection vessel. Samples were taken more frequently near the trigger followed by a gradual reduction (sample intervals at the beginning every 15 min, than every 30, 60, 90 and 120 mm). Number of investigations: 2
Eldridge et al., 2009	Australia	On-field investigation was carried out on the occurrence of *E. coli* and nutrients in the **surface runoff** after surface spreading of 2 types of dewatered sludges (irradiated, non-irradiated granulated biosolids) and manure (poultry) on a silty clay loam soil covered by turf. Sludges and manure were spreading on the surface. The applied sludges were a high temperature dried sludge (at a rate of 4500 kg/ha, DMB1), a high temperature dried sludge that received gamma irradiation (pathogen-free, at a rate of 4500 kg/ha, DMB2), and a poultry litter (at a rate of 5150 kg/ha). Investigation fields consisted of three replicates plots for four scenarios, resulting in 12 microplots (1 m x 2 m= 2 m^2 each) in a field of a slope of 10%. The four scenarios were: untreated control plot; poultry application, sludge DMB1 application and DMB2 application. Rainfall simulations (potable water at a rate of 90 mm/h for 30 min) take place after 7 days from the application; 2 samples collected for each plot: one for the first 3 L of runoff (first flush) and another for the total runoff volume. Number of investigations: 3 + control
Giudice and Young, 2011	USA	An investigation was carried out on the occurrence of endocrine disrupting compounds and heavy metals in the **surface runoff** after sludge application for approximately 31 d. Dewatered (and thermally dried) anaerobically digested sludge was applied on a sandy loam soil at a rate of 22500 kg/ha and mixed with the top 7÷15 cm of the soil bed. The investigation fields: consist of 3 replicates plots (2m x 1m x 0.38m depth each) which were built in a field of a slope of 3,5÷4% and used for control (before sludge application) and runoff analysis (after sludge application, for three different simulated rain events) as well as leachate analysis. Rainfall simulations (carbon filtrated well water at a rate of 60 mm/h until runoff occurred) took place before 5 days (control) and after 3, 9, 24 days from application in the three plots. Six runoff samples (4 L) were collected after each rainfall simulation, and their cumulative volume was investigated for the analytes of interest. A single 2.5-L leachate sample was withdrawn at the end of each simulation from the composite reservoir collecting the generated leachate (**tile drain** depth = 0,38 m; space between tiles = 0,025 m). Number of investigations: 2
Gottschall et al., 2012	Canada	On-field investigation on the occurrence of 26 PPCPs in **tile drainage** (PPCPs in the soil matrix, groundwater and wheat grain which was grown on the field) after sludge application was monitored for approximately 365 days. Dewatered (centrifugated) anaerobically digested sludge was applied to a loam soil at a rate of 22000 kg/ha and mixed with the top 20 cm of the soil bed. Investigation fields consist of 2 macroplots (3 ha each) in an agricultural field located in Ontario, Canada, fallow the year before the investigation. Tiles were placed 1,1÷1,2 m below the soil surface and spaced of 15 m each one by the other. 1 macroplot represents the control system and the second one, the treatment system (where sludge is applied). Real rainfall occurred in the study period with a total depth of 1070 mm. Samples have been collected at the bottom of the tiles time proportionally when a trigger (adjusted depending on weather and soil water content) occurs. A total of 10 hydrograph event samples were selected for analysis. Number of investigations: 1
Gottschall et al., 2013	Canada	Commercial field-scale investigations were carried out on the occurrence of 17 PPCPs (hormones), 3 pathogens and 10 sterols in **tile drainage**, in surface soil core, DMB aggregates mixed with the soil, groundwater and wheat grain uptake) after sludge application in a real agricultural field (the same of Gottschall et al., 2012) for approximately 365 days. The aim was to study the long-term persistence of the selected compounds in the environmental matrices and to correlate the occurrence of faecal bacteria with sterols. Dewatered (centrifugated)

Reference		Main characteristics of the investigations
		anaerobically digested sludge was applied to a loam soil at a rate of 22000 kg/ha and mixed with the top 20 cm of the soil bed. Investigation fields consist of the same 2 macroplots (3 ha each) in the agricultural field described in Gottschall et al., 2012, with tiles that are posed 1,1÷1,2 m below the soil surface and spaced of 15 m each one by the others. One of them was isolated and used for control investigation. Real rainfall occurred in the study period with a total depth of 1070 mm. Samples have been collected at the bottom of the tiles time proportionally when a trigger (adjusted depending on weather and soil water content) occurs. A total of 8 hydrograph event samples were selected for analysis. Number of investigations: 1
Gray et al., 2017	USA	On-field investigations were carried out on the occurrence of a broad spectrum of anthropogenic waste indicators (including 6 PPCPs) in the **surface runoff** after sludge application on an agricultural field in Colorado for approximately 40 d. The site had not been previously treated with biosolids. Dewatered anaerobically digested sludge was applied on a loamy sand soil at a rate of 3500 kg/ha and mixed with the top 15 cm of the soil bed. The investigation fields consist of 5 microplots (6 m^2 each) in a field of a slope of 2,1÷3%; each one used both for treatment and control investigation. Rainfall simulations (application of hormone-free well water at a rate of 65 mm/h, corresponding to a 100-year simulated rain event) took place before 5 days (control plot) and after 1, 8, 35 days from application in three plots. In the remaining two plots, rainfalls were conducted only at day 35 to evaluate the fate of compounds in the absence of repeated rainfall events. The same plots and similar operational conditions were used in a previous investigation by Yang et al., 2012. Number of investigations: 2
Healy et al., 2017	Ireland	An investigation was carried out on the occurrence of 2 PPCPs (TCS and TCC) in the **surface runoff** after sludge application in a field experiment for approximately 15 d. In order to compare the different behaviour, three differently treated sludges were spread on the surface of a loam soil: an anaerobically digested sludge (at a rate of 6727 kg/ha), a thermally dried sludge (at a rate of 2683 kg/ha), and lime stabilised one (at a rate of 29536 kg/ha). The investigation fields consist of replicated (n=3) hydraulically isolated microplots (0.4 m x 0.9 m= 0,36 m^2 each) in a field of a slope of 2,8÷3,7% (without control ones). Each microplots was equipped with a channel collecting all the runoff during a rain event. Rainfall simulations (at a rate of 11 mm/h) took place after 1, 2, 15 days from sludge application in the same plots. Each rainfall lasted 30 minutes from the time of the first occurrence of surface runoff. Number of investigations: 3
Lapen et al., 2008a	Canada	In field investigation carried on the occurrence of 11 PPCPs in **tile drainage** after sludge application was carried out in Ontario, Canada, for approximately 46 d. Liquid anaerobically digested sludge was applied on a silty clay loam soil at a rate of 93500 L/ha and mixed with soil bed with two different approaches (to compare the influence on the tile drain quality): tilling with the top 10 cm of the soil (subsurface spreading) and one-pass aeration tilling with the top 11 cm (surface spreading). Most of the selected PPCPs were spiked in the sludge before its soil application as their concentration was found below detection limits. The investigation fields consist of three plot replications for each application type with one control bed, for a total of 8 plots (740 m^2 each) in a field with tiles posed 0,8 m below the soil surface and spaced of 15 m each one by the others. Real rainfall occurred in the study period with a total depth of 124 mm. Samples were collected time proportionally when a trigger occurs, at the bottom of the tiles. Number of investigations: 2
Lapen et al., 2008b	Canada	In field investigations on the occurrence of *E. coli* and *C. perfringens* as well as nutrients in **tile drainage** after sludge application was carried out in Ontario, Canada, for approximately 46 d. Liquid anaerobically digested sludge was applied on a silty clay loam soil at a rate of 93500 L/ha and mixed with soil bed with two different methods (to compare the influence on the loss of microorganisms): tilling with the top 10 cm of the soil and one-pass aeration tilling with the top 11 cm. The investigation fields consist of three plot replications for each application type with one control bed, for a total of 8 plots (740 m^2 each) in a field with tiles posed 0,8 m below the soil surface and spaced of 15 m each one by the others. Real rainfall occurred in the study period with a total depth of 124 mm. Samples were collected time proportionally when a trigger occurs, at the bottom of the tiles. Number of investigations: 2
Peyton et al., 2016	Ireland	Investigations on the occurrence of 2 microorganisms, nutrients and metals in the **surface runoff** after sludge application was carried out in Ireland, for approximately 15 d. Five different sludges were applied on the surface of loam soil. The resulting runoff was compared: an anaerobically digested sludge from the UK (at a rate of 6775 kg/ha), anaerobically digested sludge

Reference		Main characteristics of the investigations
		from EIRE (at a rate of 6727 kg/ha), thermally dried sludge (at a rate of 2683 kg/ha), a lime stabilised one (at a rate of 29536 kg/ha) and a dairy cattle slurry (at a rate of 80000 kg/ha). The investigation fields consist of 30 microplots (0.9mx0.4m=0,36 m^2 each) in a field of a slope of 2,8÷3,7% for comparing six different scenarios (treatment with one sludge type + control). For this reason, 6 of them were isolated and used for control investigation. Rainfall simulations (at a rate of 11 mm/h) took place after 1, 2, 15 days from application in the same plots. The first and the last 50 mL of runoff occurring on each plot have been collected (2 samples per plot). Number of investigations: 5
Sabourin et al., 2009	Canada	An infield investigation on the occurrence of 13 PPCPs in the **surface runoff** after sludge application was carried out in Ontario, Canada, for approximately 36 d. Dewatered (centrifugated) anaerobically digested sludge was applied on a silt loam soil at a rate of 8000 kg/ha and mixed with the top 15 cm of the soil bed. The investigation fields consist of 30 microplots (2 m x 3 m=6 m^2 each) in a field of a slope of 7 %. 5 of them were isolated and used for control investigation (no sludge applied to them). A group of 5 (+1 control) microplots was considered for each rain event which took place after 1, 3, 7, 21 and 36 d from the sludge application. Rainfall simulations consisted of ozonated groundwater at a rate of 4,1 mm/min. One runoff sample for each plot was collected. Number of investigations: 1
Topp et al., 2008	Canada	Investigations on the occurrence of 9 PPCPs in the **surface runoff** after sludge application was carried out in Ontario in a real field for approximately 266 d. Liquid anaerobically digested spiked with pharmaceuticals were applied on a silt loam soil (slope 5 %) at a rate of 93500 L/ha and mixed with soil bed. Sludge was amended to the soil by two different approaches: by tilling with the top 15 cm of the soil and by subsurface injections at a depth of 10 cm. Spiked ozonated groundwater was added to the plots only by subsurface injection at a depth of 10 cm. The investigation fields consisted of 75 microplots (2 m x 1 m =2 m^2 each) in a field of a slope of 5%. The “control” plots were the 25 ones receiving spiked water, but none of the plots has been tested without PPCPs sources. Rainfall simulations (ozonated groundwater) took place after 1 or 3 or 7 or 21 or 36 or 266 days from application in plots that have never received rainfall before to investigate the degradation/adsorption effects. Rainfall simulated events lasted until a minimum of 10 l of runoff was collected in each microplot. Number of investigations: 3
Wallace et al., 2014	USA	An investigation on the occurrence of microorganisms and nutrients in the **surface runoff** after dewatered anaerobically digested sludge application was carried out in Missouri for approximately 54 d. Sludge and mineral fertiliser were applied (to compare the results) on a silt loam soil and on the same soil but with a vegetation strip buffer (to compare the buffer ability in reducing key compound losses in runoff) with surface spreading application. Four investigations occurred: untreated control plot; low rate (1664 kg/ha) of biosolids with 1 m of a vegetative filter; low rate (1664 kg/ha) of biosolids without filter, high rate of biosolids with 1 m of vegetative filter (3328 kg/ha). The investigation fields consist of operating and control plots (1.5mx2x=3 m^2 each) in a field of a slope of 3÷6%. For each experiment, four replicates were carried conducted. Rainfall simulations (deionised water at a rate of 70 mm/h) take place immediately after application. One sample per plot has been collected. Number of investigations: 5
Yang et al., 2012	USA	In field investigation was carried out in Colorado on the occurrence of 17 PPCPs (hormones) and 2 sterols in the **surface runoff** after sludge application for approximately 40 d. Dewatered anaerobically digested sludge was applied on a loamy sand soil at a rate of 3500 kg/ha and mixed with the top 15 cm of the soil bed. An analysis of the hormone partitioning between dissolved phase and suspended-particle bond phase was reported. The investigation fields consist of the microplots (6 m^2 each) in a field of a slope of 2,1÷3%; used for both treatment and control investigation. Rainfall simulations (hormone-free well water at a rate of 65 mm/h) took place before 5 days (control) and after 1, 8, 35 days from application in the same plots. 3 composites samples (early, middle, and late rain event) were collected per plot for control (5 days before sludge application) and on the first, 8th and 35th day. As at day 35 only two plots were monitored, a total of 33 composites samples were available. Number of investigations: 1

5.2. Literature findings on runoff of PPCPs and microorganisms from treated sewage sludge-amended soils

The sludge applied in the reviewed experiments was commonly anaerobically digested and, in many cases, dewatered. Exceptions were found for Eldridge et al., 2009, which adopted gamma irradiation as sludge treatment, and Healy et al., 2017 and Peyton et al., 2016 which used lime stabilised sludge.
Hereinafter, the sludge will also be referred as liquid municipal biosolid (LMB) or dewatered municipal biosolid (DMB) depending on its solid content, which, according to Sabourin et al., 2009, is less than 18% for LMB and higher than 18% for DMB.
The concentration of micropollutants in the sludge before the experiments is reported below in Table 5.3, together with ranges of concentrations found in literature as reported above in Chapter 3, and reviewed in Verlicchi and Zambello, 2015.

Table 5.3. Concentrations of PPCPs in treated sludge applied on the soil in the investigations reported in Table 5.2, together with ranges reported in the literature. Number in italics correspond to concentrations found below the limit of detection (LOD).

Class	**Compound (Literature concentration of municipal digested sludge)^a**	**This study [ng g^{-1}]**	**This study [ng L^{-1}]**	**Reference**
Analgesics/ anti-inflammatories	Acetaminophen (0,006-419 ng g^{-1})	18,7		Gottschall et al. 2012
		24		Edwards et al. 2009
		28,6±11,4		Sabourin et al. 2009
		1164		Lapen et al. 2008b
			>100000^b	Topp et al. 2008a
	Ibuprofen (0,006-1274 ng g^{-1})	63,6		Gottschall et al. 2012
		657±334		Sabourin et al. 2009
		750		Edwards et al. 2009
		1524		Lapen et al. 2008b
			NA	Topp et al. 2008a
	Naproxen (0,001-354 ng g^{-1})	6		Gottschall et al. 2012
		394±35,5		Sabourin et al. 2009
		470		Edwards et al. 2009
		477		Lapen et al. 2008b
			>10000^b	Topp et al. 2008a
Antibiotics	4-Epitetracycline	334		Gottschall et al. 2012
	Ciprofloxacin (24,4-6300 ng g^{-1})	3260		Gottschall et al. 2012
	Ofloxacin (68-2000 ng g^{-1})	1400		Gottschall et al. 2012
	Oxytetracycline	7,34		Gottschall et al. 2012
	Sulfamethoxazole (0,6-178 ng g^{-1})	12,4±1,6		Sabourin et al. 2009
		15		Edwards et al. 2009
		22±1		Lapen et al. 2008b
			>10000^b	Topp et al. 2008a
	Sulfapyridine (5-38 ng g^{-1})	25		Lapen et al. 2008b

Class	Compound (Literature concentration of municipal digested sludge)[a]	This study [ng g^{-1}]	This study [ng L^{-1}]	Reference
	Tetracycline	513		Gottschall et al. 2012
Antifungals	Miconazole (39-970 ng g^{-1})	341		Gottschall et al. 2012
Antihistamines	Diphenhydramine	689		Gottschall et al. 2012
Antiseptics	Triclocarban (81-63000 ng g^{-1})	50		Healy et al. 2017
		<2400		Healy et al. 2017
		4940		Gottschall et al. 2012
		8000		Edwards et al. 2009
		8194±28,3		Sabourin et al. 2009
		17600		Giudice and Young 2011
	Triclosan (90-46000 ng g^{-1})	270		Healy et al. 2017
		1007±54		Lapen et al. 2008b
		4900		Healy et al. 2017
		7066±29,3		Sabourin et al. 2009
		9140		Gray et al. 2017
		14000		Gottschall et al. 2012
		15900		Giudice and Young 2011
			NA	Topp et al. 2008a
Beta-blockers	Atenolol (0,44-22 ng g^{-1})	1,6±0,6		Sabourin et al. 2009
		22		Edwards et al. 2009
		43±6		Lapen et al. 2008b
			>10000[b]	Topp et al. 2008a
Fragrances and PCPs ingredients	3-Menthyl-1H-Indole (or Skatole)	372		Gray et al. 2017
	Galaxolide (HHCB) (1900-81000 ng g^{-1})	3350		Gray et al. 2017
	Indole	458		Gray et al. 2017
	Menthol	458		Gray et al. 2017
	Tonalide AHTN (120-16000 ng g^{-1})	191000		Gray et al. 2017
Hormones	11-Ketotestosterone	1,24		Yang et al. 2012
	17-α-estradiol (or Alfatradiol)	0,3		Yang et al. 2012
		0,48		Yang et al. 2012
		23,7		Gottschall et al. 2012
	17-beta-estradiol (Estradiol) (0,71-836 ng g^{-1})	0,3		Yang et al. 2012
		0,48		Yang et al. 2012
		23,7		Gottschall et al. 2012
	α-Dihydroequilin	23,7		Gottschall et al. 2012
	Androstenedione	15,59		Yang et al. 2012
		28,25		Yang et al. 2012
		66,6		Gottschall et al. 2012
	Androsterone	194		Gottschall et al. 2012
	Cis-androsterone	15,74		Yang et al. 2012
		19,14		Yang et al. 2012
	Desogestrel	32,7		Gottschall et al. 2012
	Diethylstilbestrol	0,3		Yang et al. 2012
		0,48		Yang et al. 2012
	Dihydrotestosterone (Stanolone)	3,41		Yang et al. 2012
		6,48		Yang et al. 2012
	Epitestosterone	1,52		Yang et al. 2012
		2,38		Yang et al. 2012
	Equilenin	0,79		Yang et al. 2012
		1,91		Yang et al. 2012

Class	Compound (Literature concentration of municipal digested sludge)[a]	This study [ng g^{-1}]	This study [ng L^{-1}]	Reference
		4,73		Gottschall et al. 2012
	Equilin	1,52		Yang et al. 2012
		2,38		Yang et al. 2012
		43,7		Gottschall et al. 2012
	Estradiol Benzoate	43,7		Gottschall et al. 2012
	Estriol (2,18-79,8 ng g^{-1})	0,79		Yang et al. 2012
		1,24		Yang et al. 2012
		29,4		Gottschall et al. 2012
	Estrone (3,16-22512 ng g^{-1})	72,43		Yang et al. 2012
		83,78		Yang et al. 2012
		160		Gottschall et al. 2012
	Ethinyl Estradiol (1,5-483 ng g^{-1})	2,3		Yang et al. 2012
		3,17		Yang et al. 2012
		29,6		Gottschall et al. 2012
			NA	Giudice and Young 2011
	Mestranol	0,3		Yang et al. 2012
		0,48		Yang et al. 2012
		9890		Gottschall et al. 2012
	Norethindrone	0,3		Yang et al. 2012
		0,48		Yang et al. 2012
		7,89		Gottschall et al. 2012
	Norgestrel (Levonorgestrel)	9,06		Gottschall et al. 2012
	Progesterone (90 ng g^{-1})	12,81		Yang et al. 2012
		21,69		Yang et al. 2012
		81		Gottschall et al. 2012
	Testosterone	0,3		Yang et al. 2012
		0,48		Yang et al. 2012
		4,61		Gottschall et al. 2012
Lipid regulators	Gemfibrozil (5-118 ng g^{-1})	24		Edwards et al. 2009
		31±2,2		Sabourin et al. 2009
		57		Gottschall et al. 2012
		461		Lapen et al. 2008b
			>10000[b]	Topp et al. 2008a
Stimulants	Caffeine (7,6-350 ng g^{-1})	35,4±12,8		Sabourin et al. 2009
	Cotinine	1,3		Edwards et al. 2009
		1,8		Sabourin et al. 2009
		9,4		Gottschall et al. 2012
		111±9		Lapen et al. 2008b
			NA	Topp et al. 2008a
Psychiatric drugs	Buproplan	NA		Gottschall et al. 2012
	Carbamazepine (0,01-735 ng g^{-1})	6,7±0,6		Sabourin et al. 2009
		9		Edwards et al. 2009
		183		Gottschall et al. 2012
		697±24		Lapen et al. 2008b
			NA	Topp et al. 2008a
	Citalopram (95-3294 ng g^{-1})	114,2		Gottschall et al. 2012
	Desmethyl Citalopram	NA		Gottschall et al. 2012
	Desmethyl Sertraline	NA		Gottschall et al. 2012
	Desvenlafaxine	NA		Gottschall et al. 2012
	Fluoxetine	109		Gottschall et al. 2012

Class	Compound (Literature concentration of municipal digested sludge)[a]	This study [ng g^{-1}]	This study [ng L^{-1}]	Reference
	(34-339 ng g^{-1})			
	Norfluoxetine (8,9-93 ng g^{-1})	NA		Gottschall et al. 2012
	Norvenlafaxine	NA		Gottschall et al. 2012
	Paroxetine (11-89 ng g^{-1})	NA		Gottschall et al. 2012
	Sertraline (203-913 ng g^{-1})	11,2		Gottschall et al. 2012
	Venlafaxine (289-2735 ng g^{-1})	19,1		Gottschall et al. 2012

[a] literature range concentration data found in Verlicchi and Zambello, 2015

[b] supplementary concentration added to the sludge and concentration already into the sludge not measured.

As represented in Figure 5.2, in the well-known soil textural classification triangle, the experiments were conducted in soil with various textures, allowing to compare the results in terms of ground characteristics.

The surface runoff investigations took place in plots whose size was in the range of 0.36-6 m^2, whereas, except for Giudice and Young, 2011 (who employed 2 m^2 wide plots) tile drainage investigation were conducted in fields or large area (from 740 m^2 to 3 ha).

Regarding the artificial irrigation – carried out in 30 out of 38 investigations – different patterns and intensity were identified. Rain intensity was simulated in the range of 11-90 mm h^{-1}, corresponding to rain with a specified return period, typical of the country where the investigation took place.

Details about rainfall duration were not always provided, as in most of the cases the irrigation was simulated until runoff or tile drainage *phenomena* occurred, in order to collect a specific water volume.

Some studies (among them Sabourin et al., 2009 and Topp et al., 2008) tested the runoff event in the day 0 (before sludge application) and after defined days (as long as 266), to monitor the attenuation of concentration of pollutants in runoff samples following the alternation between dry and wet periods.

In five studies (Edwards et al., 2009; Gottschall et al., 2013, 2012; Lapen et al., 2008a, 2008b) referring all to tile drainage experiments, the investigation took place on a real field. Thus, the rainfall was not simulated, but real, and the authors provided in detail the weather conditions reported in the period under study.

Overall, the authors provided a good amount of information about rainfall type, intensity, and pattern, allowing to compare the results based on this aspect. All the details are reported in Table S1 of Ghirardini and Verlicchi, 2019, and here excluded for brevity.

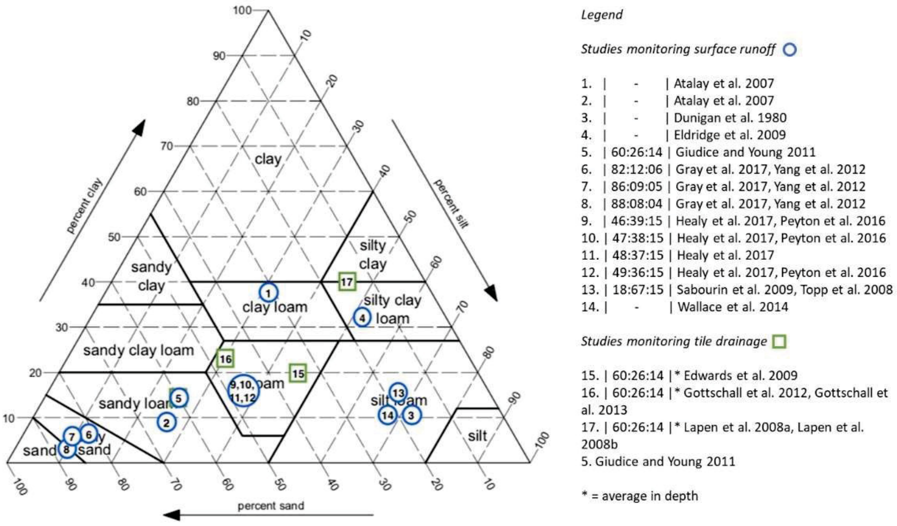


Figure 5.2. Soil texture of the plots employed in surface runoff and tile drainage investigations. Ratios between sand:silt:clay is reported in brackets, when provided by the studies.

5.2.1. Occurrence of PPCPs in land runoff and tile drainage

Concerning surface runoff, the occurrence of 33 compounds was investigated. Among them, the hormones ethynyl estradiol was the most studied (37 measures), followed by other hormones (among them estradiol, estriol, estrone, progesterone, and testosterone, with 33 values each one) and the antiseptics triclosan and triclocarban (26 values each).

As represented in Figure 5.3a, the measured occurrence variability range varied from 1 to 3 orders of magnitude for the studied PPCPs, and the highest reported concentration was found for ibuprofen (1477 ng L^{-1}) in the experiment of Topp et al., 2008 (carried out with LBM in silt loam soil, Table 5.2). The same study also observed the highest concentrations for tonalide and gemfibrozil.

The ranging values of concentration showed in Figure 5.3a are due to the fact that surface runoff was sampled in the same plot during different days of the experiment. In this context, as remarked by Sabourin et al., 2009, the temporal patterns of micropollutants transport were different for each compound. For instance, for some of them (namely triclosan, atenolol, acetaminophen, and sulfamethoxazole), the highest concentration was observed on the first day of the experiment, immediately after sludge application. While, for some others (namely, naproxen, triclocarban, carbamazepine, and caffeine), the peak of concentration was measured in the rainfall applied 2 to 7 days after the sludge amendment. This *phenomenon* is probably due to the sequestration onto soil particles, which act as a barrier from the mobilisation of PPCPs during rainfall events.

A brief statistical description of PPCPs concentration values (min, max, average, standard deviation) and background concentrations, which were measured before the sludge application, are reported in Table R1 of Appendix 3, together with a comparison between these measured values and those found in the literature for different water flows (such as surface water or WWTP effluent).

Regarding tile drainage, concentration values were provided for 46 compounds, but only 33 of them were found to occur above the limit of detection and therefore, were here included in Figure 5.3b.

The most studied compounds were the antiseptic triclosan (90 data), the nicotine metabolite cotinine (85 data) and the anticonvulsant carbamazepine (80 data). The observed concentration varied within 2-3 orders of magnitude, in which the highest concentration values were found by Lapen et al., 2008b which experimented the injection of LMB in silty clay loam (Table 5.2). In particular, the authors observed the highest concentrations for ibuprofen (4,117 ng L^{-1}), naproxen (1,045 ng L^{-1}), triclosan (3,676 ng L^{-1}), gemfibrozil (1,040 ng L^{-1}), and carbamazepine (1,136 ng L^{-1}).

Further details and statistical description (min, max, average, standard deviation, and the number of data available) about the occurrence of PPCPs in tile drainage are provided in Table R2 of Appendix 3.

Overall, it was observed that, focusing on each micropollutant, its average and maximum concentrations in tile drainage are generally higher (up to 1 order of magnitude) than those in surface runoff (Figure 5.3, Table R1, and Table R2 of Appendix 3). Nevertheless, it is not possible to state that tile drainage flow may contribute more to surface water pollution compared to land runoff, as the statistical analysis was consistently driven by the concentration data reported one particular study (Lapen et al., 2008b). In this specific case, the authors tested the influence of the presence of soil macropores in enhancing the concentration of micropollutants in tile drainage. Furthermore, the sludge adopted was particularly liquid, and, as reported by the authors, it could be directly intercepted by tile drains. This special case study demonstrated that, as better explained below in section 5.2.2, the type of sludge (liquid or dewatered) and the soil characteristics may consistently influence the mobilization of PPCPs in soil water flows. Excluding the results of Lapen et al., 2008b, it emerges that the average and maximum concentrations of each PPCP in surface runoff and tile drainage are in the same order of magnitude, suggesting that the characteristics which influence the water flow have more impact of the type of flow *per se*, in terms of resulting concentration of PPCPs in the outcome.

Finally, is worth noting that the observed concentrations, in both surface runoff and tile drainage, were generally lower than those observed in previous studies regarding WWTP effluent (Verlicchi et al., 2012), surface water (Galindo-Miranda et al., 2019) or in runoff from arable land irrigated with reclaimed wastewater (Pedersen et al., 2005).

Furthermore, as remarked by Gottschall et al., 2013 the observed concentrations of hormones are unlikely to result in significant environmental impacts, even in case of amendment with a considerable amount of sludge (the authors applied 22 Mg ha^{-1} of DMB, which is 3 times higher than the amount commonly applied in the other studies, around 8 Mg ha^{-1}).

These findings suggest that the concentrations of PPCPs in runoff and tile drainage are, except for specific exceptions, generally lower than those reported as acute toxicological endpoints, but may cause anyway chronic effects on aquatic biota. In this context, only lacking data and information are yet available to discuss the subject further.

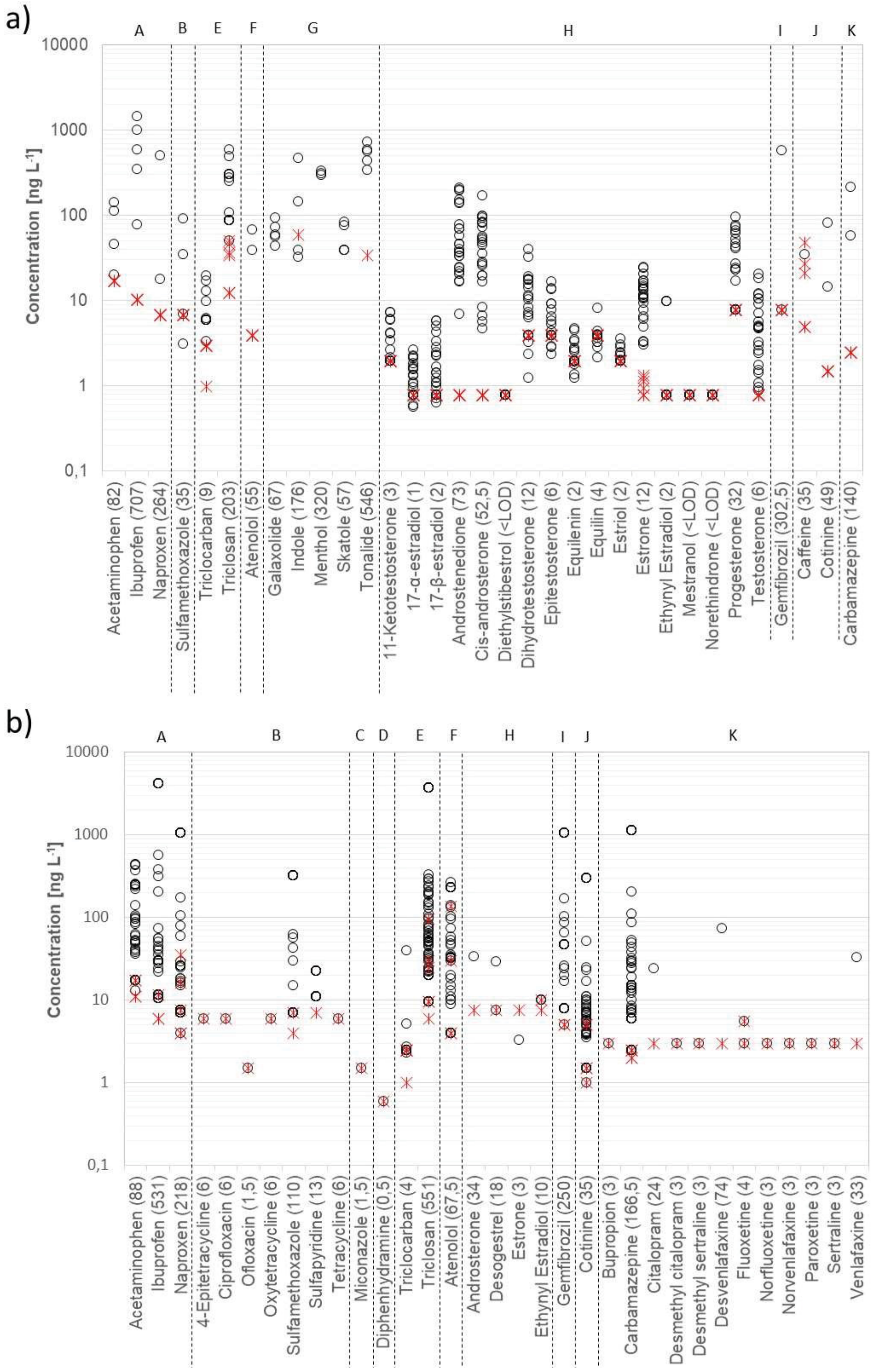


Figure 5.3. Occurrence in **surface runoff** (a) and **tile drainage** (b), of analgesics and antiinflammatories (A), antibiotics (B), antifungals (C), antihistamines (D), antiseptics (E), beta-blockers (F), personal care products (G), hormones (H), lipid regulators (I), stimulants (J), and psychiatric drugs (K), from arable land in which sewage sludge was applied (MEC, ○) and not applied yet (background concentrations, ✻). Data from: Edwards et al., 2009; Giudice and Young, 2011; Gottschall et al., 2013, 2012; Gray et al., 2017; Healy et al., 2017; Lapen et al., 2008a; Peyton et al., 2016; Sabourin et al., 2009; Topp et al., 2008; Yang et al., 2012.

5.2.2. Factors affecting PPCPs occurrence in soil water flows

Due to the complexity of the soil-water system, it is difficult to correlate the behaviour of a substance to a factor strictly. Nevertheless, focusing on the studies which investigated runoff and tile drainage varying one parameter of the experiment at a time (*e.g.*, different soils, or different rainfall patterns), it was possible to discuss the influence of these parameters.

Firstly, regarding the **physicochemical characteristics of a compound**, it was found that the mobilisation in aqueous runoff or tile drainage may be correlated with the K_{OW} of the substance. For instance, Sabourin et al., 2009 observed that chemicals with a $LogK_{OW}$ lower than 2.45 (*e.g.*, atenolol, carbamazepine, cotinine, caffeine, and acetaminophen) tended to be transported in the runoff, while those with a $LogK_{OW}$ higher than 3.18 (*e.g.*, gemfibrozil, naproxen, and ibuprofen) were more likely retained in the soil particles.
Similarly, Gottschall et al., 2012 found that compounds with consistent values of $LogK_{OW}$ (at least equal to 4, as for fluoxetine, miconazole, and tetracycline) were only partially mobilised in tile drainage, suggesting that they remained bounded in sludge or soil particles.
This is not surprising bearing in mind that K_{OW} gives a measure of the lipophilicity/hydrophilicity of a compound, thus entailing to estimate its tendency to be transported with water flow.
Despite this, some exceptions were observed by Sabourin et al., 2009. This is the case, for example, of sulfamethoxazole, which presents a very low $LogK_{OW}$ (equal to 0.89) but only the 0.5% of its initial mass (measured in the sludge before the experiment) was transported in the aqueous runoff. Another example is represented by the two antiseptics triclocarban and triclosan, which, even if they exhibited a similar $LogK_{OW}$ (4.7 and 4.9, respectively), it was found that about 40-times more triclosan was exported in the runoff.
Similar results were reported by Giudice and Young, 2011, that explained these different behaviours considering the pK_a of the compounds. In particular, as the soil-sludge system had an average pH of 8, and runoff pH varied between 7.8 and 8, triclosan is more likely to dissociate (pK_a = 8.1) compared to triclocarban (pK_a = 12.7), being thus more prone to leachate.
Mobilisation of PPCPs in surface runoff and tile drainage is also greatly influenced by the **soil characteristics**. Primarily, the more the soil receive water, the more it becomes compact, entailing a higher runoff instead of percolation. Thus, as one can expect, the moisture content influences the water flow, and *mutatis mutandis*, the time the chemicals are available for sorption onto the soil, degradation, and transformation. Consecutively, all the parameters these processes belong (such as temperature, soil pH, compound physicochemical characteristics, soil carbon content, and so forth, which are described in detail in Monteiro and Boxall, 2009) become particularly important.
In this context, it is interesting to highlight that macropores, which are very common in agricultural land due to worm burrows, can favour the rapid gravity flow of water (and contaminants) to tile drains or groundwater. This phenomenon was clearly observed by Lapen et al., 2008a, which – as already mentioned above in Section 5.2.1. – measured considerably higher concentrations of PPCPs in tile flow. On the contrary, in case of

micropores, due to previous tillage and mixing of the soil, microcontaminants remain in the first 20-30 cm of the soil, being thus less available for percolation, and more for surface runoff (Gottschall et al., 2013).

In this regard, great attention should also be paid on **sludge type** (DMB or LMB): in case of LMB, the sludge may easily percolate through macropores, entailing the occurrence of PPCPs in tile drainage or groundwater (Lapen et al., 2008a). On the contrary, in the case of DMB, sludge may be retained for a prolonged period on the soil surface, causing an environmental risk of PPCPs in surface runoff (Edwards et al., 2009).

For instance, Lapen et al., 2008b measured that the time the liquid sludge may take to reach tile drains in case of macropores ranges between 3 and 39 minutes. Whereas, employing dewatered sludge (as in Edwards et al., 2009), PPCPs can take more than 8 days to be measured in tile drainage.

Overall, considering both the last considerations and those made for soil characteristics, it is possible to infer that, for both surface runoff and tile drainage flow, the application of biosolid should be made in an adequately mixed soil. In fact, micropores allow adequate oxygenation of the sludge-soil system, enhancing micropollutants sorption and degradation in the ground, before being available for mobilisation in the aqueous phase.

This assumption was confirmed by the studies of Lapen et al., 2008a, 2008b and Topp et al., 2008 which thoroughly investigated the influence of **sludge application method**, and found that the so-called *one-pass aeration tilling* practice (described in Table 5.1) may help to mix the soil just before the sludge amendment properly. Furthermore, sludge injection can be adopted (in case of LMB) instead of one-pass aeration tilling, obtaining similar results concerning surface runoff, but paying attention in case tile drains (or shallow groundwaters) are present in the field (Topp et al., 2008).

One-pass aeration tilling may also contribute to better diffuse the oxygen in sewage sludge-amended soil, especially in the case of DMB application. In facts, the lack of oxygen in the DMB aggregates may result in greater persistence of PPCPs, which would be more prone to degradation in case of LMB application (Sabourin et al., 2009).

Finally, in addition to physicochemical characteristics of the compound, soil properties, sludge type, and sludge application method, also the **rainfall characteristics** (intensity, pattern, volume) may play an important role in the occurrence of PPCPs in surface runoff and tile drainage.

In this context, it was observed in many studies (among them Gray et al., 2017; Lapen et al., 2008a; Yang et al., 2012 and Topp et al., 2008) that concentrations of PPCPs moved in surface runoff and tile drainage are generally attenuated during subsequent rain events, but can persist even after 266 days. Of course, the worst-case scenario for PPCPs occurrence in the water environment is represented by a consistent rainfall event which causes surface runoff immediately after sludge amendment. For this reason, many countries avoid sludge and manure application during the wet season or rainfall periods (Kelessidis and Stasinakis, 2012).

5.2.3. Occurrence of microorganisms in land runoff and tile drainage

Occurrence of pathogens and indicators in surface runoff and tile drainage is represented in Figure 5.4 and statistically described in Table R1 and R2 of Appendix 3.

Due to the different unit of measure used in the experiments, it was not possible to compare all the results. Thus, in Figure 5.4, only those microorganisms for which more than 12 data in the same unit are available were included.

It emerged that, except for Eldridge et al., 2009, the application of sewage sludge onto soil always led to an increment of 3-5 orders of magnitude in the content of microorganisms in runoff and tile drainage.

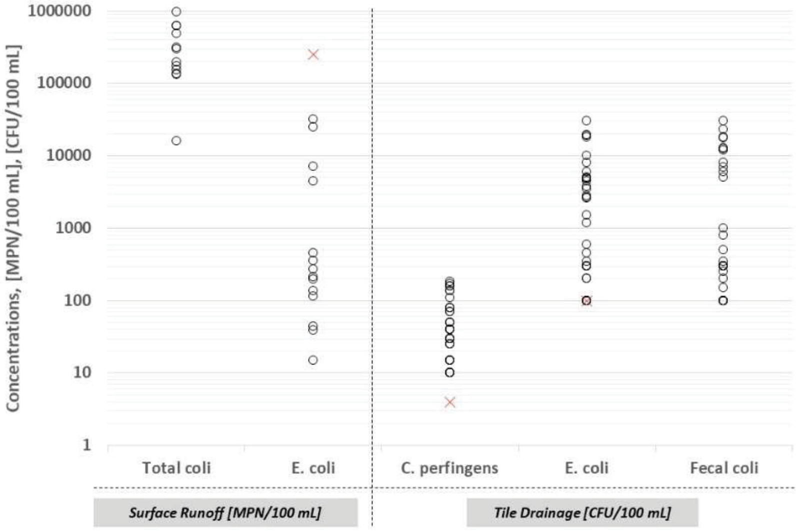


Figure 5.4. Occurrence of microorganisms in surface runoff and tile drainage from sludge-amended soils. Data from: Eldridge et al., 2009; Gottschall et al., 2013, 2012.

The rising concentrations were not correlated with the rainfall application as in the case of PPCPs. On the contrary, in some cases, bacteria could also find good soil characteristics which favour their development.

For this reason, it is difficult in the case of microorganisms to find strict correlations between their content in runoff and tile drainage and soil characteristics, sludge type, rainfall pattern, and so forth. Nevertheless, it was observed that the amendment with biosolids which underwent gamma irradiation treatment – which entail the almost complete elimination of microorganisms from sludge aggregates – did not result in further water contaminations (Eldridge et al., 2009). The same reduction in microorganisms content may be monitored in case of application of thermal dried or disinfected sewage sludge, suggesting that the application of these biosolids, not only avoid a further increase of pathogens and indicators in the soil but also may entail stressful conditions for the growth of the bacteria already present in the ground.

In any case, in the soil environment the primary removal mechanisms for microorganisms are due to desiccation, oxygen content, organic matter content, texture and ultraviolet light (Gondim-Porto et al., 2016), thus the application method plays a particularly important role. Therefore, enhancing contaminants sorption/retention in the soil with the disruption of surface macropores with *one-pass aeration tilling* may be helpful in contrast the optimal soil characteristics for bacteria growth (Lapen et al., 2008a).

These considerations were also confirmed by the findings of Atalay et al., 2007, which observed that soil texture and tilling depth (the authors incorporated the sludge at 5 cm and 10 cm soil depth) were the main factors affecting the occurrence of microorganisms in surface runoff.

In this context, also the soil water content – and thus rainfall application – gain significance in these phenomena. For instance, Peyton et al., 2016 found that bacteria counts in surface runoff may increase after the first day of rainfall application due to their regrowth during the redrying of the soil. Unfortunately, different results were found by Dunigan and Dick, 1980, which observed that counts of microorganisms decreased as the soil become drier.

Thus, overall, these contrasting results confirm that due to the significant variability of microorganisms species, it is not possible to find a single behaviour in such a complex environment as the sludge-amended soil is.

Main conclusions

- *Pharmaceutical and personal care products occur in surface runoff and tile drainage from sewage sludge-amended soil at ng* L^{-1} *- µg* L^{-1} *concentration levels.*

- *The observed concentrations, in both surface runoff and tile drainage, were generally lower than those observed in previous studies regarding WWTP effluent, surface water, and runoff from arable land irrigated with reclaimed wastewater.*

- *The average concentration of micropollutants observed in surface runoff is similar to that observed in tile drainage.*

- *Among the parameters which mainly influence the mobilization of micropollutants in surface runoff and tile drainage are worth of mention the physicochemical characteristics of the compounds (e.g.,* K_{OW} *and* pK_a*), the sludge type (liquid or dewatered), the sludge application method (e.g., surface spreading, tilling, and subsurface injection), and rainfall intensity and pattern.*

- *Only lacking - and difficult to compare – data were found regarding the concentration of microorganisms in surface runoff and tile drainage.*

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Chapter 6

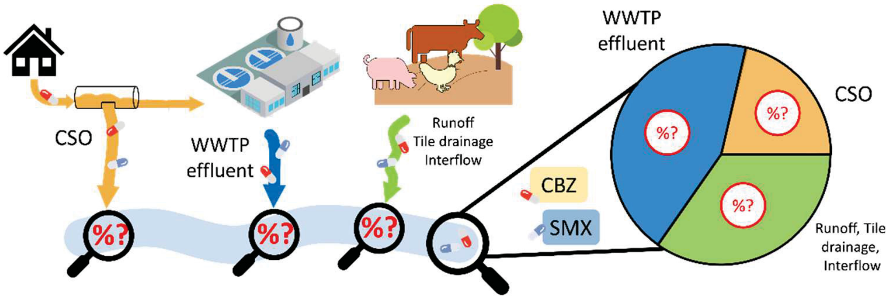
EVALUATION OF THE DIFFERENT SOURCES AND EMISSION PATHWAYS OF PHARMACEUTICALS ON A CATCHMENT SCALE

Table of contents

Aims of the chapter, in a nutshell

- *The main sources (human and animal consumption) and emission pathways (treated effluent, combined sewer overflow, and soil water flows) of pharmaceuticals in the water environment, on a catchment scale, were discussed.*
- *The main modelling tools used to predict the behaviour of pharmaceuticals in different environmental compartments were addressed.*
- *The main parameters influencing the occurrence of pharmaceuticals in the receiving water bodies, and the evaluation of their uncertainty, were discussed.*
- *The issues addressed were applied on a specific case-study, in which the main sources and emission pathways of carbamazepine and sulfamethoxazole in an Austrian catchment were evaluated, on yearly basis.*
- *The main results of this chapter were published in 2021 in Science of the Total Environment, 751, 142328 with the title: Most relevant sources and emission pathways of pollution for selected pharmaceuticals in a catchment area based on substance flow analysis (Ghirardini et al., 2021).*

Graphical abstract



6.1. Evaluation of pharmaceutical load in the different environmental compartments

In a watershed context, it is possible now to identify two main *sources* of PhACs, namely humans and animals. Regarding the first one, once consumed, a PhAC and its metabolites, may enter in the water environment through its discharge in the sewer system. Here, it is conveyed to the wastewater treatment plant (WWTP) in which, as already mentioned in Chapter 1, it can be transformed, degraded, or discharged as it is in surface water bodies through WWTP effluent.

Otherwise, in case of high-intensity rainfall events, wastewater may be directly discharged in the receiving water body without further treatments *via* combined sewer overflow (CSO), and thus releasing consistent concentrations of micropollutants in the water environment.

Regarding the animal source of PhAC in the environment, as already described in Chapter 4, once consumed, these can reach the agricultural land after manure application and pose an environmental risk in case of surface runoff, tile drainage and percolation to the receiving water stream.

Therefore, three main *emission pathways* of PhACs in the water environment can be outlined: WWTP effluent, CSO and soil water flows (e.g. surface runoff, tile drainage and percolation) from agricultural land amended with manure and/or treated sewage sludge.

In this background, this chapter aims to outline a procedure for the assessment of PhACs behaviour from their consumption to their release in surface water bodies, considering the two sources and the three emission pathways mentioned above. To do this, the subject will be dissected in different aspects concerning different environmental compartments, through the description of a case study in which the occurrence of two compounds (namely carbamazepine (CBZ) and sulfamethoxazole (SMX)) in a watershed was investigated.

The two PhACs considered in this study were selected for different reasons. In particular, CBZ was chosen for its high persistence during its passage in conventional WWTPs (Verlicchi et al., 2012) and in the agricultural environment (Kodešová et al., 2016), which makes it an ideal anthropogenic marker (Clara et al., 2004). SMX was selected because it is used as an antibiotic both for humans and animals, thus it could be found in treated sewage sludge (Verlicchi and Zambello, 2015) as well as in different manure types (as thoroughly discussed in Chapter 4 and reported in Ghirardini et al., 2020). Among all the antibiotics used both by human and animals, SMX was chosen as a higher amount of data (e.g., local human consumption, range of concentration in manure, and so forth) was available. No metabolite or transformation product of CBZ and SMX was considered in the analysis.

From now on, the description of the potential ways to estimate the behaviour of PhACs in the environment will thus be coupled with the actual procedure followed for the case study, as for example and model illustration.

Of course, some specific considerations regarding PhACs pathways are strictly site-specific and are thus avoided in this general discussion. For any further detail, the reader is anyway referred to the case study article, here cited as Ghirardini et al., 2021.

Briefly, the aim of Ghirardini et al., 2021 was to elaborate a predicting tool from which to investigate the main sources and emission pathways of CBZ and SMX in the river of an East Austrian watershed (site description is reported below) in a one-year time window. The study also estimated which of the model parameters is mainly affected by uncertainty, and which parameter mainly influence the model results.

The compounds flow (in g year^{-1}) was studied in the context of a Substance Flow Analysis (SFA), which corresponds to a comprehensive evaluation of flows and stocks of materials limited to a specific *system* defined in terms of space and time, as a systematic mass balance. The terms and definitions adopted hereinafter thus refer to those of the SFA terminology as reported in Brunner and Rechberger, 2016. In particular, within a *system*, a *compartment* is defined as a point where the substance flow splits (node), or where the substance is stored (stock). The term compartment has been preferred to the term *process*, more commonly used in SFA, to avoid confusion with the treatment processes to which wastewater and sludge are subjected. A compartment can be composed of multiple nodes or stocks, which are termed *sub-compartments*.

Compartments are linked by *flows*, expressed as mass per time, which represent the potential direction a substance may take in the system. A flow of a substance entering the system is called *input*, while a flow exiting the system is called *output*.

In addition to the basic terms necessary to analyse substance flows and stocks, the term *emission pathway* has been used herein to identify the specific flows that directly contribute to the final output flow.

Finally, from an overall point of view, a *parameter* is defined as a value which will be assumed in the SFA, such as the fraction of the flow that splits in a node as well as the substance load taken as the input.

Therefore, in Ghirardini et al., 2021 case study, the *flow* of CBZ and SMX was studied taking as *inputs* the human PhAC consumption and PhAC content in manure applied on arable land. The *output* is thus represented by the load of CBZ and SMX that reaches the river endpoint of the catchment.

The SFA was performed with the software STAN (Cencic and Rechberger, 2008), that allows balancing the system with nonlinear data reconciliation based on the conventional weighted least-squares minimisation approach and Gaussian error propagation (Cencic, 2016).

Case-study site description

Before describing the methodology, which can be used for the evaluation of PhACs load into the different compartments, a brief parenthesis should be dedicated to the case-study site description, in order to provide the context of the study, and the reason of different decision which will be made in the following sections.

The watershed under study is in the eastern part of Austria, and it extends for 404.1 km^2. Here, more than half of the total surface is covered by arable land (50.4%), followed by forests (40.5%), small urban areas (5.5%) and grasslands (2.5%). The annual average rainfall is about 573 mm, the yearly average temperature is about 10.2°C (with a minimum monthly average temperature of 5.8°C and a maximum average temperature of 14.3°C), and the mean solar radiation is 11,797 kJ m^{-2} day^{-1} (Fick and Hijmans, 2017). As represented in Figure 6.1, the area was schematically divided into four sub-catchments (called SC1, SC2, SC3 and SC4, respectively). Each of them is characterised by similar urban area coverage (5.3% of the total surface area).

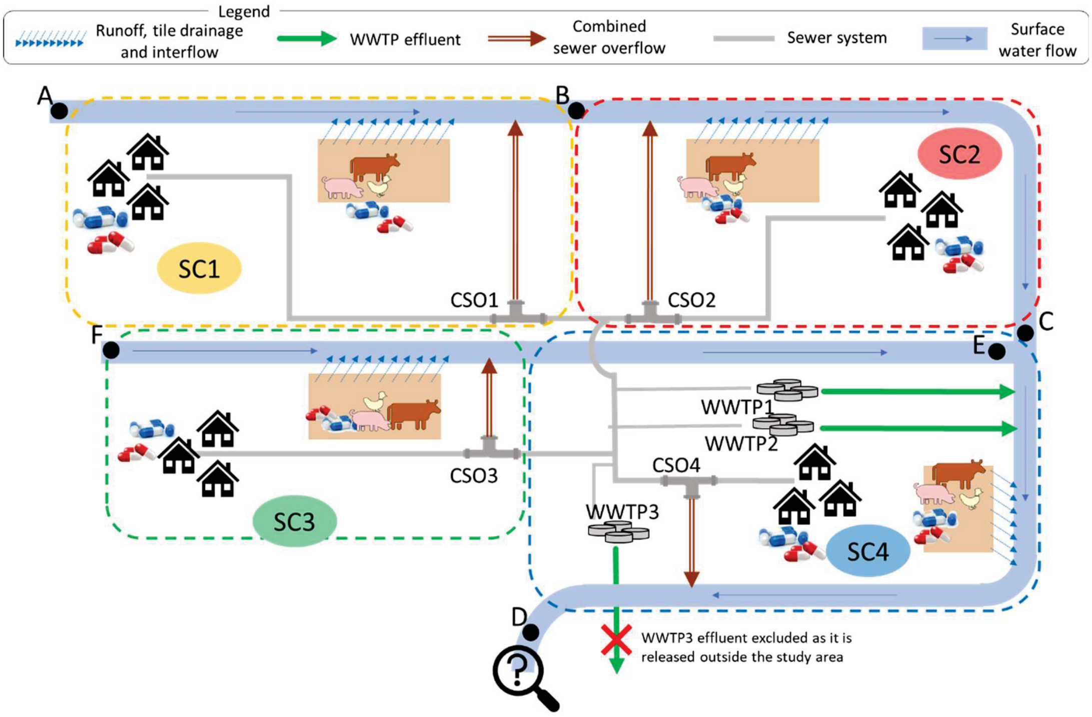


Figure 6.1. Schematic representation of the different water flows (raw wastewater, treated effluent, CSOs and surface water) in the catchment under study.

The population was approximately 108,879 in 2017 (of which 21,579 in SC1; 42,717 in SC2; 20,570 in SC3 and 24,013 in SC4). The local sewer system is combined and includes some sewer overflow outfalls.

As to the receiving surface water body, the river starts its flow in SC1 (point A in Figure 6.1) and, after 11 km, reaches SC2 (point B), in which the flow continues for 23 km towards SC4 (C). Here, the river flows for 56 km until reaching the catchment outlet (D). Also, another small tributary starts in SC3 (F) and reaches the main river in SC4 after 16 km (E). The travel time from the starting point of the river in SC1 (A) to the end in SC4 (D) is approximately 24–25 hours (surface water velocity could be assumed around 1 m s^{-1}). The annual average river discharge at the outlet of the sub-catchments is about 0.23 m^3 s^{-1} at SC1, 0.53 m^3 s^{-1} at SC2, 0.09 m^3 s^{-1} at SC3 and 1.15 m^3 s^{-1} at SC4.

In SC4 there are three WWTPs (WWTP 1, 2 and 3) which treat the wastewater of the population resident in the whole catchment, serving respectively 22%, 58% and 20% of the population. There is no significant industrial activity in the studied area. Thus, the sewer network dominantly conveys domestic wastewater and rainwater. According to Council Directive 81/271/EEC, the mixing of domestic and drained rainwater is called urban wastewater (EC, 1991) and this term will be generally used in the following also when referring to dry periods.

The three WWTPs have a capacity of 42,000 person equivalent (PE), 110,000 PE and 66,500 PE, respectively, and consist of pre-treatments, primary sedimentation and secondary treatment based on conventional activated sludge (nitrification and denitrification stages working at 0.06 kg BOD kgSS^{-1} d^{-1} with bubble aeration, a hydraulic retention time (HRT) of 24 hours and a sludge retention time (SRT) of more than 15 days. Phosphorus is removed by precipitation with aluminium and iron coagulants).

Between 2009 and 2016, the 3 WWTPs had the following average flow rates: 3,977,604 m^3 year^{-1} (WWTP1), 10,016,108 m^3 year^{-1} (WWTP2) and 2,995,752 m^3 year^{-1} (WWTP3). The WWTP1 and WWTP2 effluents are directly released into the surface water body inside SC4. In contrast, the WWTP3 discharges into a watercourse outside the catchment area and for this reason its effluent is not considered in this SFA.

WWTP2 treats the sewage sludge produced during its treatment as well as that produced in WWTP1, whereas sludges from WWTP3 are treated locally. In both WWTP2 and WWTP3, sludge treatment consists of dewatering followed by anaerobic digestion carried out at mesophilic conditions (35°C) with a minimum SRT of 20 days.

6.1.1. Household contribution

Human-consumed load of pharmaceuticals in a specific period can be evaluated considering the *per capita* consumption of the selected compound and the population number.

Unfortunately, as pharmaceuticals consumption strongly vary depending upon regions and time, *per capita* consumption data are often lacking, or outdated. In this context, examples of national and regional consumption, concerning household and hospitals can be found in Verlicchi et al., 2014 and Verlicchi and Zambello, 2016 respectively, also underlying the significant variability of this type of data.

Once consumed, the compound is only partially metabolised, and the remaining part is excreted via urine and faeces, thus reaching the sewer system.

This percentage of PhAC amount consumed, which is actually excreted in faeces and urine, is known as excretion factor (%) and can be easily found in the literature for the most studied compounds. A literature review of excretion factor values for some selected pharmaceuticals belonging to the most common classes (among them analgesics and anti-inflammatories, antibiotics, lipid regulators and psychiatric drugs) is available in Verlicchi and Zambello, 2016.

In the specific case studied in Ghirardini et al., 2021, data about SMX and CBZ *per capita* released loads in the sewer system were found in Clara et al., 2013, who measured the influent of two WWTPs in Styria, an Austrian region presenting similar characteristics to those in the studied catchment.

The per capita load released into sewer system was equal to 4.7 mg inhabitant^{-1} year^{-1} for SMX and 118.6 mg inhabitant^{-1} year^{-1} for CBZ, and, due to the method used for its measurement, they already consider the excretion factor, as well as further degradation which can occur in the sewer system from the household to the WWTPs. The resulting total load released in the sewer system due to human consumption in SC1, SC2, SC3 and

SC4 was 102 g year^{-1}, 203 g year^{-1}, 98 g year^{-1} and 114 g year^{-1} for SMX respectively, and 2,560 g year^{-1}, 5,067 g year^{-1}, 2,440 g year^{-1} and 2,845 g year^{-1}for CBZ respectively. Further calculations details are reported in Table S2 of the Supplementary Material of Ghirardini et al., 2021.

6.1.2. Animal contribution

As already described in Chapter 4, once consumed, PhACs may be excreted in animal manure which can be applied onto the soil, and then reach the water environment through surface runoff, tile drainage or percolation.

Thus, an estimation of the animal contribution to micropollutants occurrence in the environment may be done following the same scheme:

Firstly, the overall PhAC amount consumed by animals in a defined catchment can be assessed multiplying the number of head of different animals in the area with the *per-capita* consumption data referring to a specified period.

Then, only a part of this total amount is excreted with animal faeces and urine, resulting in a reduction of the potential PhAC mass which can reach the arable land *via* manure spreading. The excretion factor strongly varies among the different compounds and animals which have consumed them. Here, as an example, available values of excretion factors for a group of selected antibiotics are reported in Appendix 4 with the corresponding literature references.

Finally, as already mentioned in Chapter 4, before the application onto the soil, pharmaceutical compounds may be partially degraded during manure storage and treatment. This process is strictly correlated to the environment conditions, such as temperature, pH, microbial enzymes present and microorganisms developed in the system (Ezzariai et al., 2018).

Although an in-depth description of the phenomena is out of the scopes of this thesis, the reduction of a selected compound concentration in manure due to degradation can be assessed by means of different kinetics in which the coefficients are calibrated according to experimental results.

As an example, in Appendix 5 are reported the half-life times and the *DT90* (Disappearance Time, meaning the time needed to reach a compound concentration reduction of 90%) for different antimicrobials and two hormones. These values must be considered in the context of the specific kinetic model adopted in the compound concentration reduction assessment. For instance, the simplest and commonly used method refers to the calculation of the concentration function of time (c_t) following a single first-order kinetic equation (SFO):

$$c_t = c_0 e^{-kt} \qquad \text{(eq. 1)}$$

Where the constant k is assessed based on observed experimental data. In this case, the time within which the concentration of the tested substance is reduced by a defined percentage x (also called disappearance time, DT), can be calculated with the following equation:

$$DT_x = \frac{\ln\frac{100}{100-x}}{k} \qquad \text{(eq. 2)}$$

In this context, a very common parameter that can be found in literature, the so-called half-life time, refers to this last equation specified for a reduction of the substance of 50%.
Otherwise, more elaborate models can be adopted in the compound concentration reduction assessment. Among these, it is worth to mention the following 3:

1. The first order multi-compartment kinetic (FOMC) (Berendsen et al., 2018):

Defined by the following equation:

$$c_t = c_0\left(\frac{t}{k_2}+1\right)^{-k_1} \qquad \text{(eq. 3)}$$

In which there are two adjustable parameters, *k_1* and *k_2*. In this case, the endpoint (also referred above as disappearance time, *DT*) of a substance can be calculated following the equation:

$$DT_x = \ k_2\left[\left(\frac{100}{100-x}\right)^{\frac{1}{k_1}} - 1\right] \qquad \text{(eq. 4)}$$

Where x defines the time (in days), the substance endpoint is evaluated for (*e.g.* DT90).

2. The double first order in parallel or biexponential (DFOP) (Berendsen et al., 2018):

This model uses the sum of two first-order equations, in order to better conform the observed data of an experiment. In this case, the concentration as a function of time is expressed by the equation:

$$c_t = c_0 g e^{-k_1 t} + c_0(1-g)e^{-k_2 t} \qquad \text{(eq. 5)}$$

In which there are three adjustable parameters (*k_1*, *k_2* and *g*), where g is the fraction of degradation occurring under rate constant *k_1*. Here, because of its two exponentials, there is no closed-form equation for the evaluation of DT, which must be calculated employing an iterative procedure.

3. The Hickey Stick bi-phasic first-order kinetic (HS) (Berendsen et al., 2018):

This model consists of two sequential first-order curves:

$$\begin{cases} c_t = c_0 e^{-k_1 t} \\ c_t = c_0 \times e^{-k_1 t_b} \times e^{-k_2(t-t_b)} \end{cases} \qquad \text{(eq. 6)}$$

In which the substance concentration initially declines according to an SFO with a rate constant k_1 and, after a time t_b (breakpoint), the rate constant changes to k_2.
The endpoints are thus two, and can be calculated with the following equations:

$$\begin{cases} DT_x = \dfrac{\ln \dfrac{100}{100-x}}{k_1} & for\ t \leq t_b \\ DT_x = t_b + \dfrac{\left[\ln \dfrac{100}{100-x} - k_1 t_b\right]}{k_2} & for\ t > t_b \end{cases} \qquad \text{(eq. 7)}$$

Further information about the concentration reduction kinetics of the substances can be found in the literature cited in Appendix 5 regarding the degradation of pharmaceuticals and hormones during manure storage.

In addition to storage, zootechnical wastes treatment practices (*e.g.* composting, anaerobic digestion, lagooning, and so forth) locally employed in the catchment under study should be considered in the evaluation of animal PhAC load contribution, as they also can lead to a reduction of chemical substances sequestered in manure. Here, as already mentioned in Chapter 4, this further degradation strictly depends on the treatment type and conditions, and it is difficult to make here a proper synthesis providing a comprehensive analysis taking into account all the different examples.
On the contrary, each specific situation, concerning local most common treatment practices, must be assessed on a case-by-case basis.

As can be seen in this paragraph discussion, the assessment of micropollutant concentration applied onto arable land with manure amendment requires many data and information: number of animal present in the catchment, type of animals, pharmaceutical consumption data, details regarding local manure stock and treatment practices, and finally, half-life time or DT90 of the considered compound in manure (during storage, or a defined treatment).
All these details may be difficult to find, especially regarding consumption data, which are in some cases confidential, lacking, or even absent.
Thus, a different approach was adopted in Ghirardini et al., 2021, in which the evaluation of the amount of SMX added in arable land via manure amendment was calculated by multiplying the tons of different manure produced every year in the catchment by the corresponding SMX range of potential occurring concentration.
In particular, the tons of manure produced in the catchment was estimated given the number of cattle, swine and poultry heads provided by the Austrian Federal Ministry of Sustainability and Tourism (Integriertes Verwaltung – und Kontrollsystem, available at https://www.bmnt.gv.at/land/direktzahlungen/Invekos.html), and the average tons of manure produced by each type of animal, considering different types of manure depending on the stall type. Here, data about the average volume of manure produced by each animal were found in BMLFUW, 2017, and typical manure values of bulk density and dry matter were taken from Lfl, 2018.

The obtained amounts of animal manure produced in the whole catchment were 501 t DM year^{-1} of cattle slurry, 21,842 t DM year^{-1} of cattle solid manure, 7927 t DM year^{-1} of poultry solid manure, 181,672 t DM year^{-1} of swine slurry and 1088 t DM year^{-1} of swine solid manure.

Detailed calculations are reported in Table S4 the Supplementary Material of Ghirardini et al., 2021.

The tons of manure obtained with these considerations were then multiplied by the minimum, average and maximum SMX concentration potentially occurring in the different zootechnical effluent, as reviewed in Ghirardini et al., 2020 (further information are reported in Table S5 and S6 of the Supplementary Material of Ghirardini et al., 2021).

Finally, considering that manure is uniformly applied over all the catchment arable land, the obtained load of SMX (g year^{-1}) was divided into the four sub-catchments proportionally to the arable land surface of each one of them. The average SMX mass load input was equal to 3,762 g year^{-1} for SC1, 15,884 g year^{-1} for SC2, 7,752 g year^{-1} for SC3, and 11,305 g year^{-1} for SC4 (see Table S6 of the Supplementary Material of Ghirardini et al., 2021).

Of course, this second way to evaluating the animal contribution to PhACs load in a catchment represents a simplified version of the first one mentioned above. On the other hands, considering the SMX minimum and maximum concentrations in manure as reviewed in Ghirardini et al., 2020 allows estimating an overall SMX occurrence range, which revealed to be helpful in the sensitivity analysis. Furthermore, with this method, it is possible to take into account the value of SMX concentration in the specific manure type considered in the analysis, avoiding the estimation of compound reduction in manure storage and treatment. For instance, in estimating the load of a PhAC applied with a specific amount of treated manure, it is not necessary to assess the compound removal during the treatment, but simply estimate (considering the acceptable variability range) the potential occurrence of the compound in the specified treated manure.

Following a conservative approach, no treatments were considered in Ghirardini et al., 2021 as, in the studied catchment, it is an uncommon practice, for which only limited information can be found.

6.1.3. Pharmaceutical flow in the sewer network – Combined sewer overflow

Once in the sewer system, wastewater may be conveyed to the treatment plant, or, in case of intense rainfall events, may be discharged through combined sewer overflow (CSO) outfalls. The latter are typically located before the WWTPs to hydraulically protect the plant from high peaks of wastewater flow rate, by discharging the excess wastewater in a receiving water body, such as a river or a stream.

CSO may thus contain micropollutants residues, or microorganisms, which negatively impact the water bodies quality (Al Aukidy and Verlicchi, 2017; Phillips et al., 2012). For this reason, it is important to estimate the amount of PhACs which, once released in sewer network from households, can be discharged in surface water without further treatments.

To this end, the following method, which takes into account WWTPs wastewater influent and CSO discharge volumes, has been developed.

Firstly, data about the annual flow rate of the effluent (assumed equal to the influent) of the three WWTPs ($V_{WWTP1,2,3}$) were collected from the Austrian official register of emission into surface waters (BMLFUW, 2017b). Data about annual CSO volumes ($V_{CSO\text{-}SC1,2,3,4}$) were estimated for the whole of the Austrian territory based on Clara et al., 2014 and are reported in Clara et al., 2020 and in Table S7 of the Supplementary Material of Ghirardini et al., 2021.

Then, focusing on each SC, assuming that PhACs follow the wastewater flow passively and do not degrade in the sewer network, their concentration in the CSOs may be calculated with the following equation:

$$c_{PhAC,CSO} = \frac{\dot{m}_{PhAC,in}\Big|_{yearly} \times \frac{t_{CSO}}{1\ year}}{\left(V_{WW,dry} + V_{RAIN,overflow}\right) \times \frac{t_{CSO}}{1\ year}} \qquad \text{(eq. 8)}$$

Where, $\dot{m}_{PhAC,in}$ is the PhAC load annually released into the sewer system from the household (g year^{-1}) evaluated above, t_{CSO} is the time of the overflow duration (h), $V_{WW,dry}$ the wastewater volume discharged into the sewer system by the household in dry periods (m^3 year^{-1}) and $V_{RAIN,overflow}$ the rainfall volume due to urban runoff which led to the CSOs (m^3 year^{-1}). Here, $V_{WW,dry}$ can be assumed equal to 150 L inhabitant^{-1} day^{-1} as a standard value for peri-urban areas (Metcalfe and Eddy, 2014), and $V_{RAIN,overflow}$ can be calculated as follows:

$$V_{RAIN,overflow} = d \times V_{WW,dry} \qquad \text{(eq. 9)}$$

In which d is the factor of dilution of $V_{\mathrm{WW,dry}}$ which, according to technical principles and norms applied in Austria, was estimated to be equal to 50 on average annually, with a minimum of 30 and a maximum of 100 in this area (ÖWAV, 2007; Fenz, 2002).

Thus, the percentage of PhAC load entered into the sewer system and then discharged *via* the CSOs can be evaluated with the equation:

$$\%_{PhAC\ discharged\ via\ CSO} = \frac{c_{PhAC,CSO} \times V_{CSO}}{\dot{m}_{PhAC,in}}\Bigg|_{yearly} \qquad \text{(eq. 10)}$$

as the numerator corresponds to the PhAC load discharged via the CSOs ($\dot{m}_{PhAC,CSO}$) as shown in the equation:

$$\dot{m}_{PhAC,CSO} = c_{PhAC,CSO} \times V_{CSO} \qquad \text{(eq. 11)}$$

In this context, removing t_{CSO} from equation 8, as it appears both in the numerator and denominator, and including equation 8 in equation 11, it is possible to write equation 9 as follows:

$$\%_{PhAC\ discharged\ via\ CSO} = \frac{V_{CSO}}{(V_{WW,dry} + V_{RAIN,overflow})} \qquad \text{(eq. 12)}$$

In which $\dot{m}_{PhAC,in}$ was removed, for the same reason as t_{CSO}.

Equation 12 represents the percentage of PhAC load released in the sewer network after human excretion, which is actually discharged via CSO without further treatments, on an annual basis.

Respectively, the percentage of PhAC load contained in sewer wastewater which is conveyed to the treatment can be calculated by:

$$\%_{PhAC\ conveyed\ to\ WWTP} = 100 - \%_{PhAC\ discharged\ via\ CSO} \qquad \text{(eq. 13)}$$

The resulting percentage of the PhAC load that moved into the surface water through the CSOs ranges from 0.9% to 1.2% in SC2 and SC4, respectively, and thus about 99% of the PhAC annual load is conveyed to the treatment plant. Further details and calculations are reported in Table S8 of the Supplementary Material of Ghirardini et al., 2021.

6.1.4. *Evaluation of the fate of pharmaceuticals in a conventional wastewater treatment plant*

6.1.4.1. Removal of pharmaceuticals during water treatment

As already discussed in Chapter 1, a chemical compound entering in a WWTP with wastewater may be subjected to different processes such as adsorption onto particulate matter, biological transformation, and abiotic degradation. The degradation mechanisms are itself very complex, but it has also been extensively studied in the last decades, resulting in the implementation of different models and software which are able to give an estimation of the compound removal in the water phase and its sequestration in the solid phase (sewage sludge).

In the Austrian case study, the fate of SMX and CBZ in the WWTPs was assessed with the SimpleTreat 4.0 model (Struijs, 2014), which is the improved version of the SimpleTreat 3.1 applied in the European REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) legislation (EC, 2004).

Briefly, the model evaluates the fraction of PhAC load influent released with the WWTP effluent, removed via biotransformation, and adsorbed onto sewage sludge. It requires the compound physicochemical characteristics (namely, molecular weight, octanol-water partition coefficient K_{OW}, dissociation constant pK_a, vapour pressure v_p, solubility in water S, chemical classification based on its ionised form – base, acid or neutral, and biodegradation constant) as well as the WWTP design and operational conditions (i.e. served inhabitants, hydraulic retention time (HRT), sludge retention time (SRT), sludge loading rate and aeration type).

In this context, as the three WWTPs in the catchment present a similar configuration, they were processed as a single plant, operating in the same conditions, and treating an influent obtained as the sum of the three influent flow rates. Table S9 of Supplementary Material of Ghirardini et al., 2021 resumes the physicochemical characteristics of SMX and CBZ and the operational condition selected for the virtual WWTP.

Finally, as WWTP3 release its effluent outside the catchment, a quote of PhAC load released *via* WWTP effluent (as modelled with SimpleTreat 4.0) equal to the WWTP contribution in effluent flow rate (17.6%, as calculated in Table S7 of Supplementary Material of Ghirardini et al., 2021) was removed.

6.1.4.2. Removal of pharmaceuticals during sludge treatment

Regarding the estimation of PhAC load reduction during sludge treatment, literature regarding the fate of CBZ and SMX in an anaerobic digester operating at similar conditions of the one present in the catchment (mesophilic conditions -35°C, for around 20 days) was reviewed.

It emerged a good agreement between the different studies (Carballa et al., 2007; Gonzalez-Gil et al., 2016; Yang et al., 2016), from which an average removal of 13.3% and 88.9% for CBZ and SMX respectively were selected.

The obtained PhAC load exiting the sludge treatment was then reduced of 40%, considering the percentage of treated sewage sludge which is not reused in agriculture (data provided by local wastewater management companies) and then divided in the four SCs proportionally to their arable land surface.

Table S10, S11 and S12 of the Supplementary Material of Ghirardini et al., 2021 report the detailed calculation of CBZ and SMX degradation during treatment, local data on treated sewage sludge usage, and division between each SC arable land, respectively.

6.1.5. Evaluation of the fate and mobilisation of pharmaceuticals into soil

Once manure and sewage sludge are applied on soil, chemical compounds may be sequestered in soil particles or mobilised in the aqueous phase, as already discussed in Chapter 5.

Due to the complexity of soil matrix environment, many aspects should be considered in modelling micropollutants fate inside it. Among them, it is worth mentioning sorption in soil particles, degradation and chemical reactions, plant uptake, remobilisation in soil sediments, remobilisation in soil water flows (surface runoff, tile drainage, groundwater), degradation in groundwater, and so forth.

All these mechanisms, combined together, make it difficult to simulate the behaviour of a compound, especially considering that also physicochemical characteristics may strongly differ from a chemical to another.

This subject has been extensively studied in the past decades focusing on pesticides, which since the 1960s showed to potentially pose environmental risks to soil and water microorganisms, flora and fauna (Crawford et al., 1973). And as a result, different models were developed for the simulation of pesticides flow and behaviour in the soil after their application, taking into account hydrological aspects, physicochemical reactions and biological processes. An interesting and comprehensive discussion of these models and their characteristics is provided in the two reviews by Köhne et al., 2009a, 2009b, that also highlighted how the topic has been deepened in its central issues, and which aspects need further study.

On the other hand, these models (among them RZWQM, MACRO and HYDRUS) requires a large amount of data, and their complexity is not always balanced with the cohesion between modelled and measured results, especially when tested with compounds of emerging concern, such as pharmaceuticals and hormones. In this context, noteworthy are the studies of Knäbel et al., 2016, Larsbo et al., 2008 and Larsbo et al., 2009, which demonstrate the great potential of these prediction tools, but also their limits, and the difficulty to adapt them to pharmaceuticals or other compounds of emerging concern.

Against this background, the aim of this section is to provide a brief description of the possible methods, and past solutions, found in the literature to overcome this problem. The approach hereinafter followed concerns the cited above processes regarding PhAC fate in soil, one by one.

Of course, this will result in many simplifications that are taken as an unavoidable choice and are discussed separately, in order to open the discussion to future solutions.

Firstly – as adopted in the Austrian case study for the assessment of CBZ and SMX behaviour in soil matrix – the load of PhACs applied onto the soil with sludge and manure amendment can be divided into two fractions: the one which remains sequestered in the solid phase of the soil (M_S, g year^{-1}), and the one that could be mobilised in the water phase (M_A, g year^{-1}), following the approach of Langdon et al., 2010:

$$\frac{M_S}{M_A} = \frac{K_d \times \rho_b}{v_0} \qquad \text{(eq. 14)}$$

Where ρ_b is the bulk density of the soil (g cm^{-3}), v_0 the porosity of the soil and K_d the partition coefficient, mentioned in Chapter 1.

While ρ_b and v_0 are commonly studied data in soil science, which can be easily measured or estimated from manuals (e.g. Marshall et al., 1996) based on the soil type under investigation, the evaluation of the K_d coefficient should be better discussed:

A first way to assess the K_d may be based on the pharmaceutical's K_{OW} value by means of different empirical formulas. For example, Matter-Müller et al., 1980 studied that:

$$\log K_d = 0.67 \times \log K_{OW} + 0.39 \qquad \text{(eq. 15)}$$

And Dobbs et al., 1989 found that:

$$\log K_d = 0.58 \times \log K_{OW} + 1.14 \qquad \text{(eq. 16)}$$

In which all the logarithms have a decimal base.

A second way to study K_d is considering the soil characteristics with the equation (Langdon et al., 2010):

$$K_d = K_{OC} \times f_{OC} \qquad \text{(eq. 17)}$$

In which K_{OC} is the carbon water partition coefficient, which is defined as the ratio between the compound concentration in the organic carbon *[compound]*$_{OC}$ and water *[compound]*$_{water}$:

$$K_{OC} = \frac{[compound]_{OC}}{[compound]_{water}} \qquad \text{(eq. 18)}$$

Here, for its part, also K_{OC} can be estimated (if not already known) with empirical equations.

An example is offered by the Kenaga and Goring equation (Kenaga and Goring, 1980):

$$\log K_{OC} = 0.544 \times \log K_{OW} + 1.377 \qquad \text{(eq. 19)}$$

And f_{oc} is the is the fraction of organic carbon in the soil, which is a commonly studied data in soil science, and, if not known from a dedicated measurement, could also be estimated based on soil characteristics or soil matrix (Marshall et al., 1996).

Finally, in the K_d evaluation, mention should be made of the following equation 20 and 21 by Fetter et al., 2017 and Jones et al., 2002 respectively, which allows one to override the K_{OC} estimation:

$$K_d = f_{oc} \times \frac{10^{0.72 \times \log K_{OW} + 0.49}}{1000} \qquad \text{(eq. 20)}$$

$$K_d = f_{oc} \times 0.41 \times K_{OW} \qquad \text{(eq. 21)}$$

In this context, it is difficult to compare the different methods to evaluate the K_d coefficient, and thus the splitting between the solid and aqueous phase of the soil. On the contrary, since they were made empirically, they simply provide an indication of the potential values K_d may range.

In the Austrian case studied in Ghirardini et al., 2021, the second method was chosen for its practicality. Still, the other methods may also represent a valid alternative and provide similar results. In particular, for the calculation of K_d with Equation 17, f_{OC} was assumed to be 1.51% as suggested by Gerzabek et al., 2005 for east Austrian agricultural loamy soils, and K_{OW} was set equal to 0.89 for SMX and 2.45 for CBZ (Pubchem, Kim et al., 2016). The partition coefficient was equivalent to 1.1 cm^3 g^{-1} for SMX and 7.74 cm^3 g^{-1} for CBZ.

To calculate the result of load splitting (Equation 14), a soil bulk density of 1.28 g cm^{-3} and a soil porosity of 0.52 was assumed, as suggested by Marshall et al., 1996 as typical values for agricultural loamy soils.

The ratio between M_S and M_A were equal to 2.7 and 19.0 for SMX and CBZ, respectively. This means that 27% and 5% (for SMX and CBZ respectively) of the mass applied onto soil is moved in the aqueous phase, while the remaining 73% and 95% (for SMX and CBZ respectively) remains bonded in the solid phase.

This splitting method suggested by Langdon et al., 2010 represent a worst-case scenario for PhAC occurrence in surface water and does not consider many different processes that could take place in the soil matrix. For the Austrian specific case study, as it refers to a large catchment, it is difficult to accurately define the PhAC behaviour in the soil, as agricultural land may be amended with sewage sludge and/or different types of manure from different animals. In addition, the amendment may occur at different times and amounts. Thus the assumptions of Langdon et al., 2010 were taken as an unavoidable choice, and the uncertainty of its results was then assessed in the uncertainty analysis.

Differently, the splitting results can be further deepened, considering many other processes.

In this context, Figure 6.2 schematically represents the other potential pathways PhACs may take in the soil, including the mechanisms resulting from the sequestration onto the soil (*e.g.* degradation), the mobilisation in the aqueous phase, and also the further combination between the two phases (e.g. combination of aqueous runoff and sediment runoff).

The possible way to evaluate the behaviour of PhACs due to these mechanisms is here briefly presented.

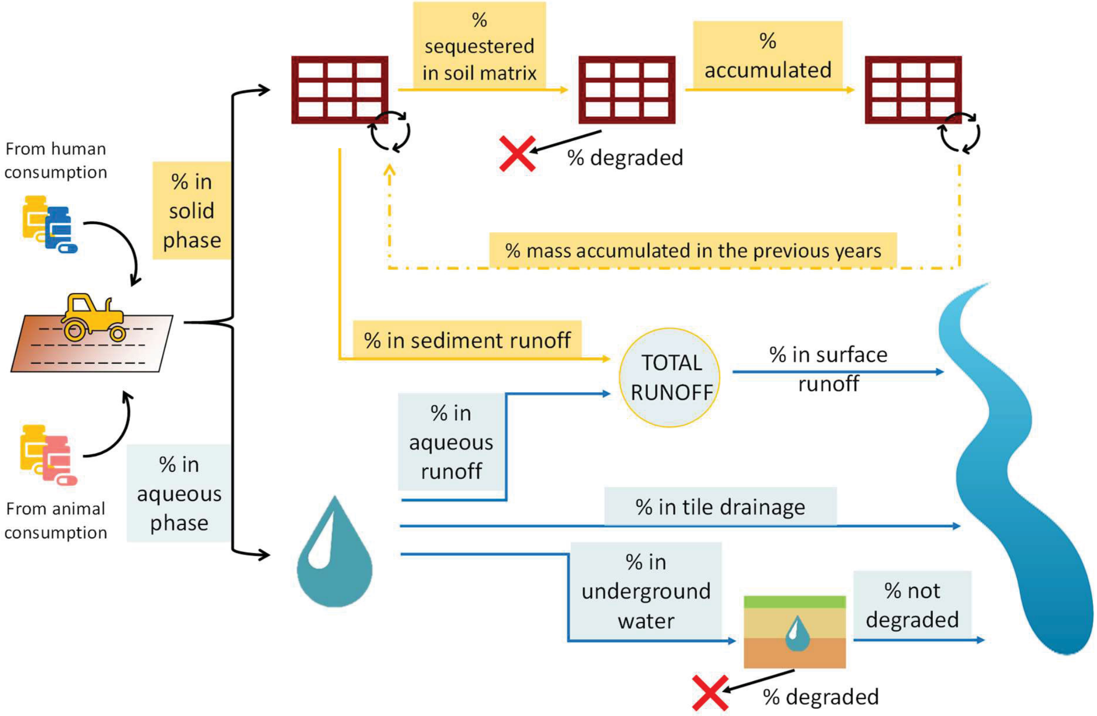


Figure 6.2. Potential pathways a pharmaceutical compound may take once applied onto arable land via treated sewage sludge and animal manure.

6.1.5.1. Fate of pharmaceuticals in soil particles

Focusing on the mass of PhACs that is sequestered in the solid phase, two additional potential routes should be mentioned: soil erosion, and storage in soil matrix with subsequent PhAC degradation (Figure 6.2).

Regarding the assessment of PhAC mass, which can be moved by soil erosion, the main difficulty is represented by the estimation of soil sediment amount (in tons) which is moved yearly in a catchment.

Fortunately, this topic has been extensively studied in the past, and nowadays many manuals and books provide easy and/or more complex method to estimate soil erosion in a river catchment, depending on the soil characteristics and weather conditions. A comparison of methods for the evaluation of erosion and deposition processes is out of the scopes of this PhD thesis, anyway, for an introduction to the problem, the reader is referred to Ivanova et al., 2000.

In this context, a test of evaluation of sediment erosion was carried out for the Austrian case study.

The SMX and CBZ load moved in surface water through soil erosion were calculated assuming that the compounds are uniformly distributed in the first 30 cm of soil (as the typical depth of sludge and manure tilling in the agricultural practices, Ghirardini and Verlicchi, 2019), and they can follow passively the eroded sediments. Thus, the fraction of PhAC load sequestered into the soil matrix, which is moved with soil erosion ($PhAC\ load|_{in\ sediment}$), was considered equal to the ratio between the volume of sediment eroded (V_{se}) and the total volume of the first 30 cm in depth of arable land ($V_{al,30}$), on yearly basis.

$$\frac{PhAC\ load|_{in\ sediment}}{M_S} = \frac{V_{se}}{V_{al,30}} \qquad \text{(eq. 22)}$$

Here, the volume of sediment transported in the studied catchment in a year (V_{se}) was collected from a database in which measured and estimated values are combined and compared together (the oper source MoRE database, Modeling of Regionalised Emissions, into water bodies Fuchs et al., 2017).

And, given the arable land area in the catchment (which, as already mentioned before, is included in Table S12 of the Supplementary Material of Ghirardini et al., 2021), the soil volume (in m^3) of the first 30 cm in depth can be calculated, for each SC, with the equation:

$$V_{al,30}\big|_{SCi} = S_{al}|_{SCi} \times \frac{30}{100} \qquad \text{(eq. 23)}$$

In which S_{al} is the arable land surface in m^2, referred to the i-th SC.

The resulting percentage of the upper layer of soil that is eroded in one year (and then the percentage of the load of PhACs moved in surface water from the soil *via* soil erosion) ranges between 0.001% in SC4 to 0.003% in SC2.

This process can be thus considered negligible for most of those substances that are measured in soil matrix at ng g^{-1} concentration level.

The mass that remains sequestered into soil matrix after sediment erosion, on a yearly basis, can be thus defined with the equation:

$$M_{S'} = M_S - PhAC\ load|_{in\ sediment} \qquad \text{(eq. 24)}$$

Focusing now on $M_{S'}$, as represented in Figure 6.2, this PhAC load should be considered as subjected to degradation.

In this regard, as already specified in a different context above, this process is strongly dependent on PhAC physicochemical characteristics, as well as the environmental conditions. Thus, for its evaluation, literature studies, in which on-field experiments were carried out, can be considered. As an example, half-life times of many common studied PhACs in different soils under different conditions can be retrieved in Albero et al., 2018, Biel-Maeso et al., 2019 and Salvia et al., 2014.

In the assessment of PhAC degradation in soil, particular attention should be paid on the specific test conditions. In fact, it is well known that soil characteristics, temperature, humidity, f_{OC}, etc. play an important role in compound persistence in soil (Monteiro and Boxall, 2009), so a good correspondence between experiment and case study conditions should be achieved before any evaluation.

6.1.5.2. Mobilisation of pharmaceuticals in the aqueous phase

Since the approach of Langdon et al., 2010 was made to simulate the worst-case scenario for surface runoff, the authors assumed that the total mass of PhAC moved in the aqueous phase (M_A) is totally transferred in the land runoff. Otherwise, M_A may be divided into different flows, also simulating tile drainage and percolation to groundwater (Figure 6.2).

In this context, with the assumption that chemicals follow the water flow passively, it is possible to split the PhAC load in different fractions proportionally to the volumes of surface runoff, tile drainage and groundwater reaching the studied surface water body on an annual basis.

In the specific Austrian case study, for example, annual data referring these three volumes were available in the cited above MoRE database (Fuchs et al., 2017). Conversely, if only limited data are available, the three water flows can be evaluated by means of many hydrological models which are commonly used nowadays in different contexts.

Water infiltration modelling has been developed extensively in the last century for its use in various fields, including hydrology, hydraulic works, agriculture, and transport of pollutants (Morbidelli et al., 2018). As a result, soil water flows can be estimated by means of simple equations or more complex models taking into account rainfall data and soil characteristics, in homogeneous or layered soils. Definitely worthy of mention are the empirical equations of Philip (Philip, 1957a, 1957b, 1957c) and Horton (Horton, 1939, 1933), or the point infiltration model for vertically uniform or non-Uniform soil "Green-Ampt" (Chow, 2010).

A description of these hydrological models based on the well-known Darcy law (Buckingham, 1907) is out of the scope of this thesis, and several mathematic and hydrological prerequisites would be necessary to explore it further. For a better understanding of the issue, the reader is referred to the well-known book of Von te Chow on Applied Hydrology (Chow, 2010), or the recent review of Morbidelli et al., 2018.

To test the possibility of evaluating soil water flows in the Austrian case study, the cited above Green-Ampt model was implemented in the Hydrologic Engineering Center-Hydrologic Modelling System (HEC-HMS, Srinivasa Raju and Nagesh Kumar, 2018).

Briefly, Green-Ampt method evaluates soil moisture as a function of depth in defined time steps, simulating an ideal saturated soil surface and a wetting front that is moving down into dry soil. When the water infiltration capacity of the soil is reached (ponding time), the exceeding rainfall is considered to start moving on the soil surface, generating the surface runoff (Chow, 2010). In this context, soil characteristics are assumed homogeneous and constant throughout the whole considered depth, but further discretisation in different soil layers are possible (Morbidelli et al., 2018). The model requires data about rainfall depth in a defined unite of time (e.g. on an hourly or daily basis) and soil properties (namely, the soil water content in saturated conditions, soil suction and soil hydraulic conductivity).

In the Austrian case study, the soil water content in saturated conditions was assumed to be equal to the porosity (0.52), soil suction and soil hydraulic conductivity were set equal to 16.68 cm and 0.68 cm h^{-1} as suggested by Maidment, 1993 for silty loam soil with a porosity ranging from 0.42 and 0.58.

Before the implementation of the model in HEC-HMS, local daily rainfall depth data in each SC were adjusted adding 0.1 mm every day from May to August to consider the irrigation that takes place in the summer period. The evaluation of the irrigation amount was made according to Vanham, 2012 that reviewed the water consumption for agricultural purposes in Austria.

Unfortunately, the model results underestimated the volume runoff of around 45%, compared to the available data collected by MoRE. This may be due to the large discretisation of time (made on a daily basis), as it imposed to set a very low value for irrigation water volume, which is expected, in the real case, to occur on limited times of the day and at a more intense rate, instead of distributed in the whole day. This estimation was anyway excluded from the main model, and it was used only as a test of the different modelling possibilities.

Finally, since land runoff and tile drainage are almost instantaneous phenomena, degradation of PhACs in these flows may be neglected. On the contrary, this process should be considered in the underground flow.

Unfortunately, very limited data regarding PhACs degradation during groundwater flow are nowadays available in the literature. One possible solution to overcome this issue is to consider studies on riverbank filtration. In this case, attention should be paid on the riverbank filtration experiment conditions (soil characteristics, water flow discharge and water travel time). As an example, Kruć et al., 2019 provided useful results on the degradation of selected PhACs in different seasons (and thus in different temperature and climate conditions), different distances for the river and the bank filtrate wells (5-250 m), different travel times (1-150 days) and for both horizontal and vertical wells. Unfortunately, to the author knowledge, not better solution were found nowadays. Therefore, the assumption of groundwater flow as a river bank filtration, (if adopted) has to be taken as an avoidable choice, making sure to proper evaluate the uncertainty of its results in the uncertainty analysis.

6.1.6. Evaluation of the fate of pharmaceuticals in surface water

Once in surface water, micropollutants may be biodegraded, adsorbed in sediment particles, degraded by photolysis, or flow in the river as it is, and be released in agricultural fields through land irrigation (Maldonado-Torres et al., 2018).

The behaviour of PhACs in the surface water is thus complex to estimate and may consistently vary from a point of the river to another.

Nowadays, many studies focused on the three main degradation mechanisms of chemical compounds during water flow (photolysis, biodegradation, and adsorption in sediments) taken separately or taken together, in a laboratory or field experiments.

To name a few: Aymerich et al., 2016 studied the attenuation of 8 PhACs (among them, carbamazepine, diclofenac, ibuprofen, and paracetamol) and 11 their transformation products, in a dedicated sampling campaign in the Ebro River, obtaining interesting results on the fate of those selected compounds in a natural system. Baena-Nogueras et al., 2017 studied the degradation of a wide number (33) of PhACs in a laboratory, focusing on photolysis and biodegradation in different types of water (freshwater and seawater), considering different experiment conditions (pH and solar irradiation). And Liu et al., 2019 experimented the attenuation of 14 antibiotics in a recirculating flume study and investigated which one of the three degradation mechanisms was mainly involved in the process by batch attenuation experiments.

In this context, as observed by Charuaud et al., 2019, biodegradation is often limited in antibiotics concentration reduction. On the contrary, photolysis appears to be the major degradation/transformation pathway of many veterinary and human PhACs in natural waters.

For this reason, in the Austrian case study, the assessment of CBZ and SMX in surface water was carried out considering the findings of Aymerich et al., 2016, which studied PhAC fate under natural sunlight (instead of artificial light) exposure in similar environmental conditions.

The authors reported almost no degradation for CBZ and an overall half-life time of 15.5 h for SMX. The fraction of SMX reduced during surface water flow was thus evaluated assuming a first-order kinetic degradation and considering the river travel time of each branch in the corresponding SC, and, following a conservative approach, no reduction was assumed for CBZ.

Finally, SMX reduction resulted equal to 25% and 50% (considered from the initial point to the endpoint of the river branch of each SC) in SC2 and SC4 respectively and was assumed equal to 0% in SC1 and SC3. Further details and calculation can be retrieved in Table S13 of the Supplementary Material of Ghirardini et al., 2021.

6.2. Evaluation of the uncertainty of the adopted assumptions

A substance flow analysis takes into account and elaborates a number of different data which are often specific to a certain geographical frame, time-specific, roughly estimated or collected after a literature review.

For this reason, SFA must be paired with an adequate uncertainty analysis which is able to evaluate the uncertainty of each entered number (Do et al., 2014).

In this context, Laner et al., 2014 distinguished three types of uncertainty assessment for SFAs, regarding (1) qualitative and semiquantitative approaches, (2) approaches based on data classification, and (3) statistical approaches.

Methods of the first group aim to express the confidence interval of the SFA overall result, without considering the specific input data uncertainty.

On the contrary, the second group refers to approaches in which typically focus on input data quality quantification, but without including rigorous mathematical procedures to propagating the data uncertainties through the model.

Statistical approaches combine the first two, by means of input data uncertainty evaluation and uncertainty propagation, and assessment of the quality of the results.

An example of this third option is provided in the SFA carried out for the Austrian case study (Ghirardini et al., 2021). Here, as the overall result uncertainty in STAN is estimated using Gaussian error propagation and data reconciliation (Cencic, 2016), only the input data uncertainty must be evaluated. This was carried out following the method of Laner et al., 2016, which was explicitly designed for data quality evaluation in SFA context, and was already tested in STAN environment.

Briefly, the method firstly evaluates the quality of each parameter defining a *score* (between 1 and 4, being 1 the best and 4 the worst evaluation) concerning defined *indicators* (namely: reliability, completeness, temporal and geographical correlation, and others, such as technical correlation). These indicator scores are established according to the scheme reported in Table 6.1 (pedigree matrix). Then, the model translates each score into a *coefficient of variation (CV)* by the use of an exponential-type equation:

$$CV = a \times e^{b \times score} \qquad \text{(eq. 25)}$$

Where a and b are user-defined coefficients which provide the quantification of the sensitivity of the indicator under study (low L, medium M and high sensitivity H), with respect of each input parameter.

According to Laner et al. (2016), a was set equal to 0.375 for low sensitive indicators, 0.75 for medium sensitivity and 1.5 for high sensitivity and b was set equal to 1.105 in all the cases. The CV may be evaluated by choosing one of the three equations for each indicator (according to the sensitivity level defined by the user), with the exception of reliability which is always translated as a medium sensitive indicator (M).

Equation 24 is continuous, allowing the translation of both integer and irrational scores.

Finally, the overall uncertainty of the specific parameter is determined using equation 25:

$$CV_{tot} = \sqrt{CV^2_{reliability} + CV^2_{completeness} + CV^2_{geogr.corr} + CV^2_{temp.corr} + CV^2_{other\ corr.}} \qquad \text{(eq. 26)}$$

The CV coefficient can be then entered directly in STAN software as the standard deviation of each parameter.

Table 6.1. Pedigree matrix for the definition of data quality indicators and qualitative criteria for the application of the scores, as reported in Laner et al., 2016.

Indicator	Definition	Score: 1	Score: 2	Score: 3	Score: 4
Reliability	Focus on the data source: documentation of data generation, e.g. assessment of sampling method, verification methods, reviewing processes.	Methodology of data generation well documented and consistent, peer-reviewed data.	Methodology of data generation is described, but not fully transparent; no verification.	Methodology not comprehensively described, but the principle of data generation is clear; no verification.	Methodology of data generation unknown, no documentation available.
Completeness	Composition of the date of all relevant mass flows. Possible over or underestimation is assessed.	Value includes all relevant processes/flows in question.	Value includes quantitatively main processes/flows in question.	Value includes partial important processes/flows, certainty of data gaps.	Only fragmented data available; important processes/mass flows are missing.
Temporal correlation	Congruence of the available data and the ideal date with respect to time reference.	Value relates to the right time period.	Deviation of value 1 to 5 years.	Deviation of values 5 to 10 years.	Deviation more than 10 years.
Geographical correlation	Congruence of the available date and the ideal date with respect to geographical reference.	Values relates to the studied region.	Values relates to socioeconomically similar region.	Socioeconomically slightly different region.	Socioeconomically very different region.
Other correlation	Congruence of the available date and the ideal date with respect to technology, product, etc.	Value relates to the same product, the same technology, etc.	Values relate to similar technology, product, etc.	Values deviate from technology/product of interest, but rough correlations can be established based on experience or data.	Values deviate strongly from technology/product of interest, with correlations being vague and speculative.

In the context of SFAs, field measurement regarding data of concentration (or load) of the selected compounds in the output (for example, in the receiving river) are often considered, in order to give to the software (e.g., STAN) support in the data reconciliation step. In this case, also the uncertainties regarding the analytical methods must be considered. A proper assessment of the analytical uncertainty regards the evaluation of the robustness of the method, its precision, and the recovery of the measuring system (van der Jagt, 2019). An in-depth discussion on quality control procedures and the estimation of the uncertainties associated to the adopted analytical method are out of the scope of this thesis, and, for further information, the reader is referred to Subedi, 2019 and van der Jagt, 2019.

6.3. Evaluation of the sensitivity of the adopted parameters

Another essential tool which should be implemented in an SFA, as well as any other numerical analysis, is the sensitivity analysis.

With this analysis, it is possible to quantify the importance a parameter has in influencing the model results, providing the measure of how the output changes following a defined variation in one input.

The sensitivity analysis can be complicated and deepened in different ways, depending on the aims of the study and the type of model one is using or implementing. One of the most common approaches for the assessment of the sensitivity of a model's parameters remains the one-at-time (OAT) sensitivity analysis (Pianosi et al., 2016).

Briefly, the analysis is carried out changing the value of one parameter at a time and evaluating the corresponding variation in the model output.

The influence of a parameter x_i on the output is thus represented by the Sensitivity index (S_i):

$$S_i = \frac{100}{p} \times \frac{OUT_i - \beta_0}{\beta_0} \qquad \text{(eq. 27)}$$

Where p is the percentage of parameter variation, OUT_i the new output value calculated after x_i variation, and $\beta 0$ the output value before any parameter variation (also called *base solution*).

In this context, p can be varied assuming a fixed value (for instance ±10% of x_i) as in Delli Compagni et al., 2020, or defined on the basis of extreme values reported in the literature, as in Verlicchi and Zambello, 2016. This last case aims to also consider the physical meaning of a parameter, instead of the only mathematical one. For example, many parameters value may not have a physical sense if decreased or increased of 10% (or another fixed value). Due to its normalisation, S_i index is not influenced by p value.

For this reason, the evaluation S_i with a variation of p according to the second method was carried out in the Austrian case-study, simulating a *real* case scenario. In particular, as extensively described in Ghirardini et al., 2021:

- the maximum CBZ and SMX load entering the system with human consumption was evaluated considering the consumption data reported in Clara et al., 2005, corresponding to 795 mg inhabitant^{-1} year^{-1} and 121 mg inhabitant^{-1} year^{-1} for CBZ and SMX, respectively. In this context, only 31% and 28% of the total load, for CBZ and SMX, respectively, was considered to reach the sewer system, according to the values of excretion factor derived from the analysis in Verlicchi and Zambello, 2016 (average of the literature values). The maximum load of CBZ and SMX resulting from these calculations is reported in Table S5 and Table S6 of the Supplementary Material of Ghirardini et al., 2021. Due to the lack of data about local consumption, it was not possible to evaluate the minimum value to set for CBZ and SMX. Thus, the minimum PhAC consumption was assumed equal to 0 in both cases;

- the minimum and maximum SMX load in manure was evaluated by multiplying the amount as assessed above, by the corresponding minimum and maximum concentrations reported in the review by Ghirardini et al., 2020 (Table S5 and Table S6 of the Supplementary Material of Ghirardini et al., 2021);
- the minimum and maximum percentage value of wastewater discharged *via* CSO was assessed as described before, assuming different values of dilution (*d*). In particular, as reported in Table S8 of the Supplementary Material of Ghirardini et al., 2021, the maximum percentage of wastewater discharged via the CSOs (1.8% in SC1, 1.4% in SC2, 1.6% in SC3 and 2.1% in SC4) corresponds to the lowest value of dilution (d=30), and the minimum percentage of wastewater discharged via the CSOs (0.6% in SC1, 0.4% in SC2, 0.5% in SC3 and 0.6% in SC4) corresponds to the highest value of dilution (d=100);
- the minimum and maximum percentages of load released with WWTP effluent were set to 10% Göbel et al., 2007 and 80% (Suárez et al., 2005) for SMX and 60% (Joss et al., 2005) and 95% (Jelic et al., 2011) for CBZ, according to the literature data reviewed in Verlicchi et al., 2012;
- the minimum and maximum percentages of PhAC that degraded during sewage sludge treatment was set equal to 0% (Yang et al., 2016) and 40% (Gonzalez-Gil et al., 2016) for CBZ and 79% (Gonzalez-Gil et al., 2016) and 99% (Carballa et al., 2007) for SMX;
- the variability range of PhAC mass moved in the soil aqueous phase after sludge and/or manure amendment was evaluated as reported above in the section regarding the evaluation of PhAC load splitting onto the soil, but considering different values of soil characteristics (namely organic carbon fraction (f_{OC}), bulk density (ρ_b) and porosity (v_0)). In particular, the K_d minimum value was calculated assuming wet clay soil (ρ_b equal to 1.1 g cm^{-3} and v_0 equal to 0.58 according to Marshall et al., 1996) and an f_{OC} of 0.92 as the minimum value found in Austrian arable lands (Gerzabek et al., 2005). The K_d maximum value was calculated assuming sandy loam soil compacted by heavy traffic of agricultural vehicles (ρ_b equal to 1.9 g cm^{-3} and v_0 equal to 0.28 according to Marshall et al., 1996) and a f_{OC} of 3.62 as the maximum value found in Austrian arable lands (Gerzabek et al., 2005);
- the minimum and maximum percentages of PhAC that degraded once in the surface water body were assessed as reported above in the section regarding PhAC behaviour in surface water, considering their maximum and minimum half-life found in the literature. As to SMZ, they were assumed equal to 17.8 days (Liu et al., 2019) and 3.7 hours (Baena-Nogueras et al., 2017). Regarding CBZ, it was already discussed that a negligible degradation might occur in the water compartment (i.e. half-time undefined) and thus only the scenario corresponding to a potential minimum half-life of 100 days (Andreozzi et al., 2003) was considered.

Further details are provided in Table S15 and Table S16 of the Supplementary Material of Ghirardini et al., 2021.

6.4. Results of the Austrian river catchment case-study

By way of illustration, some of the results of the case-study mentioned so far are briefly summarised in this paragraph. For a complete description of the SFA findings, the reader is anyway referred to Ghirardini et al., 2021.

Figure 6.3 schematically shows what is reported in a simplified way in Figure 6.1: it represents the final STAN map with all the flows from the input to the output in the surface water body in each SC. Each flow is marked with a name like SC#_F$ where SC# corresponds to the sub-catchment area to which it belongs, and F$ corresponds to the number assigned following the same order in which they are described in the sections of Ghirardini et al., 2021. In addition, only for manure, the systems make a distinction between four different types of animals producing it and this corresponds to a further number added after the flow number SC#_F2.§ (with § = 1, 2, 3, 4). Simple compartments are represented as white rectangles while the compartments consisting of multiple sub-compartments are represented as grey rectangles (arable land, WWTP and sludge treatment, and sludge stock).

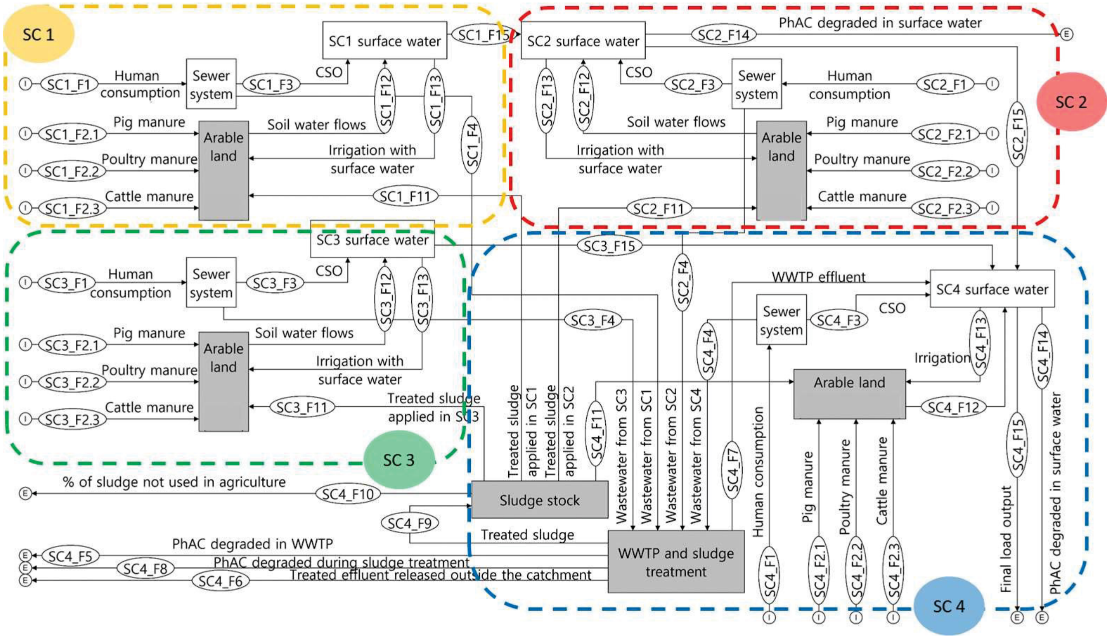


Figure 6.3. STAN representation of the flows which may contain CBZ and SMX in the studied catchment.

The parameter entered in the model are reported in Appendix 1 of Ghirardini et al., 2021 and corresponds to the load of CBZ and SMX entering the system as input (from human consumption and animal manure, marked by an I in Figure 6.3) and the splitting fractions the entering loads, evaluated as reported above.

The total annual load of CBZ and SMX released in the surface water body through the different emission pathways resulted equal to 9.5 kg year^{-1} and 10.6 kg year^{-1} respectively. In this context, it is important to remark that not all the entire of the amount released in surface water reaches the endpoint of the river (point D in Figure 6.1) because of degradation during the water flow.

Regarding CBZ, as represented in Figure 6.4, out of 9.5 kg year^{-1} of CBZ released into the catchment surface water, around 98.5% was due to WWTP effluent (9.4 kg year^{-1}), 1.5% to the CSOs (132 g year^{-1}, of which 28 g year^{-1} from SC1, 46 g year^{-1} from SC2, 24 g year^{-1} from SC3 and 34 g year^{-1} from SC4) and only a negligible percentage was from the vadose zone flow (3.7 g year^{-1}, of which 0.4 g year^{-1} from SC1, 1.5 g year^{-1} from SC2, 0.7 g year^{-1} from SC3 and 1.1 g year^{-1} from SC4).

Different results were found for SMX (Figure 6.5), for which out of 10.6 kg year^{-1} released in the river, 10.4 kg year^{-1} were related to the vadose zone flow, representing around 98% on the total released amount. WWTP effluent contributed to 229 g year^{-1} representing the remaining 2% of the entire load released, and the CSO contribution was only negligible (5.3 g year^{-1} of which 1.1 g year^{-1} from SC1, 1.8 g year^{-1} from SC2, 1.0 g year^{-1} from SC3 and 1.4 g year^{-1} from SC4).

Bearing in mind that CBZ is supplied in the system only from human consumption, and SMX is also entered with animal manure, these differences in terms of contribution of emission pathways to the load of PhACs in the receiving water body highlighted the potential variation of the results that the application of manure may make.

Further details about the PhACs load in the different compartments of the catchment are provided in Table S22 of the Supplementary Material of Ghirardini et al., 2021.

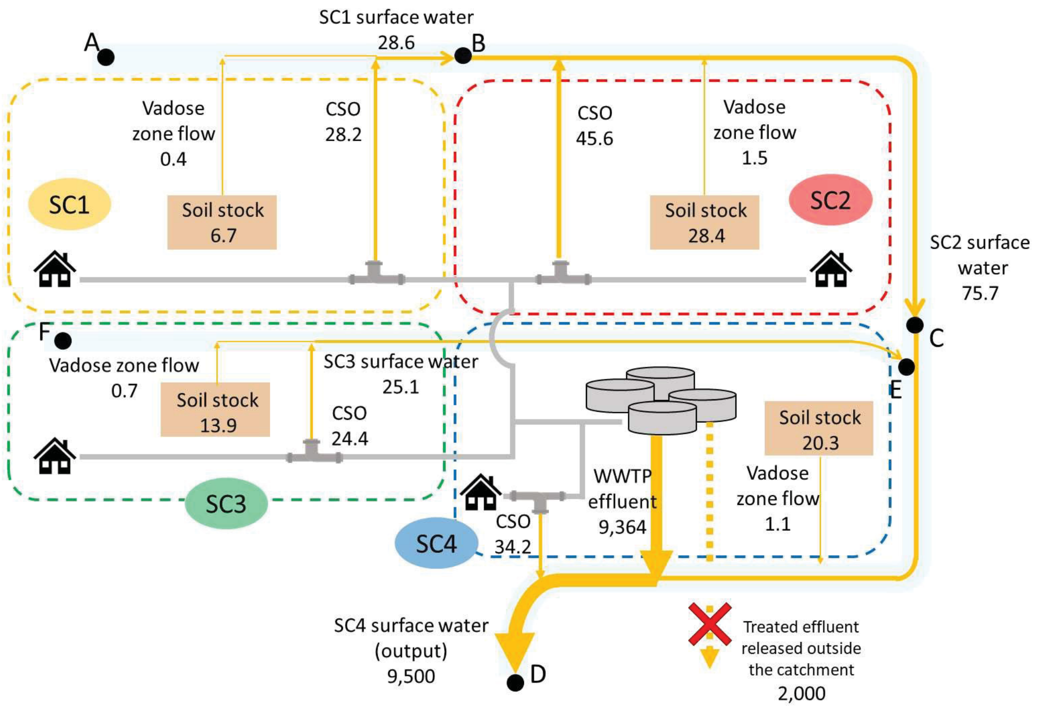


Figure 6.4. Load (g year-1) of CBZ in the emission pathways and surface water of each SC of the catchment under study.

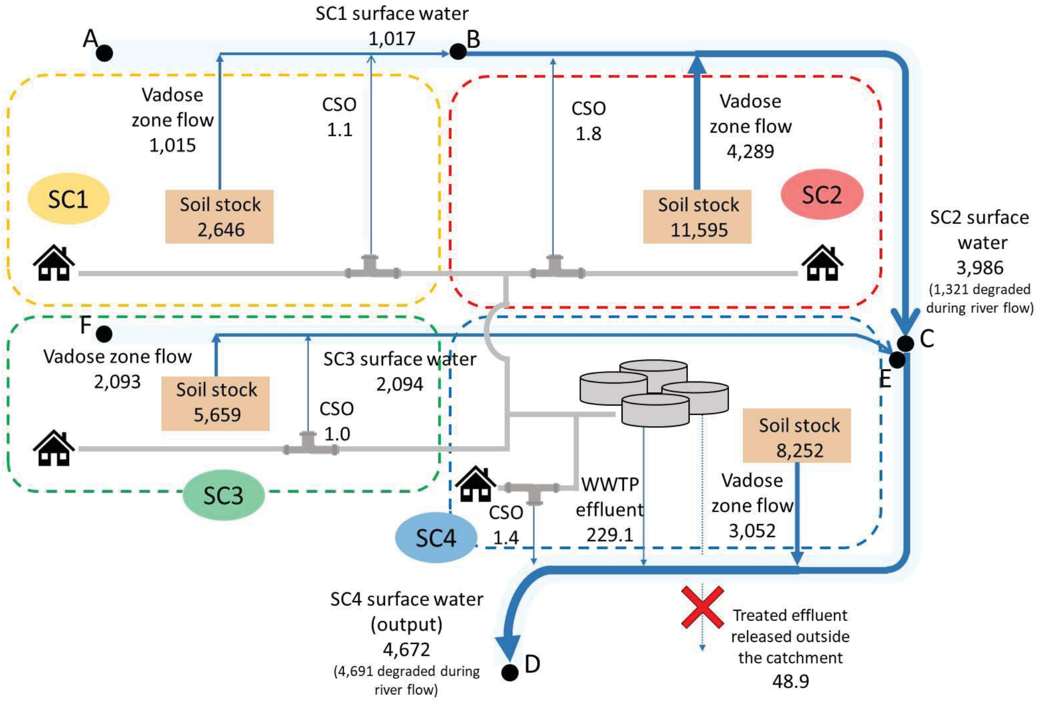


Figure 6.5. Load (g year-1) of SMX in the emission pathways and surface water of each SC of the catchment under study.

Regarding the uncertainty analysis, the uncertainty of the parameters involved in the SFA is represented, in terms of their coefficient of variation (*CV*, as defined above in Section 6.2) in Figure 6.6, and also reported in detail in Table S23 and S24 (for CBZ and SMX respectively) of the Supplementary Material of Ghirardini et al., 2021.

It emerged that the highest *CV*, and therefore the highest uncertainty, was found for the percentage of PhAC load that was moved into the aqueous phase of the soil (*CV* = 65.8). This result is not surprising, as the evaluation of this parameter did not consider many important processes occurring in the soil matrix (as already mentioned above in Section 6.1.5).

High values of *CV* were also found for the PhAC consumption data (*CV* = 41.8), percentage of wastewater conveyed to the treatment and to CSO (*CV* = 43.8) and the percentage of PhAC that degraded in surface water (*CV* = 43.8).

For the other parameters, the *CV* ranges from 14.7 (for the percentage of PhAC that degraded in WWTP, sequestered into sewage sludge or released with treated effluent) to 32.1 (for the percentage of PhAC that degraded during sewage sludge treatment).

Finally, the overall uncertainty of the PhACs load in the endpoint of the river (model output) was evaluated by STAN using Gaussian error propagation, resulting equal to 45% and 113% for CBZ and SMX, respectively.

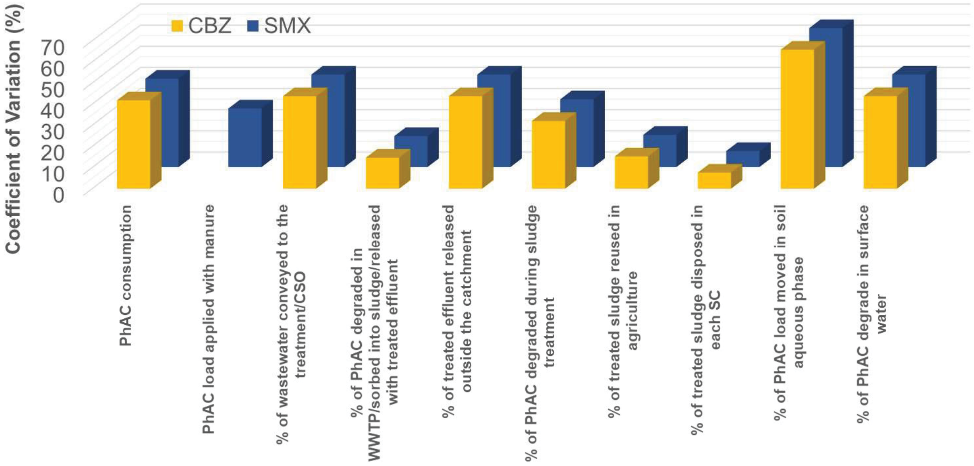


Figure 6.6. Uncertainty of the parameters involved in the uncertainty analysis reported in terms of coefficient of variation (*CV*), as defined above in Section 6.2.

Finally, the sensitivity analysis revealed that the parameters which mainly influence the results are different for the two PhACs studied.

Regarding CBZ, the highest S_i range, and therefore the highest significance was found for CBZ removal in WWTP ($S_{i,min}$=-110%, $S_{i,max}$=54.7%), followed by degradation in surface water, for which only the maximum value can be evaluated (due to the assumption of no degradation, for the minimum value) ($S_{i,max}$=109%).

On the contrary, regarding SMX, degradation in surface water was the most significant ($S_{i,min}$=-72.2%, $S_{i,max}$=131%), followed by soil characteristics (represented by the percentage of SMX moved in the aqueous phase, as evaluated above in Section 6.1.5) ($S_{i,min}$=-72.2%, $S_{i,max}$=131%).

The differences in parameter influence between CBZ and SMX is driven by the addition of manure in the second one, which entails a significant amount of compound in the soil comportment, and thus a consistent influence of soil-related parameters, instead of wastewater-related parameters, as in CBZ.

Table 6.2. Summary of the results of the case study reported in Ghirardini et al., 2021.

	CBZ			**SMX**		
Load in SC4 river endpoint ± overall uncertainty	9.5 ± 45% kg year^{-1}			4.6 ± 113% kg year^{-1}		
Emission pathway contribution	WWTP effluent	CSOs	Runoff, tile drainage, interflow	WWTP effluent	CSOs	Runoff, tile drainage, interflow
	98.6%	1.4%	<< 1%	2.1%	<< 1%	97.8%
Most uncertain parameters	Flow splitting in soil system (*CV* = 65.8) PhAC degradation in surface water (*CV* = 43.8) Flow splitting in the sewer system (*CV* = 43.8) Treated effluent released outside the catchment (*CV* = 43.8) PhAC human consumption (*CV* = 41.8)					
Most significant parameters	Human consumption CBZ removal in WWTP CBZ degradation in surface water Treated wastewater released outside the catchment			SMX degradation in surface water Soil characteristics SMX amount added with manure amendment		

The results of the models, which are summarised in Table 6.2, anyway, were only partially verified by a dedicated sampling campaign in a different point of the river and in different days of the year (further details regarding the sampling methods and analysis are reported in a dedicated section of Ghirardini et al., 2021). In particular, if for CBZ the modelled results (in terms of compound concentration, evaluated considering the annual average values of river water flow) were very similar to the one measured in surface water, for SMX they were overestimated of 2 order of magnitude (Figure 6.7). This may be due to the approach followed for the evaluation of SMX load in soil water flows from manure-amended arable land, which has been done considering no degradation of the compound both in manure and in manure-amended soil. In this context, as already explained in Section 6.1.5, the evaluation of PhAC load splitting in the soil compartment should consider many different mechanisms which are excluded in the assumptions of Langdon et al., 2010.

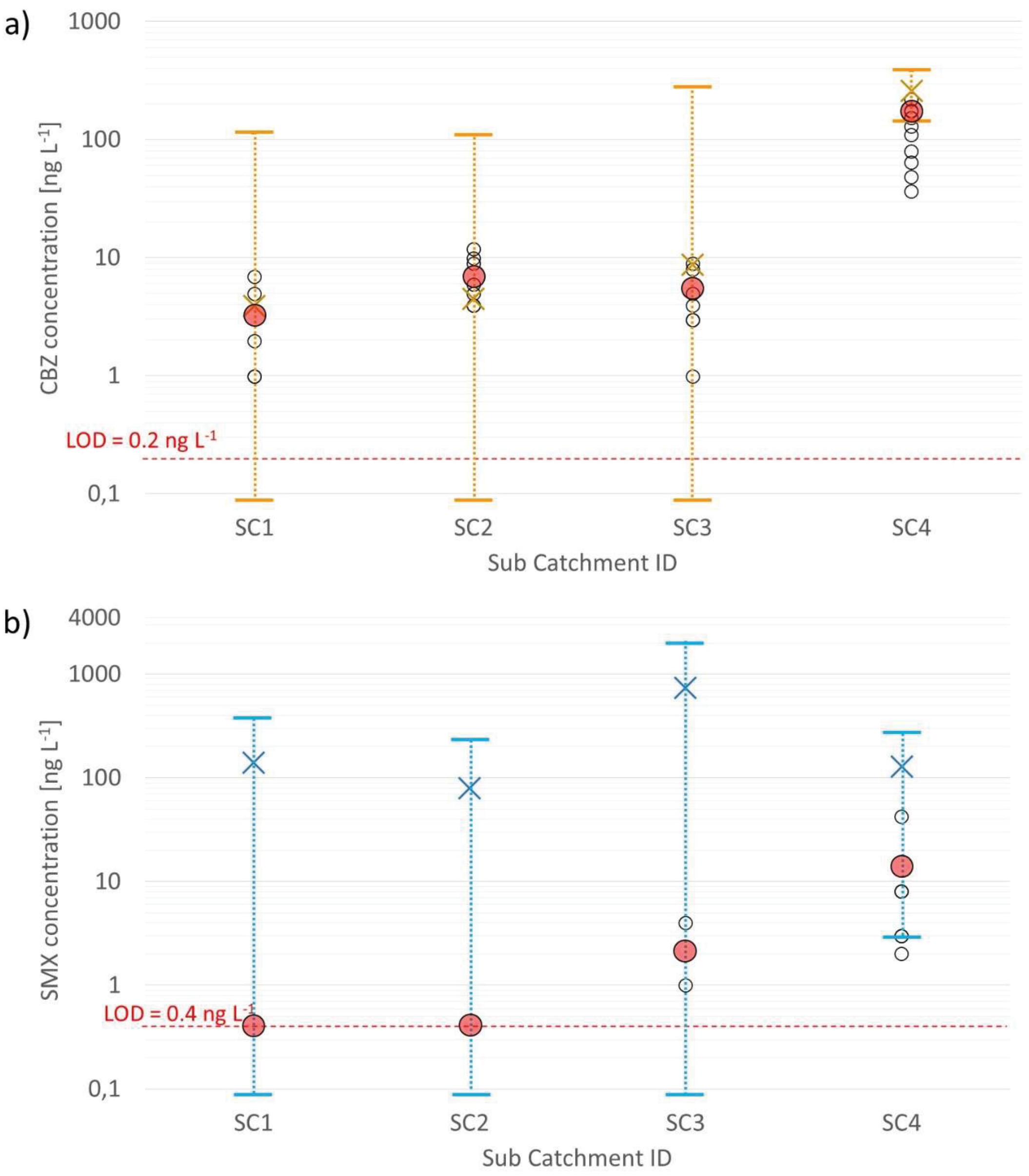


Figure 6.7. Comparison between the case-study modelled and measured results for CBZ (a) and SMX (b). Measured concentrations and the average measured concentration are represented by empty black dots and red dots, respectively, while the average modelled concentrations and uncertainty range are represented by crosses and scatters, respectively.

The case-study offers the possibility of evaluating all the methods proposed above for the assessment of PhACs behaviour in the environment on a catchment scale, resulting in theoretical considerations which serve as starting point for an in-depth analysis. Further research would thus consider:

- PhAC fate and mobilisation in the soil environment, considering the PhAC physicochemical properties and the soil characteristics;
- PhAC fate in the surface water environment, investigating the potential degradation pathways depending on water conditions, such as turbidity, salinity, temperature, and other physical and chemical properties;
- monitoring campaigns which exhaustively investigate the main emission pathways of pharmaceutical compounds.

Overall, SFA proved to be a valuable tool for the evaluation of micropollutants behaviour in the aquatic environment on a catchment scale, especially when numerous and reliable data are available.

Main conclusions

- *Substance Flow Analysis proved to be a valuable tool for the estimation of micropollutant occurrence in the water compartments of a catchment, especially when a large and reliable set of data is available.*

- *Wastewater treatment plant effluent showed to be the most relevant emission pathway of human-consumed pharmaceuticals (e.g., carbamazepine) in the receiving water body.*

- *Soil water flows (such as surface runoff, tile drainage, and groundwater flow) also became a relevant emission pathway in case of pharmaceuticals consumed by both human and animals (e.g., the antibiotic sulfamethoxazole).*

- *Combined sewer overflow was, in any case, a secondary emission pathway of pharmaceuticals in the environment, compared to the other two considered. Nevertheless, it can be considered as a point source, which may release a consistent amount of micropollutants in the receiving river without any treatment.*

- *The most important parameters of the analys are related to WWTP characteristics and degradation in surface water in the case of human-consumed pharmaceuticals, while are related to soil characteristics and degradation in surface water in the case of human- and animal-consumed pharmaceuticals.*

- *The most uncertain parameters in the analysis are related to the modelling of the fate and transport of substances in the soil system and in the surface water.*

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Chapter 7

INSIGHTS ABOUT TOXIC EFFECTS ON CROP DUE TO IRRIGATION WITH SURFACE WATER CONTAMINATED WITH PHARMACEUTICALS AND PERSONAL CARE PRODUCTS

Table of contents

Aims of the chapter, in a nutshell

- *The results, in terms of concentrations of pharmaceuticals, of several sampling campaigns were clustered to make a limited number of mixtures, representative of a real-case scenario of surface water used for crop irrigation.*

- *The obtained representative mixtures were used to provide a laboratory high throughput ecotoxicological assessment of PhAC exposure using garden cress (Lepidium Sativum) as the model species.*

- *The measured effects on garden cress growth regarded root length, shoot height, biomass weight, number of seeds germinated, and number of days needed to reach the 4-leaf stage.*

7.1. Introduction

Pharmaceutical and personal care products (PPCPs) are nowadays commonly detected in surface water bodies all around the globe (Voulvoulis et al., 2016). As already mentioned in the previous chapter of this thesis, after human and animal consumption, these compounds may reach the water courses through many different pathways, such as wastewater treatment plant (WWTP) effluents, combined sewer overflows (CSOs), hospital effluents and also surface runoff, tile drainage and groundwater from arable land amended with sewage sludge or animal manure.

Once in surface water, PPCPs may enter in the agro-ecosystem through crop irrigation, and potentially be uptaken and accumulated in plant tissue (Miller et al., 2016). This process could not only adversely affect plant growth but also pose the risk of PhAC exposure to animals and humans that consume those agricultural products (Carter et al., 2019).

To date, information concerning the effects of these micropollutants on crops irrigated with contaminated water are missing or incomplete (Poustie et al., 2020). In this regard, some studies (among them Godoy et al., 2018) investigated the PhAC phytotoxicity on plants focusing on one compound at a time and in mg L^{-1} to g L^{-1} concentration level. If in one hand this type of studies appropriately contributes to fill important knowledge gaps regarding acute toxicity of micropollutants and help to define the well-known lethal and effective concentrations (LC and EC) which are commonly used in the environmental risk assessment established by the European Medicines Agency (European Medicines Agency, 2006), on the other hand, they only occasionally provide information about antagonistic, synergistic and additive effects between different PhAC, which are expected to occur in the environment as a complex mixture instead of a single substance (Bártíková et al., 2016).

In this regard, mixture effects demonstrated to occur in laboratory plant toxicity experiments, suggesting the importance of also involving PhAC mixture in future investigations (Timmerer et al., 2020).

Also, as highlighted in some recent studies (among them Di Baccio et al., 2017), plant growth may be negatively affected not only by PhACs spiked at considerable concentrations, but also at environmentally relevant concentrations (ng L^{-1} to µg L^{-1}).

Bearing all these points in mind, this chapter aims to illustrate the effects (in terms of biomass growth, root length and shoot height elongation) on crops – focusing specifically on garden cress (*Lepidium sativum*), an edible plant commonly cultivated in northern Europe – of PhAC mixtures which are taken as representatives of real case scenarios of crop irrigation with surface water.

To do this, data about the occurrence of PhACs in surface water all around the world were considered, and 7 specific mixtures related to 7 sampling campaigns carried out in different countries were selected as representative for many others in terms of PhAC occurrence ratio. This was done through a specific cluster analysis which helped to create 7 groups containing the 133 sampling campaigns considered and providing not only the representative mixtures of these groups but also their minimum and maximum level of PhAC occurrence, allowing integrating the plant growth experiment with a dedicated exposure test.

The experiment discussed in the following sections was carried out during the visiting period in the University of York (from September to December 2019), under the supervision of the Professor A. Boxall of the department of Environment and Geography. Nowadays, the findings of the investigation are not yet published.

L. Sativum was chosen for its remarkable adaptability to different environmental conditions and its sensitivity to phytotoxic substances that make it suitable for quick and reliable ecotoxicological tests (Janecka and Fijalkowski, 2008).

7.2. Selection of representative mixtures of PPCPs and set-up of the plant growth experiment

7.2.1. Selection of representative mixtures of PPCPs for the irrigation experiment: The Cluster Analysis

For the selection of the mixtures of PPCPs which can be representative of various observable surface waters contaminated, a large dataset of measured concentrations of PPCPs in rivers and lakes all around the globe has been considered.
The starting dataset refers to the findings of a study, nowadays in preparation by Dr Wilkinson of the Department of Environment and Geography of the University of York, which groups the results of 133 sampling campaigns, carried out in 102 different countries, about concentrations of 62 PPCPs.
In this context, in order to obtain clusters that may be representative of mixtures with different *ratios* of PPCPs occurrence, all the available concentrations were normalized with the total concentration of the referring sapling campaign. Normalization is also suggested before a cluster analysis to standardize the dataset values and to reduce outliers which may drive the analysis (Mohamad and Usman, 2013). The PPCPs that were never detected in all the sampling campaigns have been removed from the dataset, as well as for the sampling campaigns in which less than five compounds were detected, in order to avoid for non-detected compounds to drive the cluster analysis. The final adopted dataset is reported in Appendix 6 in Table I1 and Table I2. It consists of average concentration data of 47 PPCPs referring to 102 sampling campaigns, from now on called *data points*.

The cluster analysis was carried out in MATLAB with the well-known *k*-means algorithm (Hennig et al., 2016), which has proven to be effective in case of a dataset in which data points appear to be qualitatively grouped due to their similarities (*e.g.*, similar patterns of PhAC occurrence ratio in different sampling campaigns) (Meilă, 2006).
Briefly, *k*-means is a partitional clustering algorithm that leads creating K groups (clusters, c_k) of data points x_i in which the within-cluster sum of squares S (*i.e.*, variance, typically the squared Euclidean distance) between the data points and their empirical mean (centroid, μ_k) is minimized (Hennig et al., 2016):

$$S = min \sum_{k=1}^{K} \sum_{x_i \in c_k} \|x_i - \mu_k\|^2 \qquad \text{(eq. 1)}$$

The *k*-means algorithm requires three user-specified parameters: *cluster initialization*, *distance metric*, and the *number of clusters K*.
As MATLAB default, *cluster initialization* was here carried with the kmeans++ method (Arthur and Vassilvitskii, 2007) and the *distance metric* was set as squared Euclidean.
The determination of the *number of clusters* that better describe the whole dataset is often tricky and does not exist a predefined criterion for its evaluation (Jain, 2010).

To help overcome this issue, in this study to well-known methods, combined together, were carried out: The Elbow and the Silhouette methods (Kassambara, 2017).

The Elbow method help to graphically identify the number of clusters (hereinafter referred as *partitioning configuration*) so that adding another cluster does not lead consistently to a further reduction of *S*. In this study, the value of *S* function of *K* was calculated running the *k*-means algorithm for values of *K* starting from 2 and ending with 102.

The Silhouette method was then applied to evaluate the consistency of the solutions proposed by the interpretation of the Elbow method results.

In particular, the Silhouette value is a measure of how a specific data point is well matched to a specific cluster. It ranges between -1 and + 1, where the lowest and the highest value indicates that the data point is poorly and well-matched, respectively. Here, the average between the silhouette values of the whole dataset (Sil_{ave}) gives an overall assessment of the consistency of the carried-out clustering (Kassambara, 2017). In this context, Sil_{ave} has been evaluated for the data set considering the number of clusters suggested by the Elbow method results.

The number of clusters that better represent the entire dataset was then evaluated according to the highest value of Sil_{ave} observed among the different solutions.

Finally, once the clustering algorithm was run, the sampling campaign closest to each cluster centroid (evaluated with Euclidean distance) was chosen to represent the specific cluster.

The graphical result of the Elbow method is represented in Figure 7.1. It emerges that the number of clusters the dataset may be divided into (and thus the *partitioning configurations*) ranges between 7 and 12.

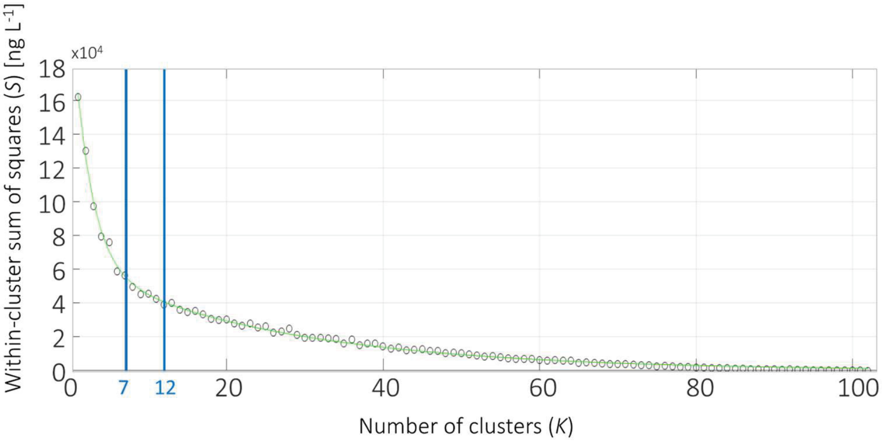


Figure 7.1. Graphical results of the elbow method.

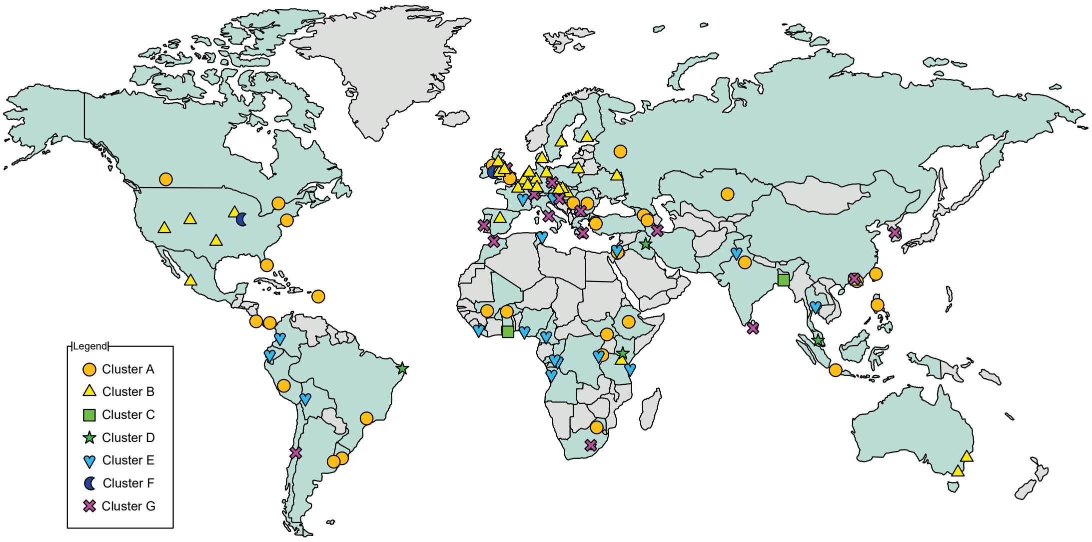


Figure 7.2. Graphical representation of the cluster related to each sampling campaign. Countries in which at least one sampling campaign was carried out are coloured in light green, while countries in which no sampling campaign was carried out are coloured in light grey.

Among the potential partitioning configurations, the division in 7 clusters showed the highest cohesion between each data point and its assigned cluster with a Silhouette value of 0.55 (Table 7.1).
Therefore, the dataset was divided into 7 clusters (hereinafter indicated with the respective alphabet letter, from A to G) containing 33, 29, 2, 4, 17, 2, and 15 sampling campaigns (datapoints) respectively (Table 7.2).

Table 7.1. Silhouette value of the dataset clustered in different partitioning configurations.

Number of clusters in the partitioning configuration	Average Silhouette value	number of clusters included in the partitioning configuration containing more than 4 campaigns
7	0.55	5
8	0.51	5
9	0.47	4
10	0.52	6
11	0.48	5
12	0.39	4

Table 7.2. Description and details of the clusters adopted for the generation of the mixtures.

Cluster	Data points contained	PhAC mainly occurring in the sample (concentration ratio)	Details about the sampling campaign contained in the cluster		PhAC sum of concentration [ng L^{-1}]	Mixture ID
A	33	caffeine (40.2%); metformin (25.4%); paracetamol (12.3%); sulfamethoxazole (6.8%)	Sampling campaign closest to the cluster centroid:	Pretoria (South Africa)	12,272	A mid
			Sampling campaign with the lowest PhAC sum of concentration within the cluster:	Miami (USA)	464	A low
			Sampling campaign with the highest PhAC sum of concentration within the cluster:	Addis Ababa (Ethiopia)	52,365	A high
B	29	metformin (18.5%); cimetidine (11.2%); sitagliptin (8.8%); caffeine (7.1%)	Sampling campaign closest to the cluster centroid:	Kai Tak River (Hong Kong)	7,965	B mid
			Sampling campaign with the lowest PhAC sum of concentration within the cluster:	Melbourne (Australia)	329	B low
			Sampling campaign with the highest PhAC sum of concentration within the cluster:	Madrid (Spain)	23,846	B high
C	2	metronidazole (73.4%); metformin (9.5%); ranitidine (10.2%)	Sampling campaign closest to the cluster centroid:	Barisal (Bangladesh)	10,052	C mid
			Sampling campaign with the lowest PhAC sum of concentration within the cluster:	Barisal (Bangladesh)	10,052	C mid^{A}
			Sampling campaign with the highest PhAC sum of concentration within the cluster:	Accra (Ghana)	21,564	C high
D	4	fexofenadine (68.8%); metformin (22.6%)	Sampling campaign closest to the cluster centroid:	Kajang (Malaysia)	14,176	D mid
			Sampling campaign with the lowest PhAC sum of concentration within the cluster:	Natal (Brazil)	2,226	D low
			Sampling campaign with the highest PhAC sum of concentration within the cluster:	Nairobi (Kenya)	40,317	D high
E	17	paracetamol (54.3%); metformin (14.7%); nicotine (7.8%)	Sampling campaign closest to the cluster centroid:	Bangkok (Thailand)	2,992	E mid
			Sampling campaign with the lowest PhAC sum of concentration within the cluster:	Ljubljana (Slovenia)	237	E low
			Sampling campaign with the highest PhAC sum of concentration within the cluster:	La Paz (Bolivia)	108,828	E high
F	2	gabapentin (43.2%);	Sampling campaign closest to the cluster centroid:	Missouri - St. Louis (USA)	241	F mid

Cluster	Data points contained	PhAC mainly occurring in the sample (concentration ratio)	Details about the sampling campaign contained in the cluster		PhAC sum of concentration $[\mathrm{ng\ L^{-1}}]$	Mixture ID
🟦		caffeine (36.0%); codeine (11.8%)	Sampling campaign with the lowest PhAC sum of concentration within the cluster:	Missouri - St. Louis (USA)	241	F mid[A]
			Sampling campaign with the highest PhAC sum of concentration within the cluster:	Dublin (Ireland)	694	F high
G	15	metformin (58.6%); caffeine (15.1%); paracetamol (8.3%)	Sampling campaign closest to the cluster centroid:	Lisbon (Portugal)	2,823	G mid
			Sampling campaign with the lowest PhAC sum of concentration within the cluster:	Rudbar (Iran)	252	G low
			Sampling campaign with the highest PhAC sum of concentration within the cluster:	Sofia (Bulgaria)	12,822	G high

[A] Clusters C and F only contain 2 data points. The sampling campaign with the lowest sum of concentration corresponds to the closest to the centroid campaign.

A rapid glance of Figure 7.2 shows that the clusters B and F contain sampling campaigns in high-income countries, with only two exceptions Arusha (Tanzania) and Puerto Vallarta (Mexico), while clusters C and D have sampling campaigns carried out in low and middle-income countries. Cluster E contains sampling campaigns mainly belonging to the Mediterranean country, central Africa, and Eastern South America, while Cluster G is mainly related to sampling campaigns carried out in Europe and some Asian countries (among them China and South Korea). Cluster A contains a broad spectrum of sampling campaigns belonging to different countries without a defined pattern. This is not surprising as it grouped the samples in which caffeine (CAF) occurs with the highest concentration ratio and bearing in mind that this micropollutant is widely consumed all over the world (López-Pacheco et al., 2019).

For the laboratory experiment, the concentrations of the cluster-representative campaign were used to define the main mixture, and its concentration ratios were multiplied with minimum and maximum sum of concentration related to the sampling campaigns belonging to the cluster, to obtain two additional mixtures with which to carry out the exposure test.

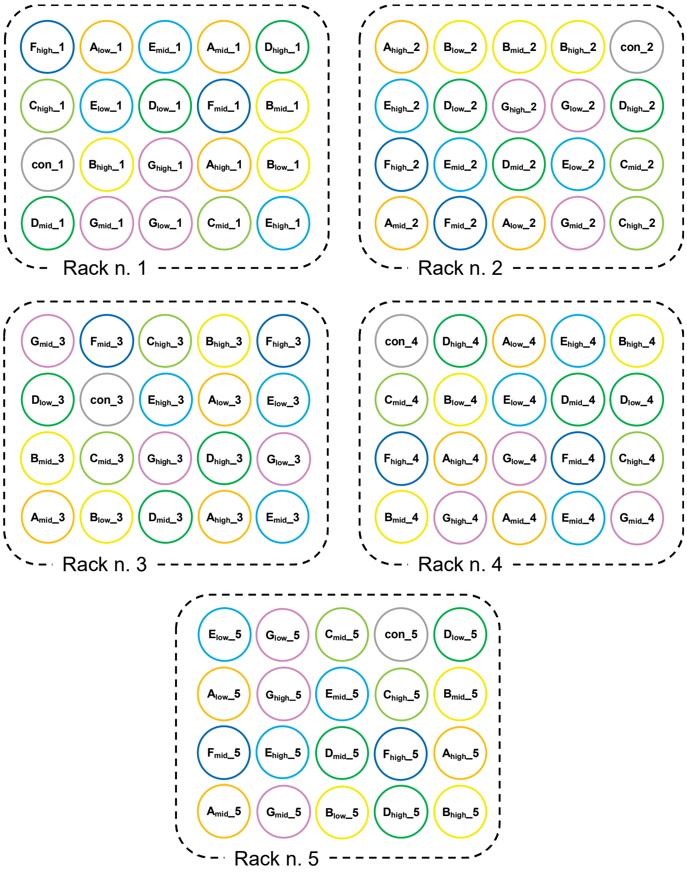
Table I3 of Appendix 6 reports the ratios of concentration of the sampling campaign representing the centroid of each cluster, as well as the mixtures obtained multiplying the concentration ratios of the centroid campaign with the minimum and maximum sum of the concentration of the sampling campaign related to each cluster, except for clusters C and F which contains only 2 data points. In these specific cases, the centroid campaign showed the lowest sum of PhAC concentration, thus only the maximum concentration-related mixture was further derived.

It should be noted that among the 47 compounds considered in the cluster analysis, 9 of them are not present in the 7 centroids. Thus, a total of 19 mixtures (3 for treatments A, B, D, E, and G, and 2 for treatments C and F) containing 38 PhACs were obtained and are available for the plant growth experiment. Here, PhACs occurring at higher concentrations are fexofenadine (27.7 µg L^{-1} in D-high), caffeine (21.0 µg L^{-1} in A-high), Metformin (16.0 µg L^{-1} in E-high) and Metronidazole (15.8 µg L^{-1} in C-high), and the treatments showing the higher PhAC content are E-high, A-high and D-high, with a total PhAC concentration of 108.8 µg L^{-1}, 52.3 µg L^{-1} and 40.3 µg L^{-1} respectively.

7.2.2. Setup of the plant growth experiment

The selected mixtures were then used to irrigate L. Sativum and monitoring the effects on its growth, in terms of biomass weight, root length, and shoot height.

For each mixture, glass test tubes (150 mm length, 16 mm Ø) containing 19.20 g of silica sand were prepared in replicates of five. Each test tube was saturated with 4 mL of the mixture under investigation or tap water (for the 5 control replicates), and immediately after, two seeds of *L. sativum* were planted at a depth of 0.5 ± 0.1 cm. The test tubes were then covered with laboratory wrapping film (PARAFILM) and incubated in an environmental test chamber under controlled conditions (50% relative humidity, 16 h light at 22°C and 8 h dark at 18°C). The resulting 100 test tubes (5 replicates of 19 mixtures + 1 control) were arranged in 5 racks with a completely randomized design (carried out with MATLAB software), to have 20 test tubes of all the mixtures in each rack (Figure 7.3).



F_{high}_1	A_{low}_1	E_{mid}_1	A_{mid}_1	D_{high}_1
C_{high}_1	E_{low}_1	D_{low}_1	F_{mid}_1	B_{mid}_1
con_1	B_{high}_1	G_{high}_1	A_{high}_1	B_{low}_1
D_{mid}_1	G_{mid}_1	G_{low}_1	C_{mid}_1	E_{high}_1

Rack n. 1

A_{high}_2	B_{low}_2	B_{mid}_2	B_{high}_2	con_2
E_{high}_2	D_{low}_2	G_{high}_2	G_{low}_2	D_{high}_2
F_{high}_2	E_{mid}_2	D_{mid}_2	E_{low}_2	C_{mid}_2
A_{mid}_2	F_{mid}_2	A_{low}_2	G_{mid}_2	C_{high}_2

Rack n. 2

G_{mid}_3	F_{mid}_3	C_{high}_3	B_{high}_3	F_{high}_3
D_{low}_3	con_3	E_{high}_3	A_{low}_3	E_{low}_3
B_{mid}_3	C_{mid}_3	G_{high}_3	D_{high}_3	G_{low}_3
A_{mid}_3	B_{low}_3	D_{mid}_3	A_{high}_3	E_{mid}_3

Rack n. 3

con_4	D_{high}_4	A_{low}_4	E_{high}_4	B_{high}_4
C_{mid}_4	B_{low}_4	E_{low}_4	D_{mid}_4	D_{low}_4
F_{high}_4	A_{high}_4	G_{low}_4	F_{mid}_4	C_{high}_4
B_{mid}_4	G_{high}_4	A_{mid}_4	E_{mid}_4	G_{mid}_4

Rack n. 4

E_{low}_5	G_{low}_5	C_{mid}_5	con_5	D_{low}_5
A_{low}_5	G_{high}_5	E_{mid}_5	C_{high}_5	B_{mid}_5
F_{mid}_5	E_{high}_5	D_{mid}_5	F_{high}_5	A_{high}_5
A_{mid}_5	G_{mid}_5	B_{low}_5	D_{high}_5	B_{high}_5

Rack n. 5

Figure 7.3. Experimental set-up of the plant growth study. The random design of the test tubes was defined using the MATLAB software.

The Parafilm™ was removed only after three days to avoid the water losses by evaporation (quantified as 0.5 ± 0.05 mL day^{-1}) in the test tubes and so providing the best conditions for seed germination (Figure 7.4). After that, the soil water content in the test tubes was maintained constant, adding 0.5 mL *per* plant daily. The racks were turned every day to ensure the same light conditions for all the test tubes. The plants were kept in the environmental test chamber for a total duration of 16 days, in which the number of days the plants took to sprout and the number of leaves that comes out was monitored. After that, the plants were thoroughly rinsed with tap water to remove the soil and to collect the plant from the tubes without damaging the roots.

Each plant was then patted dry with a paper towel, and its root length and shoot height were measured.

Finally, the plants were dried in an oven at 60°C for 9 hours, and their biomass weight was measured.

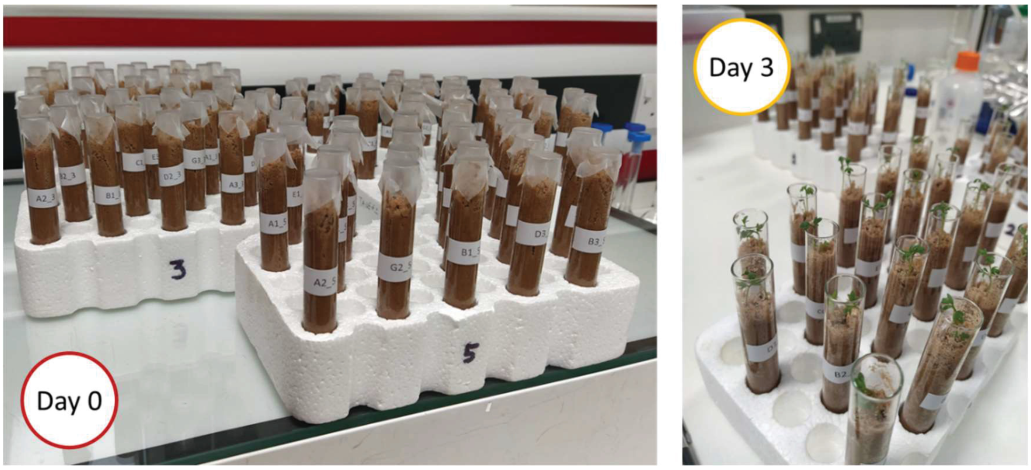


Figure 7.4. Garden cress racks, in day 0, with PARAFILM, and in day 3, after they sprout.

7.3. Toxic effects of crop irrigation with surface water containing mixtures of PPCPs

The measured effects on plants irrigated with the different mixtures are represented in Figure 7.6 and reported in Tables I4-I9 of Appendix 6, as well as the statistical analysis results. Here, no significant effects were measured about the days the plants need to sprout (Table I4 of Appendix 6). On the contrary, the growth of the leaves was significantly slowed down when the plants were irrigated with the high exposure of D treatment (p=0.003) (Table I5 of Appendix 6).

After 16 days, the growth of garden cress was affected in different aspects by the application of pharmaceutical-amended water:

As reported in Table I6 of Appendix 6, plant biomass weight was significantly (p<0.035) reduced when any mixture, except for D-mid and E-low, was applied. In this context, a maximum decrease of 55% of plant biomass between control and F-mid treated plants were measured. Remarkable effects were also determined in plants that received B-mid, D-low, E-high, G-mid, and G-high, for which a mass reduction of 45%, 47%, 45%, 49%, and 45% respectively was observed. As an example, the replicates of G-high, G-low, and control can be observed in Figure 7.5.

Figure 7.5. Garden cress plants after 16 days of growth. On the left, comparison between plants irrigated with G-high, G-low and control treatments. On the right, garden cress plants dried before the evaluation of the biomass weight.

It is worth noting that the decreasing in plant biomass was proportional to the exposure only for treatments A, C, and E. At the same time, in the other cases, the higher effects on mass reduction were observed in plants that received the medium (in case of B, F, and G) and low (in case of D) treatment exposure.

This may be due to the complexity of the processes impacting the availability of PhACs in plant roots and tissue (*e.g.*, transformation in the rhizosphere, sorption to plant roots, transformation in plant xylem or phloem), as well as interactions between PhACs, which make it difficult to infer and compare phytotoxic effects on plants (Miller et al., 2016).

Regarding root length and shoot height, no significant reductions were observed after the application of any mixture (Table I7 and I8 of Appendix 6) except for plants that received E-high, which were significantly impacted (*p*=0.048), resulting in a decrease of 41% of the root length.

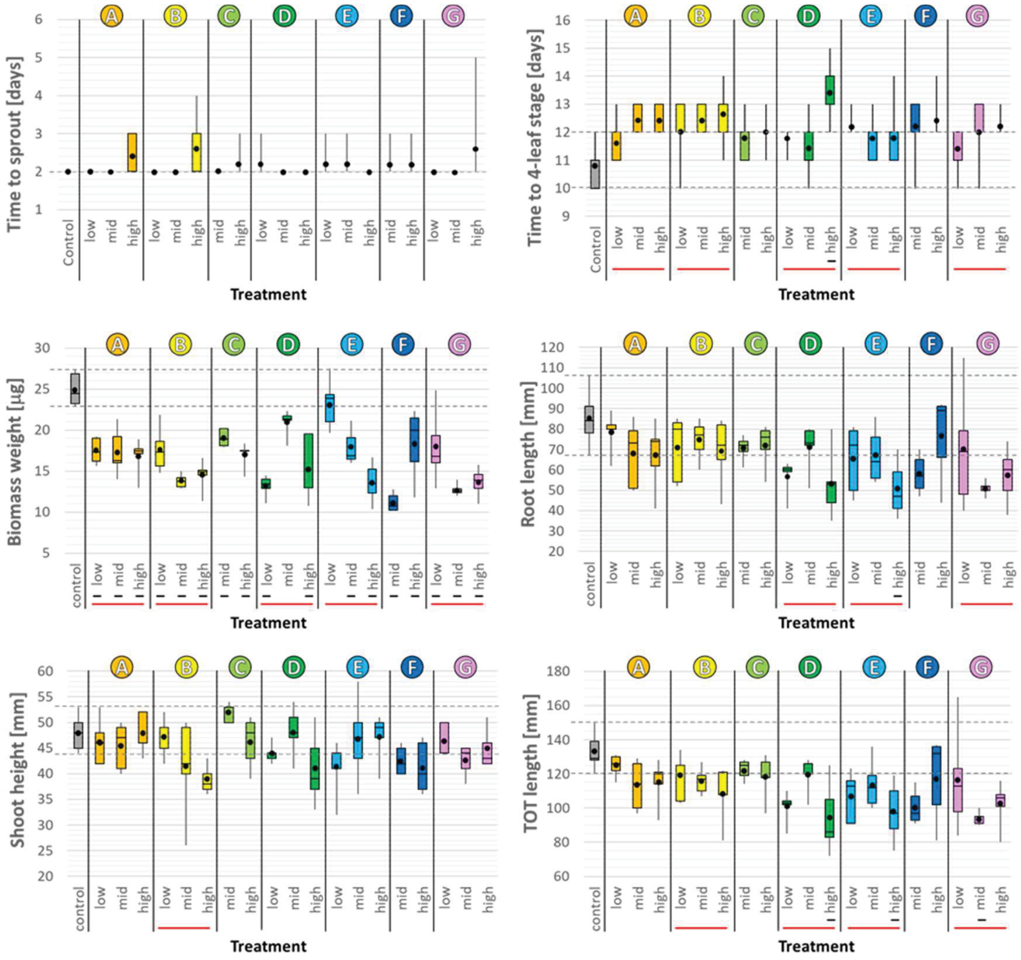


Figure 7.6. Boxplot (median, upper and lower quartile) with whiskers from minimum to maximum, representing the measured effects on plants (n=5) that received the 7 different treatments at low, medium, and high exposure, after 16 days of the experiment. Average measured values are plotted as black dots. Significant differences ($p<0.05$) between control and each mixture exposure (1-way ANOVA) are highlighted with short black dashes below the exposure label. Significant differences between control and treatments as a whole (2-way ANOVA) are highlighted with long red dashes below the exposure label.

Notwithstanding this, considering the differences in plant growth due to both treatment and exposure in the statistical analysis (two-way ANOVA), it emerges that root length was significantly affected when plants were treated with D, E, and G (*p*<0.001), and the same holds for the shoot height in plants that received B treatment (*p*=0.041). As a confirmation of these results, the difference in cress total length between the control and treated plants was found to be significant (p<0.001) when B, D, E, and G mixtures were applied, resulting in an average length decreasing of 17%, 27%, 25%, and 28% respectively (Table I9 of Appendix 6).

Overall, these findings did not lead to a single and clear interpretation.
The plant growing inhibition caused by treatments A, E, and G may be due to the considerable concentration of caffeine and paracetamol (PCT) (which were spiked at 21 μg L^{-1}, 9.6 μg L^{-1}, and 2 μg L^{-1} for CAF and, 6.5 μg L^{-1}, 60 μg L^{-1}, and 1 μg L^{-1} for PCT in the high exposure mixture of the three treatments respectively, Table I3 of Appendix 6) which revealed to be toxic for different plant species also at low concentrations. For example, Zezulka et al., 2019 found that *Zea Mays* root length growth was negatively affected by irrigation with water containing PCT at 10 μg L^{-1} and Fekete-Kertész et al., 2015 observed that 10 μg L^{-1} CAF treatment may inhibit *Lemna Minor* growth in terms of frond number and chlorophyll content. Here, it should be mentioned that the concentrations reported in these studies are both higher than those present in G-high, in which the compound that occurs at the highest concentration is metformin (MET, at 7.5 μg L^{-1}). Thus, the negative consequences on biomass and root growth of plants that received the G treatments may be due to the combination of different compounds (mixture effects), as MET generally does not show apparent acute effects on plants even at higher (mg L^{-1} level) concentrations (Briones et al., 2016).
Regarding the garden cress biomass and total length reduction caused by B and D treatments no further considerations can be done, as, to the best of the authors' knowledge, no previous studies investigated the effects of those mixtures' key PhACs (namely, cimetidine, gabapentin and sitagliptin for B treatment and fexofenadine for D treatment, Table 7.2) at environmentally relevant concentrations. However, it is impossible to strictly compare these study results with the existing literature findings, as different macrophyte species may have different phytotoxicological responses to a given chemical stressor (Hillis et al., 2011).
The effects observed in this work may also strongly differ to those observed in studies in which the plants are exposed to a single substance at a time, instead of a mixture of PhACs, due to antagonistic, synergistic, and additive effects between the different compounds (Rede et al., 2019). Furthermore, also soil characteristics may play an important role in plant sensitivity to phytotoxic substances by promoting the contaminants' bio-availability and uptake by plants (Sallach et al., 2018).

Concluding, although it is impossible to strictly correlate the observed effects on *L. sativum* growth with the exposure to a specific compound, the results mentioned above indicate that mixtures of PhACs occurring at ng L^{-1} to μg L^{-1} level may cause a reduction of plant biomass and plant elongation.
Overall, these findings highlighted that not only reclaimed wastewater irrigation may cause adverse effects on agricultural productivity due to micropollutants occurrence

(Poustie et al., 2020), but also surface water irrigation may potentially impact plant growth.

It is important to remark that *L. sativum* was here treated with tap water spiked with different PhACs at different concentrations so that to simulate real case scenario of PhAC pollution. Still, surface water, as well as reclaimed wastewater, may also contain major plant nutrients (N, P, and K) which may lead to positive impacts in terms of soil microbial activity and plant growth (Jaramillo and Restrepo, 2017).

Future research should thus evaluate the impact of crop irrigation with mixtures in which different surface water constituents (such as micropollutants, metals, salts, and plant nutrients) are present at the same time, to represent the actual common irrigation practice better.

Also, further study is required for evaluating the chronic effects of crop irrigation with PhAC at environmentally relevant concentrations, to better assess to what extent the agricultural productivity may be negatively impacted by emerging pollutants occurring in surface water, and what effects may be observed in edible plants.

Main conclusions

- *Cluster analysis proved to be a valuable tool to group a large amount of data about the occurrence of PhACs in surface water and to define a limited number of representative mixtures which can be used in ecotoxicological laboratory experiments.*

- *Seven mixtures, representative of socio-economic and regional patterns, were identified.*

- *Plant growth was negatively affected by the treatment of some mixtures, suggesting that also the irrigation with surface water contaminated with PhACs at environmentally relevant concentrations may cause adverse effects on agricultural productivity.*

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Conclusions

In conclusion, this PhD thesis aimed to elaborate a methodology to assess the occurrence of pharmaceuticals and personal care products in different environmental compartments of a catchment, from their consumption to their release into the receiving water bodies.

To do this, an in-depth literature review was conducted to have an overall view of the content of PPCPs in two wastes typically applied onto agricultural soil as amendants or fertilizers: sewage sludge (raw or treated) and animal manure (from different animals, and with different characteristics).

Furthermore, working on the findings of the recent scientific literature led to briefly investigate the various sampling strategies adopted in the monitoring of micropollutants, and estimating the potential uncertainty related to the measures.

Once considered all these elements, it was possible to start developing a model to quantify the occurrence of two selected pharmaceuticals (namely carbamazepine and sulfamethoxazole) in arable land and receiving river of an Austrian catchment. This work, carried out in collaboration with Dr Zoboli and Professor Zessner of the Institute for Water Quality and Resource Management of the TU Wien, helped to define the main aspects to bring this issue from a case-study to a general overview of the phenomena included.

Finally, to evaluate the potential risks posed by irrigating edible crops with surface water contaminated with PPCPs, a laboratory experiment was conducted in the Department of Environment and Geography of the York University, under the supervision of Professor Boxall, Dr Sallach, and Dr Wilkinson.

The answers obtained from these studies were manifold.

Firstly, it was found that zootechnical wastes may contain considerable quantities of antibiotics, hormones, and other compounds. The high occurrence observed, measurable at $\mu g\ g^{-1}$, up to $mg\ g^{-1}$, level of concentration, are caused by the significant amounts of pharmaceuticals periodically administered to animals for therapeutic uses, or growth promotion.

An exhaustive number of data was collected in this regard, leading to the publication of a dedicated review article, in Volume 707 of *Science of the Total Environment* (Ghirardini et al., 2020).

A comparison between these findings and those regarding the occurrence of PPCPs in sewage sludge reported by Verlicchi and Zambello, 2015, showed that, generally, animal manure contains higher concentrations of these compounds, even if belonging to a smaller group of classes (mainly antibiotics and hormones, as mentioned above).

The investigation of the behaviour of micropollutants when applied on arable land with sewage sludge – reviewed and published in Volume 655 of *Science of the Total Environment* (Ghirardini and Verlicchi, 2019) – led to the conclusion that, anyway, their residual concentrations in the receiving stream are strongly limited by several mechanisms occurring in the soil (*e.g.*, sorption onto soil particles, abiotic transformations, plant roots uptake, and so forth).

This fact suggests that the water flows occurring in vadose zone (such as surface runoff, tile drainage, and interflow) of soils amended with sewage sludge are only minor contributors to the occurrence of pharmaceutical and personal care products in surface water.

Despite this, due to the higher amount of pharmaceuticals commonly measured in zootechnical wastes compared to sewage sludge, a similar conclusion cannot be reached regarding soil water flows from arable land amended with animal manure.

Once studied from a modelling point of view, this issue took a different shape. In one hand the Austrian case-study confirmed that for human-related pharmaceuticals (*e.g.*, carbamazepine), the soil water flows are only marginal in contributing to their occurrence in surface water, especially compared to the wastewater treatment plant effluent.

But on the other hand, the focus on sulfamethoxazole (an antibiotic consumed both by humans and animals) resulted in the conclusion that the application of zootechnical wastes on arable land may be the principal contribute for its release in the receiving river, suggesting that, for at least some specific compounds, also the contribution of soil water flows cannot be overlooked.

However, many difficulties were reported about the modelling of the behaviour of pharmaceuticals in the different environmental compartments, on a catchment scale. Among them, the need to collect a considerable amount of data regarding climate conditions, soil characteristics, sewer network details, and design and operational conditions of the wastewater treatment plants working in the catchment, to name just a few.

For this reason, a remarkable uncertainty related to many assumptions was observed. In this context, it is worthy of mention that the estimation of the behaviour of pharmaceuticals into the soil system (once applied with sewage sludge or manure) was the most challenging issue, resulting in the highest uncertainty observed among all the parameters of the model (above 65%).

A high uncertainty was also observed for the behaviour of pharmaceuticals in the surface water flow (depending on many mechanisms, such as photolysis, sorption onto sediments, and degradation), in the sewer system (depending on the estimation of the contribution of combined sewer overflow), and human consumption (for lack of recent local data). The uncertainty was higher than 40% for all these three parameters.

In addition, it should mention that also the data used for the analysis, and obtained from various sampling campaigns, may be affected by uncertainty. As briefly described in this thesis, and better reported in a dedicated article published in Volume 11 of *Water* (MDPI)

(Verlicchi and Ghirardini, 2019), the measure of micropollutants concentration in water flow may vary by 1-30% in case of flow-proportional sampling, up to 40% in case of time-proportional.
The substance flow analysis conducted for carbamazepine and sulfamethoxazole also helped to identify the most significant parameters in modelling the behaviour of micropollutants on a catchment scale. It emerged that, for carbamazepine, due to its specific human consumption, the parameters which mostly affect the final results (in terms of its occurrence in the receiving river) were all related to wastewaters, for example, the percentage of wastewater released *via* CSO, or the fate of the pharmaceutical during the treatment.
On the contrary, for the behaviour and occurrence in surface water of sulfamethoxazole, the most significant parameters were related to the characteristics of the soils in the catchment, due to the predominance of manure application as a source of this second compound.
The results of the model were anyway satisfactory, especially regarding carbamazepine, and were published, with an in-depth discussion of its strength and weaknesses, in Volume 751 of *Science of the Total Environment* (Ghirardini et al., 2021).

The last part of this PhD work, in which the toxic effects on edible crops due to irrigation with surface water contaminated with mixtures of PPCPs, led to some interesting preliminary results, which are at the present day under discussion and preparation for the publication.
It was observed that garden cress growth eas affected, in terms of biomass weight, root elongation, and shoot height, when watered with mixtures of PPCPs simulating surface waters monitored all around the globe. This phenomenon occurred even when the compounds occurred at minimal concentrations (at ng L^{-1} level) suggesting that the mixtures of micropollutants may enhance the toxic effects, due to the synergistic and additive effects between the compounds.

The findings mentioned so far show that the occurrence of pharmaceutical and personal care products in the agricultural agroecosystem, and *mutatis mutandis* in the receiving water environment, are visible and measurable. Despite this, several efforts are needed to characterize the problem exhaustively.
For instance, further research should focus on:

(i) The study of the occurrence of micropollutants in soil water flows (*e.g.*, surface runoff and tile drainage) from arable land amended with animal manure.
In this regard, the investigations carried out in the last decades by many researchers showed a number of results which can be, in some cases, discordant or incomplete.
Therefore, further dedicated experiments should be carried out comparing the effects due to the application of different sludge and zootechnical wastes, in different soil and environmentl conditions. In addition, an in-depth review of the existing literature on this topic may thus certainly help to discuss these

results, and compare them with the same reported in case of sewage sludge application.

(ii) The investigation and the implementation of *ad hoc* models regarding the fate of PPCPs once applied on arable land. Here, due to the complexity of the problem, this issue may be overcome by studying the different mechanisms, occurring within the soil, separately.

(iii) The monitoring of the occurrence of PPCPs in various environmental compartments of a catchment, in different weather conditions, employing an exhaustive and comprehensive sampling campaign. This practice may strongly help to define the primary sources and emission pathways (*e.g.*, WWTP effluent, CSO, and surface runoff) of pharmaceuticals, and provide acceptable criteria for comparison with the results of the existing predicting tools.

(iv) The investigation with dedicated experiments on the relationship between the occurrence of specific micropollutants in surface water and the effects measured once used to irrigate the most common cultivate edible plants (*e.g.*, *Allium cepa*, *Lactuca sativa*, *Lepidium sativum*, or *Zea Mays*).

This research may help to isolate the compounds, in a complex mixture, which mainly cause toxic effects on crop, entailing their *prioritization*.

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Appendix 1

List of pharmaceuticals and personal care products included in the occurrence analysis for sewage sludge and manure (Ghirardini et al., 2020; Verlicchi and Zambello, 2015) and their main physicochemical properties.

Class	Compound	CAS#	Formula[a]	Mole weight [g mol^{-1}][a]	LogK$_{ow}$[a]	pK$_a$[a]	Charge at pH 7[b]	Occurrence data available for:	
								Sewage sludge	Manure
Analgesics and anti-inflammatories	Acetaminophen (or paracetamol)	103-90-2	$HOC_6H_4NHCOCH_3$	151.2	0.46÷1.53	9.38	Neutral	✓	✓
	Codeine	76-57-3	$C_{18}H_{21}NO_3$	299.4	1.28	8.21	Positive	✓	
	Diclofenac	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	296.1	2.16÷4.8	pka1: 4.00 pka2: 4.15	Negative	✓	
	Fenoprofen	29679-58-1	$C_{15}H_{14}O_3$	242.3	3.1	3.96	Negative		✓
	Flunixin	38677-85-9	$C_{14}H_{11}F_3N_2O_2$	296.2	4.9	pka1: 1.88 pka2: 5.38	Negative		✓
	Hydrocodone	125-29-1	$C_{18}H_{21}NO_3$	299.4	0.98÷2.45	8.48	Positive	✓	
	Ibuprofen	15687-27-1	$C_{13}H_{18}O_2$	206.3	3.97÷4.55	pka1: 4.5 pka2: 5.2	Negative	✓	✓
	Ketoprofen	22071-15-4	$C_{16}H_{14}O_3$	254.3	3.12÷3.2	3.88	Negative	✓	✓
	Mefenamic acid	61-68-7	$C_{15}H_{15}NO_2$	241.3	5.12	pka1: 3.89 pka2: -1.58	Negative	✓	✓
	Naproxen	22204-53-1	$C_{14}H_{14}O_3$	230.3	3.18	4.19	Negative	✓	✓
	Salicylic acid	69-72-7	HOC_6H_4COOH	138.1	2.26	2.79	Negative	✓	✓
	Tramadol	27203-92-5	$C_{16}H_{25}NO_2$	263.4	2.51	9.41	Positive	✓	
Antianginals	Dehydronifedipine	67035-22-7	$C_{17}H_{16}N_2O_6$	344.3			Neutral	✓	
Antiarrhythmics	Amiodarone	19774-82-4	$C_{25}H_{29}I_2NO_3$	645.3	7.75	6.5	Positive	✓	
	Lidocaine	137-58-6	$C_{14}H_{22}N_2O$	234.3	1.66÷2.44	7.86	Positive	✓	
Anticoagulants	Warfarin	81-81-2	$C_{19}H_{16}O_4$	308.3	2.7	5.87	Negative	✓	
Antihelmintics	Flubendazole	31430-15-6	$C_{16}H_{12}FN_3O_3$	313.3	2.91	pka1: 8.44 pka2: 3.93	Neut./Neg.		✓
Antimicrobials	Amoxicillin	26787-78-0	$C_{16}H_{19}N_3O_5S$	365.4	0.87	pka1: 3.23 pka2: 7.22	Neut./Neg.		✓
	Amprolium	121-25-5	$C_{14}H_{19}ClN_4$	278.8	-2.50	5.33	Positive		✓
	Anhydrochlortetracycline	65490-24-6	$C_{22}H_{21}ClN_2O_7$	460.9				✓	✓
	Anhydrotetracycline	13803-65-1	$C_{22}H_{22}N_2O_7$	426.4	-3.49	pka1: 7.2 pka2: 6.22	Neut./Neg.	✓	✓
	Atrazine	1912-24-9	$C_8H_{14}ClN_5$	215.7	2.61	pka1: 14.48 pka2: 4.2	Neutral		✓
	Azithromycin	83905-01-5	$C_{38}H_{72}N_2O_{12}$	749.0	0.44÷3.16	pka1: 8.7 pka2: 9.5	Positive	✓	✓

Class	Compound	CAS#	Formula[a]	Mole weight [g mol^{-1}][a]	LogK$_{ow}$[a]	pK$_a$[a]	Charge at pH 7[b]	Occurrence data available for:	
								Sewage sludge	Manure
	Bacitracin A	1405-87-4	$C_{66}H_{103}N_{17}O_{16}S$	1422.7	-3.3	pka1: 3.74 pka2: 13.46	Pos./Neut.		✓
	Benzylpenicillin (or Penicillin G)	61-33-6	$C_{16}H_{18}N_2O_4S$	334.4	1.83 ÷ 2.74	3.53	Negative	✓	✓
	Carbadox	6804-07-5	$C_{11}H_{10}N_4O_4$	262.2	0.13	pka1: 10.6 pka2: -1.45	Neutral		✓
	Ceftiofur (or excenel)	80370-57-6	$C_{19}H_{17}N_5O_7S_3$	523.6	0.54 ÷ 1.6	pka1: 2.52 pka2: 3.49	Negative		✓
	Chloramphenicol	56-75-7	$C_{11}H_{12}Cl_2N_2O_5$	323.1	1.14	8.69	Neut./Neg.	✓	✓
	Chlortetracycline	57-62-5	$C_{22}H_{23}ClN_2O_8$	478.9	-0.62	pka1: 3.3 pka2: 7.4 pKa: 9.3	Negative	✓	✓
	Ciprofloxacin	85721-33-1	$C_{17}H_{18}FN_3O_3$	331.3	0.4	pka1: 5.56 pka2: 8.77	Pos./Neut.	✓	✓
	Clarithromycin	81103-11-9	$C_{38}H_{69}NO_{13}$	748.0	3.16	pka1: 12.46 pka2: 9	Positive	✓	✓
	Clindamycin	18323-44-9	$C_{18}H_{33}ClN_2O_5S$	425.0	2.1		Pos./Neut.	✓	
	Cloxacillin	61-72-3	$C_{19}H_{18}ClN_3O_5S$	435.9	2.48	pka1: 2.78 pka2: -0.42	Negative		✓
	Colistin A	7722-44-3	$C_{53}H_{100}N_{16}O_{13}$	1169.5		pka1: 9.07 pka2: 10.24	Positive		✓
	Colistin B	7239-48-7	$C_{52}H_{98}N_{16}O_{13}$	1155.5		pka1: 9.07 pka2: 10.24	Positive		✓
	Cyromazine	66215-27-8	$C_6H_{10}N_6$	166.2	-0.06	pka1: 14.91 pka2: 9.36	Positive		✓
	Danofloxacin	112398-08-0	$C_{19}H_{20}FN_3O_3$	357.4	1.85	pka1: 5.49 pka2: 7.31	Pos./Neut.		✓
	Demeclocycline	127-33-3	$C_{21}H_{21}ClN_2O_8$	464.9	-1.14	pka1: 6.98 pka2: 6.17	Neut./Neg.	✓	✓
	Difloxacin	98106-17-3	$C_{21}H_{19}F_2N_3O_3$	399.4	0.89	pka1: 6.1 pka2: 7.6	Neut./Neg.	✓	✓
	Doxycycline (or vibramycin)	564-25-0	$C_{22}H_{24}N_2O_8$	444.4	-0.02	pka1: 3.5 pka2: 7.7 pKa3: 9.5	Negative	✓	✓
	Enrofloxacin	93106-60-6	$C_{19}H_{22}FN_3O_3$	359.4	1.1	6.27	Neut./Neg.	✓	✓
	Epianhydrotetracycline	4465-65-0	$C_{22}H_{22}N_2O_7$	426.4			Negative	✓	✓
	Epichlortetracycline	101342-45-4	$C_{22}H_{23}ClN_2O_8$	478.9			Negative	✓	✓
	epi-iso-chlortetracycline	14297-93-9	$C_{22}H_{23}ClN_2O_8$	478.9					✓
	epi-oxytetracycline	14206-58-7	$C_{22}H_{24}N_2O_9$	460.4					✓
	epi-tetracycline	23313-80-6	$C_{22}H_{24}N_2O_8$	444.4			Neut./Neg.	✓	✓
	Erythromycin	114-07-8	$C_{37}H_{67}NO_{13}$	733.9	3.06	8.8	Positive	✓	✓
	Erythromycin-H$_2$O	67733-56-6	$C_{37}H_{69}NO_{14}$	715.9					✓

Class	Compound	CAS#	Formula[a]	Mole weight [g mol^{-1}][a]	LogK$_{ow}$[a]	pK$_a$[a]	Charge at pH 7[b]	Occurrence data available for:	
								Sewage sludge	Manure
	Fleroxacin	79660-72-3	$C_{17}H_{18}F_3N_3O_3$	369.3	0.24	pka1: 5.45 pka2: 6.48	Neut./Neg.	✓	✓
	Florfenicol	73231-34-2	$C_{12}H_{14}Cl_2FNO_4S$	358.2	-0.04	8.49	Neut./Neg.		✓
	Flumequine	42835-25-6	$C_{14}H_{12}FNO_3$	261.3	1.6	5.81	Neut./Neg.		✓
	Furazolidone	67-45-8	$C_8H_7N_3O_5$	225.2	-0.04÷0.9				✓
	Gatifloxacin	112811-59-3	$C_{19}H_{22}FN_3O_4$	375.4			Positive	✓	
	Isochlortetracycline	514-53-4	$C_{22}H_{23}ClN_2O_8$	478.9			Negative	✓	✓
	Josamycin	16846-24-5	$C_{42}H_{69}NO_{15}$	828.0	2.39÷3.16		Positive	✓	
	Lasalocid	25999-31-9	$C_{34}H_{54}O_8$	590.8	6.74*	2.64	Negative		✓
	Leucomycin A5	18361-45-0	$C_{39}H_{65}NO_{14}$	771.9		pka1: 12.68 pka2: 8.51	Positive		✓
	Levofloxacin	100986-85-4	$C_{18}H_{20}FN_3O_4$	361.4	-0.39	6.25	Negative	✓	
	Lincomycin	154-21-2	$C_{18}H_{34}N_2O_6S$	406.5	0.29÷0.86	7.8	Pos./Neut.	✓	✓
	Lomefloxacin	98079-51-7	$C_{17}H_{19}F_2N_3O_3$	351.3	-0.3	pka1: 5.45 pka2: 8.78	Neutral	✓	✓
	Marbofloxacin	115550-35-1	$C_{17}H_{19}FN_4O_4$	362.4		pka1: 5.28 pka2: 6.69	Negative		✓
	Methacycline	914-00-1	$C_{22}H_{22}N_2O_8$	442.4	-0.3	pka1: 7.28 pka2: 5.81	Neut./Neg.		✓
	Minocycline	10118-90-8	$C_{23}H_{27}N_3O_7$	457.5	0.05	pka1: 7.35 pka2: 6.26	Neut./Neg.	✓	✓
	Monensin	17090-79-8	$C_{36}H_{62}O_{11}$	670.9	2.75÷5.43	4.24	Negative		✓
	Moxifloxacin	15109609-2	$C_{21}H_{24}FN_3O_4$	401.4	0.95		Positive	✓	
	N^4-Acetyl-sulfamethazine	100-90-3	$C_{14}H_{16}N_4O_3S$	320.4	5				✓
	Narasin	55134-13-9	$C_{43}H_{72}O_{11}$	765.0	4.9÷6.2	4.5	Negative		✓
	Nicarbazin	330-95-0	$C_{19}H_{18}N_6O_6$	426.4		10.77	Neutral		✓
	Norfloxacin	70458-96-7	$C_{16}H_{18}FN_3O_3$	319.3	-1.03÷2.1	pka1: 6.3 pka2: 8.2 -	Pos./Neut.	✓	✓
	Novobiocin	303-81-1	$C_{31}H_{36}N_2O_{11}$	612.6	2.5÷4.1	5.51	Negative		✓
	Ofloxacin	82419-36-1	$C_{18}H_{20}FN_3O_4$	361.4	0.36	pka1: 5.35 pka2: 6.72	Neut./Neg.	✓	✓
	Oleandomycin (or matromycin)	3922-90-5	$C_{35}H_{61}NO_{12}$	687.9	1.69	pka1: 12.67 pka2: 9	Positive		✓
	Ormetoprim	6981-18-6	$C_{14}H_{18}N_4O_2$	274.3	1.23	7.16	Pos./Neut.		✓
	Oxytetracycline	79-57-2	$C_{22}H_{24}N_2O_9$	460.4	-1,22 ÷ -0,9	pka1: 3.27 pka2: 7.3 pKa3: 9.1	Negative	✓	✓

Class	Compound	CAS#	Formula[a]	Mole weight [g mol^{-1}][a]	LogK$_{ow}$[a]	pK$_a$[a]	Charge at pH 7[b]	Occurrence data available for:	
								Sewage sludge	Manure
	Pefloxacin	70458-92-3	$C_{17}H_{20}FN_3O_3$	333.4	0.27 ÷ 2.4	Pka1: 5.55 Pka2: 7.01	Neutral		✓
	Penicillin V	87-08-1	$C_{16}H_{18}N_2O_5S$	350.4		2.79	Negative	✓	
	Pirlimycin	79548-73-5	$C_{17}H_{31}ClN_2O_5S$	411.0	-1.1	pka1: 12.41 pka2: 8.88	Positive		✓
	Pristinamycin (or virginiamycin)	11006-76-1	$C_{71}H_{84}N_{10}O_{17}$	1349.5	1.52	pka1: 7.33 pka2: 2.57	Neut./Neg.		✓
	Roxithromycin (or rulide)	80214-83-1	$C_{41}H_{76}N_2O_{15}$	837.1	1.7 ÷ 2.5	8.8 ÷ 9.2	Positive	✓	✓
	Salinomycin	53003-10-4	$C_{42}H_{70}O_{11}$	751.0	5.15^C÷8.53	4.45 ÷ 6.4	Negative		✓
	Sarafloxacin	98105-99-8	$C_{20}H_{17}F_2N_3O_3$	385.4	1.07	pka1: 5.55 pka2: 8.76	Pos./Neut.	✓	✓
	Sparfloxacin	110871-86-8	$C_{19}H_{22}F_2N_4O_3$	392.4			Positive	✓	
	Spiramycin	8025-81-8	$C_{43}H_{74}N_2O_{14}$	843.1	1.87	pka1: 7.88 pka2: 9.28	Positive	✓	
	Sulfacetamide	144-80-9	$C_8H_{10}N_2O_3S$	214.2	-0.96	5.4	Negative		✓
	Sulfachloropyridazine (or sulfaclozine)	80-32-0	$C_{10}H_9ClN_4O_2S$	284.7	0.31	pka1: 6.6 pka2: 2.02	Neut./Neg.		✓
	Sulfadiazine	68-35-9	$C_{10}H_{10}N_4O_2S$	250.3	-0.09	pka1: 6.36 pka2: 2.10	Neut./Neg.	✓	✓
	Sulfadimethoxine	122-11-2	$C_{12}H_{14}N_4O_4S$	310.3	1.4÷1.63	pka1: 6.91 pka2: 1.90	Negative	✓	✓
	Sulfadoxine	2447-57-6	$C_{12}H_{14}N_4O_4S$	310.3	0.7	pka1: 6.12 pka2: 3.44	Neut./Neg.	✓	✓
	Sulfaguanidine	57-67-0	$C_7H_{10}N_4O_2S$	214.2	-1.22	pka1: 10.53 pka2: 7.72	Pos./Neut.		✓
	Sulfamerazine	127-79-7	$C_{11}H_{12}N_4O_2S$	264.3	0.14	pka1: 6.99 pka2: 2	Neut./Neg.	✓	✓
	Sulfameter	651-06-9	$C_{11}H_{12}N_4O_3S$	280.3	0.41	pka1: 7.06 pka2: 1.98	Negative		✓
	Sulfamethazine (or sulfadimidine)	57-68-1	$C_{12}H_{14}N_4O_2S$	278.3	0.14÷0.89	2.65	Neut./Neg.	✓	✓
	Sulfamethizole (or sulfamethazole)	144-82-1	$C_9H_{10}N_4O_2S_2$	270.3	0.54	pka1: 6.71 pka2: 1.95	Negative		✓
	Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253.3	0.89	pka1: 6.16 pka2: 1.97	Neut./Neg.	✓	✓
	Sulfamethoxypyridazine	80-35-3	$C_{11}H_{12}N_4O_3S$	280.3	0.32	pka1: 6.84 pka2: 2.02	Neut./Neg.		✓
	Sulfamonomethoxine	1220-83-3	$C_{11}H_{12}N_4O_3S$	280.3	0.7	pka1: 7.15 pka2: 3.53	Neut./Neg.		✓
	Sulfanilamide	63-74-1	$C_6H_8N_2O_2S$	172.2	-0.62	10.58	Neut./Neg.	✓	✓
	Sulfapyridine	144-83-2	$C_{11}H_{11}N_3O_2S$	249.3	0.35	pka1: 8.4 pka2: 2.3	Neut./Neg.	✓	✓
	Sulfaquinoxaline (or sulfachinoxalin)	59-40-5	$C_{14}H_{12}N_4O_2S$	300.4	1.68	pka1: 6.7 pka2: 2.13	Neut./Neg.		✓
	Sulfathiazole	72-14-0	$C_9H_9N_3O_2S_2$	255.3	0.05	pka1: 6.93 pka2: 2.04	Negative	✓	✓

Class	Compound	CAS#	Formula[a]	Mole weight [g mol^{-1}][a]	LogK$_{ow}$[a]	pK$_a$[a]	Charge at pH 7[b]	Occurrence data available for:	
								Sewage sludge	Manure
	Sulfisoxazole	127-69-5	$C_{11}H_{13}N_3O_3S$	267.3	1.01	pka1: 5.8 pka2: 2.17	Negative		✓
	Tetracycline	60-54-8	$C_{22}H_{24}N_2O_8$	444.4	-1.19 ÷ -1.3	pka1: 3.3 pka2: 7.7 pKa3: 9.7	Negative	✓	✓
	Thiamphenicol	847-25-6	$C_{12}H_{15}Cl_2NO_5S$	356.2	-0.27	8.75	Neutral		✓
	Tiamulin	55297-95-5	$C_{28}H_{47}NO_4S$	493.7	4.75	pka1: 14.43 pka2: 9.51	Positive		✓
	Tilmicosin	108050-54-0	$C_{46}H_{80}N_2O_{13}$	869.1	3.8	pka1: 12.55 pka2: 10.16	Positive		✓
	Toltrazuril	69004-03-1	$C_{18}H_{14}F_3N_3O_4S$	425.3		7.42	Neut./Neg.		✓
	Trimethoprim	738-70-5	$C_{14}H_{18}N_4O_3$	290.3	0.73÷0.91	7.16	Pos./Neut.	✓	✓
	Tylosin	1401-69-0	$C_{46}H_{77}NO_{17}$	916.1	1.63	7.73	Pos./Neut.	✓	✓
	α-apo-oxytetracycline		$C_{22}H_{22}N_2O_8$	442.4					✓
	β-apo-oxytetracycline		$C_{22}H_{22}N_2O_8$	442.4					✓
Antidiabetics	Glibenclamide	10238-21-8	$C_{23}H_{28}ClN_3O_5S$	494.0		5.3	Negative	✓	
	Metformin	657-24-9	$C_4H_{11}N_5$	129.2	-2.64	12.4	Positive	✓	
Antiemetics	Domperidone	57808-66-9	$C_{22}H_{24}ClN_5O_2$	425.9	3.9	7.9	Positive	✓	
Antifungals	Clotrimazole	23593-75-1	$C_{22}H_{17}ClN_2$	344.8	6.26	4.1	Pos./Neut.	✓	
	Econazole	27220-47-9	$C_{18}H_{15}Cl_3N_2O$	381.7			Positive	✓	
	Fluconazole	86386-73-4	$C_{13}H_{12}F_2N_6O$	306.3	0.25	1.76	Neutral	✓	
	Ketoconazole (or xolegel)	65277-42-1	$C_{26}H_{28}Cl_2N_4O_4$	531.4	4.35	pka1: 3.96 pka2: 6.75	Positive	✓	
	Miconazole	22916-47-8	$C_{18}H_{14}Cl_4N_2O$	416.1	6.25		Positive	✓	
	Terbinafine	91161-71-6	$C_{21}H_{25}N$	291.4		7.1	Positive	✓	
	Thiabendazole	148-79-8	$C_{10}H_7N_3S$	201.3	2.47	4.64	Neutral	✓	
Antihistamines	Diphenhydramine	58-73-1	$C_{17}H_{21}NO$	255.4	3.11	8.98	Positive	✓	
	2-(Diphenylmethoxy)acetic acid (or benzyloxyacetic acid)	21409-25-6	$C_9H_{10}O_3$	166.2			Negative	✓	
Antihypertensives	Amlodipine	88150-42-9	$C_{20}H_{25}ClN_2O_5$	408.9	3	9.4	Positive	✓	
	Desacetyl diltiazem	42399-40-6	$C_{20}H_{24}N_2O_3S$	372.5			Positive	✓	
	Diltiazem	42399-41-7	$C_{22}H_{26}N_2O_4S$	414.5	2.7	8.06	Positive	✓	
	Enalapril	75847-73-3	$C_{20}H_{28}N_2O_5$	376.4	0.07	3.67÷5.28	Negative	✓	

Class	Compound	CAS#	Formula[a]	Mole weight [g mol^{-1}][a]	LogK$_{ow}$[a]	pK$_a$[a]	Charge at pH 7[b]	Occurrence data available for: Sewage sludge	Manure
	Hydrochlorothiazide	58-93-5	$C_7H_8ClN_3O_4S_2$	297.7	-0.07	7.9	Negative	✓	
	Norverapamil	67018-85-3	$C_{26}H_{36}N_2O_4$	440.6	4.59		Positive	✓	
	Verapamil	52-53-9	$C_{27}H_{38}N_2O_4$	454.6	3.7	8.92	Positive	✓	
Antineoplastics	Cyclophosphamide	50-18-0	$C_7H_{15}Cl_2N_2O_2P$	261.1	0.63		Neutral	✓	
	Ifosfamide	3778-73-2	$C_7H_{15}Cl_2N_2O_2P$	261.1	0.86		Neutral	✓	
Antiprotozoals	Quinacrine	83-89-6	$C_{23}H_{30}ClN_3O$	400.0	5.75		Positive	✓	
	Clenbuterol	037148-27-9	$C_{12}H_{18}Cl_2N_2O$	277.2	2		Positive	✓	
	Salbutamol	357636-26-9	$C_{13}H_{21}NO_3$	239.3		pka1: 9.3 pka2: 10.3	Positive	✓	
	Theophyline	58-55-9	$C_7H_8N_4O_2$	180.2	-0.02	8.81	Negative	✓	
Antiseptics	Methyl triclosan	4640-01-1	$C_{13}H_9Cl_3O_2$	303.6	5				✓
	Orthophenylphenol	90-43-7	$C_6H_5C_6H_4OH$	170.2	3.09				✓
	Pipemidic acid	51940-44-4	$C_{14}H_{17}N_5O_3$	303.3	-2.15		Positive	✓	
	Triclosan	3380-34-5	$C_{12}H_7Cl_3O_2$	289.5	4.76	8.1	Neut./Neg.	✓	✓
	Triclocarban	101-20-2	$C_{13}H_9Cl_3N_2O$	315.6	4.9	12.7	Negative	✓	
Beta-blockers	Acebutolol	37517-30-9	$C_{18}H_{28}N_2O_4$	336.4				✓	
	Atenolol	29133-68-7	$C_{14}H_{22}N_2O_3$	266.3	0.16	9.6	Positive	✓	
	Betaxolol	63659-18-7	$C_{18}H_{29}NO_3$	307.4	2.81	9.4	Positive	✓	
	Bisoprolol	66722-44-9	$C_{18}H_{31}NO_4$	325.4	1.87	9.5	Positive	✓	
	Carazolol	57775-29-8	$C_{18}H_{22}N_2O_2$	298.4	3.59		Positive	✓	
	Celiprolol	56980-93-9	$C_{20}H_{33}N_3O_4$	379.5	1.92		Positive	✓	
	Metoprolol	37350-6	$C_{15}H_{25}NO_3$	267.4	1.88	9.7	Positive	✓	✓
	Nadolol	42200-33-9	$C_{17}H_{27}NO_4$	309.4	0.81	9.67	Positive	✓	
	Propranolol	525-66-6	$C_{16}H_{21}NO_2$	259.3		9.42	Positive	✓	
	Sotalol	3930-20-9	$C_{12}H_{20}N_2O_3S$	272.4	0.24	pka1: 8.2 pka2: 9.8	Positive	✓	
Contrast medium	Iopromide	73334-07-3	$C_{18}H_{24}I_3N_3O_8$	791.1	-2.05		Pos./Neut.	✓	
Diuretics	Furosemide	54-31-9	$C_{12}H_{11}ClN_2O_5S$	330.7	2.03	3.9	Negative	✓	
Hormones	11-Ketotestosterone	564-35-2	$C_{19}H_{26}O_3$	302.4	1.296				✓

Class	Compound	CAS#	Formula[a]	Mole weight [g mol^{-1}][a]	LogK$_{ow}$[a]	pK$_a$[a]	Charge at pH 7[b]	Occurrence data available for:	
								Sewage sludge	Manure
	17α-estradiol (E2α or alfatradiol)	50-28-2	$C_{18}H_{24}O_2$	272.4	3.57÷4.14	pka1: 10.33 pka2: -0.88	Neutral		✓
	17α-estradiol-3-sulfate		$C_{18}H_{24}O_5S$	352.4					✓
	17α-ethynylestradiol (EE2 or ethinyl estradiol)	57-63-6	$C_{20}H_{24}O_2$	296.4	3.67÷4.15	pka1: 10.33 pka2: -1.66	Neutral	✓	✓
	17α-hydroxyprogesterone	68-96-2	$C_{21}H_{30}O_3$	330.5	3.04	12.7	Neutral		✓
	17α-trenbolone	80657-17-6	$C_{18}H_{22}O_2$	270.4	2.316				✓
	17β-estradiol (E2β or estradiol)	50-28-2	$C_{18}H_{24}O_2$	272.4	3.1÷4.01	Pka1: 10.33 Pka2: -0.88	Neutral	✓	✓
	17β-estradiol-3, 17-diglucuronide		$C_{30}H_{40}O_{14}$	624.6					✓
	17β-estradiol-3, 17-disulfate	3233-70-3	$C_{18}H_{24}O_8S_2$	432.5					✓
	17β-estradiol-3-glucuronide		$C_{24}H_{32}O_8$	448.5	1.46	pka1: 3.3 pka2: -0.88	Negative		✓
	17β-estradiol-3-glucuronide-17-sulfate		$C_{24}H_{32}O_{11}S$	528.6					✓
	17β-estradiol-3-sulfate	481-96-9	$C_{18}H_{24}O_5S$	352.4	2.9				✓
	17β-estradiol-3-sulfate-17-glucuronide		$C_{18}H_{24}K_2O_5S$	430.6					✓
	17β-estradiol-17-sulfate		$C_{18}H_{24}O_5S$	352.4					✓
	17β-estradiol-17-glucuronide		$C_{24}H_{32}O_8$	448.5					✓
	17β-trenbolone	10161-33-8	$C_{18}H_{22}O_2$	270.4	2.316	-0.89	Neutral		✓
	Androstadienedione	897-06-3	$C_{19}H_{24}O_2$	284.4					✓
	Androstenedione	63-05-8	$C_{19}H_{26}O_2$	286.4	2.717				✓
	Androsterone	53-41-8	$C_{19}H_{30}O_2$	290.4	3.932	-1.36	Neutral		✓
	Dienestrol	84-17-3	$C_{18}H_{18}O_2$	266.3	5.9	9.1	Neutral		✓
	Diethylstilbestrol	56-53-1	$(C_2H_5)_2SO_4$	268.4	5.07	8.63	Negative	✓	✓
	Epiandrosterone (or trans-Androsterone)	481-29-8	$C_{19}H_{30}O_2$	290.4	3.69	-1.36	Neutral		✓
	Estriol (E3)	50-27-1	$C_{18}H_{24}O_3$	288.4	2.45÷2.81	10.33	Neutral	✓	✓
	estriol-3-glucuronide	2479-91-6	$C_{24}H_{32}O_9$	464.5	0.72	3.3	Negative		✓
	estriol-3-sulfate	481-95-8	$C_{18}H_{24}O_6S$	368.4					✓
	Estrone (E1)	53-16-7	$C_{18}H_{22}O_2$	270.4	3.13÷3.62	10.33	Neutral		✓
	estrone-3-glucuronide	2479-90-5	$C_{24}H_{30}O_8$	446.5	1.9	3.3	Negative		✓
	estrone-3-sulfate	481-97-0	$C_{18}H_{22}O_5S$	350.4	0.29	-1.75	Negative		✓

Class	Compound	CAS#	Formula[a]	Mole weight [g mol^{-1}][a]	LogK$_{ow}$[a]	pK$_a$[a]	Charge at pH 7[b]	Occurrence data available for:	
								Sewage sludge	Manure
	Hexestrol	5635-50-7	$C_{18}H_{22}O_2$	270.4	5.60*	9.93	Neutral		✓
	Medroxyprogesterone	520-85-4	$C_{22}H_{32}O_3$	344.5	3.5	12.7	Neutral		✓
	Melengesterol acetate		$C_{25}H_{32}O_4$	396.5	4.2				✓
	Mestranol	72-33-3	$C_{21}H_{26}O_2$	310.4	4.61÷4.67	-1.66	Neutral		✓
	Progesterone	57-83-0	$C_{21}H_{30}O_2$	314.5	3.87	-0.88	Neutral	✓	✓
	Testosterone	58-22-0	$C_{19}H_{28}O_2$	288.4	3.32				✓
	Trendione	4642-95-9	$C_{18}H_{20}O_2$	268.4					✓
	α-zearalanol (or frideron)	26538-44-3	$C_{18}H_{26}O_5$	322.4	3.859	pka1: 8.68 pka2: -1.27	Neut./Neg.		✓
	α-zearalenol (or taleranol)	36455-71-7	$C_{18}H_{24}O_5$	320.4	4.168	pka1: 8.54 pka2: -1.25	Neut./Neg.		✓
	α-zearalenol (or zeranol)	42422-68-4	$C_{18}H_{26}O_5$	322.4	3.859	pka1: 8.68 pka2: -1.27	Neut./Neg.		✓
	β-zearalenol (or zeranol)	71030-11-0	$C_{18}H_{24}O_5$	320.4	4.168	pka1: 8.54 pka2: -1.25	Neut./Neg.		✓
Hypnotics	Zolpidem	82626-48-0	$C_{19}H_{21}N_3O$	307.4	3.85		Positive	✓	
Inhibitors	Allopurinol	315-30-0	$C_5H_4N_4O$	136.1	-0.55	pka1: 9.54 pka2: -0.45	Neutral		✓
Lipid regulators	Atorvastatin	134523-00-5	$C_{33}H_{35}FN_2O_5$	558.6	6.36	4.33	Negative	✓	
	Bezafibrate	41859-67-0	$C_{19}H_{20}ClNO_4$	361.8	4.25	3.6	Negative	✓	
	Clofibric acid	882-09-7	$C_{10}H_{11}ClO_3$	214.6	2.57	3.37	Negative	✓	✓
	Fenofibrate	49562-28-9	$C_{20}H_{21}ClO_4$	360.4	5.19		Neutral	✓	✓
	Gemfibrozil	25812-30-0	$C_{15}H_{22}O_3$	250.3	3.4	4.42	Negative	✓	✓
	Simvastatin	79902-63-9	$C_{25}H_{38}O_5$	418.6	4.68		Neutral	✓	
Non-ionic surfactants	Nonylphenol (NP)	25154-52-3	$C_{15}H_{24}O$	220.4	5.76		Neutral	✓	
	Nonylphenol monoethoxylate (NP1EO)	27986-36-3	$C_{17}H_{28}O_2$	264.4			Neutral	✓	
	Nonylphenol diethoxylate (NP2EO)	20427-84-3	$C_{19}H_{32}O_3$	308.5			Neutral	✓	
	4-tert-octylphenol (OP)	1806-26-4	$C_{14}H_{22}O$	206.3	5.25		Neutral	✓	
	4-tert-octylphenol monoethoxylate (OP1EO)	1322-97-0	$C_{16}H_{26}O_2$	250.4			Neutral	✓	
	4-tert-octylphenol diethoxylate (OP2EO)	2315-61-9	$C_{18}H_{30}O_3$	294.4			Neutral	✓	
Parabens	Methylparaben	99-76-3	$C_8H_8O_3$	152.1	1.96	8.5	Neut./Neg.		✓
	Propylparaben	94-13-3	$C_{10}H_{12}O_3$	180.2	3.04	8.5	Neut./Neg.		✓

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								Sewage sludge	Manure
Plasticizer	Bisphenol A	80-05-7	$(CH_3)_2C(C_6H_4OH)_2$	228.3	3.32	9.78	Neutral		✓
Psychiatric drugs	Alprazolam	28981-97-7	$C_{17}H_{13}ClN_4$	308.8	2.12		Positive	✓	
	Amitriptyline	50-48-6	$C_{20}H_{23}N$	277.4	4.92	9.4	Positive	✓	
	Aripiprazole	129722-12-9	$C_{23}H_{27}Cl_2N_3O_2$	448.4	5.3	7.6	Positive	✓	
	Bupropion	34841-39-9	$C_{13}H_{18}ClNO$	239.7	3.85	8.22	Positive	✓	
	Carbamazepine	298-46-4	$C_{15}H_{12}N_2O$	236.3	2.45	13.9	Neutral	✓	✓
	Citalopram	59729-33-8	$C_{20}H_{21}FN_2O$	324.4	3.74	9.78	Positive	✓	
	Dehydro-aripiprazole	129722-25-4	$C_{23}H_{25}Cl_2N_3O_2$	446.4	3.91		Positive	✓	
	Desmethyl mirtazapine (or normirtazapine)	61337-68-6	$C_{16}H_{17}N_3$	251.3			Positive	✓	
	Desmethylfluvoxamine	192876-02-1	$C_{14}H_{19}F_3N_2O_2$	304.3			Positive	✓	
	Diazepam	439-14-5	$C_{16}H_{13}ClN_2O$	284.7	2.82	3.4	Neutral	✓	
	Doxepin (or Sinequan)	1668-19-5	$C_{19}H_{21}NO$	279.4			Positive	✓	
	Escitalopram	128196-01-0	$C_{20}H_{21}FN_2O$	324.4	3.74	9.5	Positive	✓	
	Fluoxetine	54910-89-3	$C_{17}H_{18}F_3NO$	309.3		9.5	Positive	✓	
	Fluvoxamine	54739-18-3	$C_{15}H_{21}F_3N_2O_2$	318.3			Positive	✓	
	Lamotrigine	84057-84-1	$C_9H_7Cl_2N_5$	256.1			Positive	✓	
	Lorazepam	846-49-1	$C_{15}H_{10}Cl_2N_2O_2$	321.2	2.39÷3.95	pka1: 1.3 pka2: 11.5	Neutral	✓	
	Mirtazapine	61337-67-5	$C_{17}H_{19}N_3$	265.4		7.7	Positive	✓	
	N-desmethylcitalopram	62498-67-3	$C_{19}H_{19}FN_2O$	310.4			Positive	✓	
	Nordazepam	1088-11-5	$C_{15}H_{11}ClN_2O$	270.7	3.89		Neutral	✓	
	Norfluoxetine	126924-38-7	$C_{16}H_{16}F_3NO$	295.3		9.05	Positive	✓	
	Norquetiapine	753475-15-9	$C_{17}H_{17}N_3S$	295.4			Pos./Neg.	✓	
	Norsertraline (or desmethylsertraline)	91797-57-8	$C_{16}H_{15}Cl_2N$	292.2	4.82		Pos./Neg.	✓	
	Nortriptyline	72-69-5	$C_{19}H_{21}N$	263.4	4.51	10.11	Positive	✓	
	O-desmethylvenlafaxine (or desvenlafaxine)	93413-62-8	$C_{16}H_{25}NO_2$	263.4	2.72	pka1: 9.4 pka2: 10.66	Positive	✓	
	Oxazepam	604-75-1	$C_{15}H_{11}ClN_2O_2$	286.7	2.24÷3.37		Negative	✓	
	Paroxetine	61869-08-7	$C_{19}H_{20}FNO_3$	329.4	1.23	9.6	Positive	✓	

Class	Compound	CAS#	Formula[a]	Mole weight [g mol^{-1}][a]	LogK$_{ow}$[a]	pK$_a$[a]	Charge at pH 7[b]	Occurrence data available for:	
								Sewage sludge	Manure
	Pipamperone	1893-33-0	$C_{21}H_{30}FN_3O_2$	375.5	2.02		Positive	✓	
	Quetiapine	111974-69-7	$C_{21}H_{25}N_3O_2S$	383.5	3.17	pka1: 2.78 pka2: 7.46	Positive	✓	
	Sertraline	79617-96-2	$C_{17}H_{17}Cl_2N$	306.2	5.29	9.16	Positive	✓	
	Venlafaxine	93413-69-5	$C_{17}H_{27}NO_2$	277.4	3.28	10.09	Positive	✓	
Receptor antagonists	Cimetidine	51481-61-9	$C_{10}H_{16}N_6S$	252.3	0.4	6.8	Pos./Neut.	✓	
	Famotidine (or pepcidine)	76824-35-6	$C_8H_{15}N_7O_2S_3$	337.5	-0.64		Positive	✓	
	Loratadine	79794-75-5	$C_{22}H_{23}ClN_2O_2$	382.9	5.2		Neutral	✓	
	Omeprazole	73590-58-6	$C_{17}H_{19}N_3O_3S$	345.4	2.23	9.68	Neutral	✓	
	Ranitidine	66357-35-5	$C_{13}H_{22}N_4O_3S$	314.4	0.27	2.4	Positive	✓	
Sinthetic musks	Cashmeran (or DPMI)	33704-61-9	$C_{14}H_{22}O$	206.3	4.49		Negative	✓	
	Celestolide (or ADBI)	13171-00-1	$C_{17}H_{24}O$	244.4	5.7		Negative	✓	
	Galaxolide (or HHCB)	1222-05-5	$C_{18}H_{26}O$	258.4	5.9		Neutral	✓	
	Phantolide (or AHDI)	15323-35-0	$C_{17}H_{24}O$	244.4			Negative	✓	
	Tonalide (AHTN)	1506-02-1	$C_{18}H_{26}O$	258.4	5.7		Negative	✓	
	Traseolide (or ATII)	68140-48-7	$C_{18}H_{26}O$	258.4	8.1		Negative	✓	
Stimulants	Caffeine	58-08-2	$C_8H_{10}N_4O_2$	194.2	-0.07	14	Neutral	✓	
	Cotinine	486-56-6	$C_{10}H_{12}N_2O$	176.2	0.07		Positive	✓	
	Paraxanthine	611-59-6	$C_7H_8N_4O_2$	180.2			Negative	✓	
UV filter	Oxybenzone	131-57-7	$C_{14}H_{12}O_3$	228.2	3.79	7.1	Negative	✓	

Legend:
[a]retrieved from PubChem: https://pubchem.ncbi.nlm.nih.gov/
[b]retrieved from Chemicalize by ChemAxon: https://chemaxon.com/products/chemicaliz

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Appendix 2

Table of contents

Table M1. Descriptive statistical analysis of the occurrence of the selected compounds in raw cattle manure (ng g^{-1} DM). Values below LOD are underlined once, values below LOQ are underlined twice and *values graphically estimated* are written in italics, (min= minimum, max= maximum, STD= standard deviation, n.of data= number of collected data).

Class	Compound	min	max	average	STD	75th percentile	n. of data
				[ng g^{-1} DM]			#
Analgesics and anti-inflammatories	Flunixin	27.0	27.0	27.0	/	27.0	1
	Ibuprofen	5.0	5.0	5.0	/	5.0	1
	Salicylic acid	85	85	85	/	85	1
Anticonvulsants	Carbamazepine	5.0	5.0	5.0	/	5.0	1
Antihelmintics	Flubendazole	0.9	73.0	10.9	16.7	5.0	28
Antimicrobials	Amprolium	100	100	100	/	100	1
	Anhydrochlortetracycline	1.2	15.0	8.3	6.7	14.0	5
	Anhydrotetracycline	0.2	200	60.2	95.6	105	7
	Bacitracin A	5.2	5.2	5.2	/	5.2	1
	Carbadox	1.6	1.6	1.6	/	1.6	1
	Ceftiofur	5.2	5.2	5.2	/	5.2	1
	Chloramphenicol	1.2	9.7	5.4	6.0	7.6	2
	Chlortetracycline	0.8	27590	1215	3562	990.9	68
	Ciprofloxacin	1.1	29590	1451	6448	50.0	21
	Clarithromycin	2.5	2.5	2.5	/	2.5	1
	Danofloxacin	1.0	3060	872	1471	1073	4
	Difloxacin	0.7	2630	486	1060	40.3	11
	Doxycycline	1.0	1050	187	297	250	14
	Enrofloxacin	0.7	46700	2318	10176	33.4	21
	epi-chlortetracycline	23.0	75.0	52.0	22.5	70.8	6
	epi-oxytetracycline	3.4	5.0	4.2	1.1	4.6	2
	epi-tetracycline	1.5	1125	77.2	261	5.0	35
	Erythromycin	0.5	12.0	1.8	3.4	1.1	11
	Erythromycin-H2O	0.5	0.5	0.5	/	0.5	1
	Fleroxacin	0.7	2220	1122	1268	2220	4
	Florfenicol	2.0	2.0	2.0	/	2.0	1
	Furazolidone	2.0	202.7	33.4	70.7	14.6	8
	iso-chlortetracycline	33000	33000	33000	/	33000	1
	Lasalocid	3300	3300	3300	/	3300	1
	Leucomycin A5	0.6	0.6	0.6	/	0.6	1
	Lincomycin	2.2	5.0	4.9	0.5	5.0	29
	Lomefloxacin	0.4	5530	1253	2404	610	5
	Marbofloxacin	0.6	0.9	0.7	0.3	0.8	2
	Methacycline	1.0	960	312	445	420	4
	Monensin	429	6598	2434	2272	2993	6
	N4-Acetyl-sulfamethazine	50.0	50.0	50.0	/	50.0	1
	Norfloxacin	1.0	2760	348	836	53.4	12
	Ofloxacin	0.9	118.6	22.3	30.4	32.2	17
	Oleandomycin	0.4	0.4	0.4	/	0.4	1
	Ormetoprim	1.0	5.0	3.0	2.8	4.0	2
	Oxytetracycline	0.1	225000	5815	29452	166	62
	Pefloxacin	1.0	1.0	1.0	/	1.0	1
	Pirlimycin	109	154	131	22.5	142	3
	Pristinamycin	5.0	5.0	5.0	/	5.0	1
	Roxithromycin	0.5	5.0	1.2	1.3	0.8	10
	Salinomycin	100	100	100	/	100	1
	Sarafloxacin	1.6	50.0	25.8	34.2	37.9	2
	Sulfacetamide	2.6	2.6	2.6	/	2.6	1
	Sulfachloropyridazin	2.4	360	71.4	119	40.4	15
	Sulfadiazine	0.5	25.0	6.0	9.4	5.0	13
	Sulfadimethoxine	0.1	457	24.4	78.4	9.0	42
	Sulfadoxine	0.7	0.7	0.7	/	0.7	1
	Sulfaguanidine	17.0	250	106	126	150	3
	Sulfamerazine	15.5	90.0	65.2	43.0	90.0	3
	Sulfameter	0.5	0.5	0.5	/	0.5	1
	Sulfamethazine	0.5	30250	892	3849	100	69
	Sulfamethoxazole	0.5	36.0	6.8	7.4	5.0	29
	Sulfamethoxypyridazine	25.0	25.0	25.0	0.0	25.0	2
	Sulfamonomethoxine	1.0	26.5	9.9	11.3	11.1	4
	Sulfanilamide	11.5	11.5	11.5	/	11.5	1
	Sulfapyridine	0.4	0.4	0.4	/	0.4	1
	Sulfaquinoxaline	1.4	1.4	1.4	/	1.4	1

Class	Compound	min	max	average	STD	75th percentile	n. of data
		[ng g^{-1} DM]					#
	Sulfathiazole	0.4	5.0	2.7	3.3	3.9	2
	Sulfisoxazole	0.5	0.5	0.5	/	0.5	1
	Tetracycline	0.8	1200	72.0	220	26.5	56
	Tiamulin	0.6	29.0	7.9	14.1	8.3	4
	Tilmicosin	0.6	0.6	0.6	/	0.6	1
	Toltrazuril	2.3	2.3	2.3	/	2.3	1
	Trimethoprim	0.5	20.1	4.0	6.3	4.3	9
	Tylosin	0.5	438	32.3	63.6	36.1	69
	α-apo-oxytetracycline	1.8	1.8	1.8	/	1.8	1
	β-apo-oxytetracycline	0.3	0.3	0.3	/	0.3	1
Hormones	11-Ketotestosterone	0.1	0.1	0.1	/	0.1	1
	17α-estradiol	0.2	1416	141	352	88.2	17
	17α-ethynylestradiol	0.2	9.2	4.6	3.6	5.9	5
	17α-hydroxyprogesterone	0.1	6.6	0.9	2.1	0.2	9
	17α-trenbolone	0.1	55.0	6.7	14.9	3.5	16
	17β-estradiol	0.5	153	26.9	45.3	33.2	18
	17β-estradiol-3-glucuronide	1.0	1.0	1.0	0.0	1.0	2
	17β-estradiol-3-sulfate	0.5	169	42.7	70.7	16.7	5
	17β-trenbolone	0.0	52.0	3.5	12.9	0.3	16
	Androstadienedione	0.1	0.1	0.1	0.0	0.1	6
	Androstenedione	0.1	14.6	3.9	4.3	5.3	11
	Androsterone	0.3	69.0	7.2	21.7	0.3	10
	Estriol (E3)	0.1	1.9	1.4	0.7	1.9	14
	Estrone (E1)	0.1	697	98.1	192	76.0	21
	estrone-3-glucuronide	1.0	1.0	1.0	0.0	1.0	2
	estrone-3-sulfate	0.8	94.6	24.5	46.7	24.7	4
	Medroxyprogesterone	7.5	7.5	7.5	0.0	7.5	2
	Melengestrol acetate	0.2	18.5	4.7	5.1	5.9	15
	Progesterone	0.3	2103	250	634	5.0	13
	Testosterone	0.1	2.3	0.7	1.0	1.5	9
	Trendione	0.02	0.1	0.1	0.0	0.1	8
	α-zearalanol	0.5	126	37.4	45.9	66.1	10
	α-zearalenol	0.6	46.0	7.9	15.4	1.9	10
	β-zearalanol	0.6	0.6	0.6	/	0.6	1
	β-zearalenol	0.5	11.4	3.6	4.9	6.7	10
Plasticizers	Bisphenol A	4.1	10.9	8.3	3.2	10.8	4

Table M2. Descriptive statistical analysis of the occurrence of the selected compounds in in raw liquid/semi-liquid cattle manure (ng L^{-1}). Values below LOD are underlined once, values below LOQ are underlined twice and *values graphically estimated* are written in italics, (min= minimum, max= maximum, STD= standard deviation, n.of data= number of collected data).

Class	**Compound**	**min**	**max**	**average**	**STD**	**75th percentile**	**n. of data**
				[ng L^{-1}]			**#**
Analgesics/ anti-inflammatories	Ibuprofen	50.0	50.0	50.0	/	50.0	1
Anticonvulsants	Carbamazepine	5.0	5.0	5.0	/	5.0	1
Antimicrobials	Anhydrochlortetracycline	7.9	7.9	7.9	0.0	7.9	3
	Anhydrotetracycline	3.5	150	52.3	84.6	76.8	3
	Azithromycin	5.0	5.0	5.0	/	5.0	1
	Bacitracin A	13.0	13.0	13.0	0.0	13.0	2
	Carbadox	6.7	6.7	6.7	0.0	6.7	2
	Ceftiofur	19.7	19.7	19.7	0.0	19.7	2
	Chloramphenicol	1.6	10.0	4.4	4.8	5.8	3
	Chlortetracycline	3.0	5,860,000	281,281	1,247,918	473	22
	Ciprofloxacin	1.2	5.0	1.6	1.3	1.2	9
	Clarithromycin	1.4	1.4	1.4	0.0	1.4	2
	Cloxacillin	18.4	18.4	18.4	0.0	18.4	2
	Danofloxacin	2.3	2.3	2.3	0.0	2.3	2
	Difloxacin	1.8	1.8	1.8	0.0	1.8	2
	Doxycycline	10.0	440	61.0	127	21.7	11
	Enrofloxacin	2.4	5.0	2.7	0.9	2.4	9
	epi-chlortetracycline	4.0	4,110,000	766,691	1,649,564	367,517	6
	epi-iso-chlortetracycline	10.0	267,000	24,367	80,473	100	11
	epi-oxytetracycline	10.0	10.0	10.0	/	10.0	1
	epi-tetracycline	10.0	2,800	346	808	150	14
	Erythromycin	0.5	8.0	2.1	2.6	3.5	12
	Erythromycin-H2O	3.0	8.0	4.7	2.9	5.5	3
	Fleroxacin	1.6	1.6	1.6	0.0	1.6	2
	Florfenicol	4.7	4.7	4.7	0.0	4.7	2
	iso-chlortetracycline	10.0	2,360,000	280,274	687,556	1,200	13
	Leucomycin A5	2.2	2.2	2.2	0.0	2.2	2
	Lincomycin	0.5	84.4	18.9	26.2	28.3	27
	Lomefloxacin	1.4	5.0	2.6	2.1	3.2	3
	Marbofloxacin	1.0	1.0	1.0	0.0	1.0	2
	Methacycline	9.2	25.6	11.3	5.8	9.2	8
	Monensin	1.4	16,240	2,010	4,171	1,080	17
	Narasin	1.1	1.1	1.1	0.0	1.1	2
	Norfloxacin	3.1	26.9	6.0	7.9	3.1	9
	Novobiocin	5.9	5.9	5.9	0.0	5.9	2
	Ofloxacin	3.2	86.1	20.3	31.5	12.5	9
	Oleandomycin	0.7	0.7	0.7	0.0	0.7	2
	Ormetoprim	0.9	5.0	2.3	2.3	3.0	3
	Oxytetracycline	1.9	32,800	1,094	5,788	62.5	32
	Pefloxacin	3.8	3.8	3.8	0.0	3.8	2
	Pristinamycin	5.0	5.0	5.0	/	5.0	1
	Roxithromycin	0.9	5.0	2.3	2.3	3.0	3
	Salinomycin	0.8	0.8	0.8	0.0	0.8	2
	Sarafloxacin	5.0	5.8	5.5	0.5	5.8	3
	Sulfacetamide	4.5	4.5	4.5	0.0	4.5	2
	Sulfachloropyridazine	0.5	8.3	2.5	3.1	3.2	11
	Sulfadiazine	3.4	17,000	1,555	5,123	8.8	11
	Sulfadimethoxine	0.5	7,500	504	1,645	41.3	24
	Sulfadoxine	1.7	630	162	312	167	4
	Sulfamerazine	0.5	0.6	0.5	0.0	0.5	8
	Sulfameter	1.9	1.9	1.9	0.0	1.9	2
	Sulfamethazine	0.7	15,000	1,165	3,237	90.4	32
	Sulfamethizole	0.5	0.5	0.5	0.0	0.5	8
	Sulfamethoxazole	0.5	19,000	1,200	39,41	585	23
	Sulfamonomethoxine	2.2	166	71.4	69.4	137	8
	Sulfapyridine	1.9	1.9	1.9	0.0	1.9	2
	Sulfaquinoxaline	3.0	3.0	3.0	0.0	3.0	2
	Sulfathiazole	0.5	5.0	1.3	1.5	1.5	11
	Sulfisoxazole	2.8	2.8	2.8	0.0	2.8	2
	Tetracycline	2.0	6,830	449	1,330	143	32
	Tiamulin	0.5	0.5	0.5	0.0	0.5	8
	Tilmicosin	1.9	17.0	6.9	8.7	9.5	3

Class	Compound	min	max	average	STD	75th percentile	n. of data
		[ng L^{-1}]					#
	Trimethoprim	1.4	2,300	140	526	5.0	19
	Tylosin	0.5	5.0	1.5	1.4	2.5	14
Hormones	17α-estradiol	100	11,000	892	1,937	770	32
	17α-ethynylestradiol	20.0	20.0	20.0	/	20.0	1
	17α-trenbolone	400	2,900	1,128	707	1,200	9
	17β-estradiol	3.0	650	112	164	118	34
	17β-estradiol-3, 17-disulfate	5.0	320	53.2	105.0	17.0	13
	17β-estradiol-3-glucuronide	100	100	100	/	100	1
	17β-estradiol-3-sulfate	5.0	230	48.0	76.9	67.5	15
	17β-trenbolone	20.0	180	62.2	52.9	50.0	9
	Estriol (E3)	35.0	2,730	732	823	700	11
	Estrone (E1)	10.0	1,200	308	321	495	34
	estrone-3-glucuronide	100	100	100	/	100	1
	estrone-3-sulfate	12.0	180	52.6	48.2	52.0	12
	Medroxyprogesterone	47.5	47.5	47.5	/	47.5	1
	Progesterone	105	105	105	/	105	1
	Trendione	15.0	120	59.4	37.1	80.0	9
Plasticizers	Bisphenol A	229	305	267	53.7	286	2

Table M3. Descriptive statistical analysis of the occurrence of the selected compounds in in raw swine manure (ng g^{-1} DM). Values below LOD are underlined once, values below LOQ are underlined twice and *values graphically estimated* are written in italics, (min= minimum, max= maximum, STD= standard deviation, n.of data= number of collected data).

Class	Compound	min	max	average	STD	75th percentile	n. of data
				[ng g^{-1} DM]			#
Analgesics and anti-inflammatories	Flunixin	0.6	2,300	1,225	1,027	1,925	4
	Salicylic acid	12	440	283	156	403	4
Antimicrobials	Bacitracin A	5.2	320,000	28,134	85,166	2,547	14
	Benzylpenicillin (or Penicillin G)	0.6	1,956	635	979	1,382	6
	Carbadox	1.6	1.6	1.6	0.0	1.6	4
	Ceftiofur	2.3	2820	131	601	5.2	22
	Chloramphenicol	0.5	124,579	11,693	28,761	8,522	20
	Chlortetracycline	1.0	879,600	76670	176,264	78,100	49
	Ciprofloxacin	1.0	33,980	1,692	5,594	735	41
	Clarithromycin	0.1	0.5	0.4	0.2	0.5	5
	Colistin A	20.2	48,600	4,094	13,389	1140	13
	Colistin B	15.0	40,800	4391	12,803	875	10
	Danofloxacin	1.0	2,920	431	1,098	47.8	7
	Difloxacin	0.7	2,510	438	933	277	7
	Doxycycline	2.8	106,000	4,826	13,439	2,757	100
	Enrofloxacin	0.2	3,3260	1,413	5,461	635	38
	epi-chlortetracycline	53.5	44,737	7,042	12,533	6,075	12
	epi-oxytetracycline	16.7	450	141	197	252	6
	epi-tetracycline	1.5	2,179	458	628	666	12
	Erythromycin	1.0	1.0	1.0	/	1.0	1
	Erythromycin-H2O	0.5	2.0	0.6	0.3	0.5	29
	Fleroxacin	0.7	7,460	662	2,064	17.0	13
	Florfenicol	0.6	4.0	2.1	1.0	2.1	7
	Furazolidone	2.0	661	85.5	162	105	16
	Leucomycin A5	0.6	15.2	4.0	5.3	4.9	13
	Lincomycin	0.1	17,000	1,098	3,613	421	22
	Lomefloxacin	0.4	44,160	6,430	16,640	423	7
	Marbofloxacin	0.6	660	228	259	390	8
	Methacycline	1.0	5,430	474	1,562	14.4	12
	N4-Acetyl-sulfamethazine	50.0	2,600	848	1,192	1,093	4
	Norfloxacin	1.0	5,500	446	1462	35.9	14
	Ofloxacin	0.4	4,387	356	963	57.5	37
	Oleandomycin	0.4	0.4	0.4	0.0	0.4	2
	Ormetroprim	1.0	1.0	1.0	0.0	1.0	4
	Oxytetracycline	0.4	354,000	11,180	43,662	6,025	72
	Pefloxacin	1.0	1.0	1.0	0.0	1.0	3
	Roxithromycin	0.5	10.0	1.0	2.0	0.5	23
	Salinomycin	3.2	11.0	7.1	5.5	9.1	2
	Sarafloxacin	1.6	1.6	1.6	0.0	1.6	4
	Sulfacetamide	2.6	2.6	2.6	0.0	2.6	4
	Sulfachloropyridazine (sulfaclozine)	2.4	3,510	164	669	50.4	27
	Sulfadiazine	0.1	7,100	215	829	70.7	93
	Sulfadimethoxine	0.6	1,000	158	373	53.0	7
	Sulfadoxine	0.2	220	28.9	66.2	5.8	12
	Sulfaguanidine	17.0	1,550	532	881	790	3
	Sulfamerazine	15.5	140	98.5	71.9	140	3
	Sulfameter	0.5	0.5	0.5	0.0	0.5	4
	Sulfamethazine (or sulfadimidine)	0.1	20,000	1329	3,375	396	59
	Sulfamethizole (or Sulfamethazole)	6.0	2,000	685	1,139	1,025	3
	Sulfamethoxazole	0.2	840	73.4	182	34.1	30
	Sulfamethoxypyridazine	25.0	25.0	25.0	0.0	25.0	4
	Sulfamonomethoxine	0.5	20,000	906	3512	64.3	34
	Sulfanilamide	11.5	40.0	30.5	16.5	40.0	3
	Sulfapyridine	0.4	0.4	0.4	0.0	0.4	4
	Sulfaquinoxaline (or sulfachinoxalin)	1.4	14.0	4.6	5.4	4.9	9
	Sulfathiazole	0.4	12,400	1,175	3,724	135	11
	Sulfisoxazole	0.5	0.5	0.5	0.0	0.5	4

Class	Compound	min	max	average	STD	75th percentile	n. of data
		[ng g^{-1} DM]					#
	Tetracycline	0.4	98,200	4,123	13,366	2,081	59
	Thiamphenicol	2.0	10.0	6.0	5.7	8.0	2
	Tiamulin	0.4	120	30.9	40.7	43.0	9
	Tilmicosin	15.0	13,022	5,679	5,423	10,504	10
	Toltrazuril	0.5	960	490	456	770	5
	Trimethoprim	0.1	246	17.	42.0	14.1	40
	Tylosin	0.5	32,500	1,241	5,210	13.2	44
Antihelmintics	Flubendazole	230	3,400	1,715	1,528	2,800	4
Hormones	17α-estradiol (E2α or alfatradiol)	4.0	500	120	193	117	6
	17α-ethynylestradiol (EE2 or ethinyl estradiol)	0.2	10.4	4.9	5.5	9.3	4
	17β-estradiol (E2β or estradiol)	0.2	1500	121	350	3.4	19
	17β-estradiol-3-glucuronide	1.0	1.0	1.0	0.0	1.0	3
	17β-estradiol-3-sulfate	1.2	16.2	9.2	4.9	11.0	6
	Estriol (E3)	0.4	315	47.8	118	9.0	7
	Estrone (E1)	0.1	4,800	473	1,121	447	19
	estrone-3-glucuronide	1.0	1.0	1.0	0.0	1.0	3
	estrone-3-sulfate	0.5	3.8	1.6	1.4	1.6	5
	Progesterone	6.0	53.0	17.8	23.5	17.8	4
	Testosterone	1.0	9.0	4.5	3.3	5.3	4
Plasticizers	Bisphenol A	0.1	6.0	1.6	3.0	1.6	4

Table M4. Descriptive statistical analysis of the occurrence of the selected compounds in in raw swine liquid/semi-liquid manure (ng L^{-1}). Values below LOD are underlined once, values below LOQ are underlined twice and *values graphically estimated* are written in italics, (min= minimum, max= maximum, STD= standard deviation, n.of data= number of collected data).

	Compound	min	max	average	STD	75th percentile	n. of data
				[ng L^{-1}]			#
Analgesics and anti-inflammatories	Flunixin	66.0	40,000	25,689	22,241	38,500	3
	Salicylic acid	16,000	66,000	44,333	25,658	58,500	3
Antimicrobials	Amoxicillin	3,600	3,600	3,600	/	3,600	1
	Anhydrochlortetracycline	6,100	6,100	6,100	/	6,100	1
	Bacitracin A	1,910	51,200	19,000	27,904	27,545	3
	Benzylpenicillin (or Penicillin G)	1,2000	12,000	12,000	/	12,000	1
	Carbadox	6.7	6.7	6.7	/	6.7	1
	Cieftiofur	19.7	12,000	6,010	8,471	9,005	2
	Chloramphenicol	0.2	441,900	82,920	150,900	114,225	10
	Chlortetracycline	0.4	108000000	5,779,915	24,754,350	272,600	19
	Ciprofloxacin	0.3	263,100	35,854	92,037	3,665	15
	Cyromazine	2.5	150	76.3	104	113	2
	Clarithromycin	1.4	1.4	1.4	/	1.4	1
	Cloxacillin	18.4	18.4	18.4	/	18.4	1
	Danofloxacin	2.3	2.3	2.3	/	2.3	1
	Demeclocycline	7,100	7,100	7,100	/	7,100	1
	Difloxacin	1.8	1.8	1.8	/	1.8	1
	Doxycycline	25.0	5,900	1,239	2,296	562	6
	Enrofloxacin	1.9	44,500	7,786	12,352	110,00	13
	epi-anhydro-tetracycline	7,200	7,200	7,200	/	7,200	1
	epi-tetracycline	8,000	8,000	8,000	/	8,000	1
	Erythromycin-H2O	3.0	433	180	225	269	3
	Fleroxacin	1.6	1.6	1.6	/	1.6	1
	Florfenicol	117	1350	615	650	864	3
	Leucomycin A5	2.2	8.0	4.1	3.3	5.1	3
	Lincomycin	126	2,0400,000	2,923,094	7,706,599	22,500	7
	Lomefloxacin	1.7	1.7	1.7	/	1.7	1
	Marbofloxacin	1.0	4,200	1,800	1,757	2,325	4
	Minocycline	8,200	8,200	8,200	/	8,200	1
	Monensin	1.4	1.4	1.4	/	1.4	1
	Narasin	1.1	1.1	1.1	/	1.1	1
	Norfloxacin	0.4	389,200	121,653	179,931	347,775	12
	Novobiocin	5.9	5.9	5.9	/	5.9	1
	Ofloxacin	3.2	840	296	471	443	3
	Ormetoprim	0.9	0.9	0.9	/	0.9	1
	Oxytetracycline	0.3	993,800	118,278	251,580	61,925	18
	Pefloxacin	3.8	3.8	3.8	/	3.8	1
	Roxithromycin	0.9	0.9	0.9	/	0.9	1
	Salinomycin	0.8	0.8	0.8	/	0.8	1
	Sarafloxacin	5.8	5.8	5.8	/	5.8	1
	Sulfacetamide	4.5	4.5	4.5	/	4.5	1
	Sulfachloropyridazine (sulfaclozine)	8.3	8.3	8.3	/	8.3	1
	Sulfadiazine	3.4	13,100	4,372	7,559	6,556	3
	Sulfadimethoxine	1.2	1.2	1.2	/	1.2	1
	Sulfadoxine	1.7	6,300	3,151	4,454	4,725	2
	Sulfameter	1.9	1.9	1.9	/	1.9	1
	Sulfamethazine (or sulfadimidine)	0.6	11,000,000	643,048	2,585,780	2,163	18
	Sulfamethoxazole	1.6	63,600	12,726	28,439	12.5	5
	Sulfamonomethoxine	0.1	3,494,100	300,378	1,005,893	32,125	12
	Sulfapyridine	1.9	1.9	1.9	/	1.9	1
	Sulfaquinoxaline (or sulfachinoxalin)	3.0	60.0	25.2	30.5	36.3	3
	Sulfathiazole	1.0	9,700	1,831	3,866	2.2	10
	Sulfisoxazole	2.8	2.8	2.8	/	2.8	1
	Tetracycline	25.0	137,000	20,682	43,989	8,150	10
	Tiamulin	25.0	1,900	650	1,083	963	3
	Tilmicosin	75.0	7,400	2,517	4,229	3,738	3

	Compound	min	max	average	STD	75th percentile	n. of data
				[ng L^{-1}]			#
	Toltrazuril	60.0	9,900	5,287	4,949	7,900	3
	Trimethoprim	12.9	4,600	1,379	2,167	1,679	4
	Tylosin	1.3	300,000	28,402	8,292	5.7	15
Antihelmintics	Flubendazole	770	940	877	92.9	930	3
Hormones	17α-estradiol (E2α or alfatradiol)	19.0	1594.0	479.3	696.6	740.0	5
	17α-ethynylestradiol (EE2 or ethinyl estradiol)	20.0	20.0	20.0	0.0	20.0	2
	17β-estradiol (E2β or estradiol)	0.1	345	128	138	160	8
	17β-estradiol-3-glucuronide	16,000	28,300	22,775	6,411	28,225	4
	17β-estradiol-3-sulfate	364	542	465	89.4	539	4
	Estriol (E3)	40.0	772	242	241	247	8
	Estrone (E1)	31.0	2,195	475	670	490	9
	estrone-3-glucuronide	100	10,800	7,167	6,121	10,700	3
	estrone-3-sulfate	13.0	3,450	1,605	1,848	3,068	4
	Progesterone	30.0	30.0	30.0	0.0	30.0	4
	Testosterone	9.0	100	41.0	40.3	47.5	4
Plasticizers	Bisphenol A	218	446	335	115	427	4

Table M5. Descriptive statistical analysis of the occurrence of the selected compounds in in raw poultry manure (ng g^{-1} DM). Values below LOD are underlined once, values below LOQ are underlined twice and ***values graphically estimated*** are written in italics, (min= minimum, max= maximum, STD= standard deviation, n.of data= number of collected data).

Class	Compound	min	max	average	STD	75th percent ile	n. of data
				[ng g^{-1} DM]			#
Analgesics and anti-inflammatories	Acetaminophen (or Paracetamol)	1.4	225	25.3	57.9	4.4	23
	Fenoprofen	1.7	1.7	1.7	0.0	1.7	2
	Ibuprofen	0.9	1.4	1.0	0.2	0.9	23
	Ketoprofen	1.1	1.1	1.1	0.0	1.1	2
	Mefenamic Acid	1.9	1.9	1.9	0.0	1.9	2
	Naproxen	1.5	1.5	1.5	0.0	1.5	2
	Salicylic acid	1.5	2501	469	762	577	23
Anticonvulsants	Carbamazepine	1.4	109	6.1	22.4	1.4	23
Antimicrobials	Amprolium	5.6	8,492	2,714	3,370	4,163	8
	Bacitracin A	5.2	5.2	5.2	/	5.2	1
	Carbadox	1.6	1.6	1.6	/	1.6	1
	Chloramphenicol	5700	13,400	9,550	5,445	11,475	2
	Chlortetracycline	1.0	94,710	3,509	16,396	293	34
	Ciprofloxacin	19.1	45,590	5,792	14,065	2,389	10
	Clarithromycin	0.5	0.5	0.5	/	0.5	1
	Danofloxacin	1.0	2,480	644	1,224	680	4
	Difloxacin	0.7	12,380	3,117	6,176	3,128	4
	Doxycycline	1.0	78,516	10,935	22,260	9,775	47
	Enrofloxacin	0.8	1,420,760	35,774	213,817	940	44
	Erythromycin	0.5	10,580	374.5	1,963	12.4	29
	Fleroxacin	0.7	99,430	25,059	49,582	25,428	4
	Flumequine	21.0	51,912	11,834	19,582	22,652	20
	Furazolidone	2.0	15,600	1,752	4,875	506	10
	Lasalocid	100	10,600	4,570	5,421	6,805	3
	Leucomycin A5	0.6	0.6	0.6	/	0.6	1
	Lomefloxacin	0.4	7,030	1,941	3,405	2,253	4
	Marbofloxacin	0.6	0.6	0.6	/	0.6	1
	Methacycline	1.0	5,860	1,512	2,900	1,570	4
	Monensin	0.5	4,057	556	1,260	253	10
	Narasin	0.5	6,490	2,672	2,439	3,730	9
	Nicarbazin	1.0	10,950	3,952	4,542	6,688	6
	Norfloxacin	10.2	225,450	8,058	40,364	1,144	31
	Ofloxacin	1.0	4,990	475	1,432	31.8	12
	Oleandomycin	0.4	0.4	0.4	/	0.4	1
	Ormetoprim	1.0	1.0	1.0	/	1.0	1
	Oxytetracycline	0.8	416,750	13,796	72,375	1,600	33
	Pristinamycin (or Virginiamycin)	0.6	0.6	0.6	0.0	0.6	6
	Roxithromycin	0.5	89.0	9.1	24.3	7.0	13
	Salinomycin	0.2	22,000	7,844	7,449	7,900	11
	Sarafloxacin	1.6	1.6	1.6	/	1.6	1
	Sulfacetamide	2.6	2.6	2.6	/	2.6	1
	Sulfachloropyridazine (sulfaclozine)	2.4	35530	1646	6439	125	32
	Sulfadiazine	0.8	91,000	4,066	14,481	1,176	54
	Sulfadimethoxine	0.5	8.9	4.7	3.4	6.3	6
	Sulfadoxine	0.7	2.7	1.7	1.4	2.2	2
	Sulfaguanidine	10.0	570	199	321	294	3
	Sulfamerazine	15.5	660	259	350	380	3
	Sulfameter	0.5	9.8	5.9	4.0	8.7	6
	Sulfamethazine (or sulfadimidine)	0.8	6,040	284	1,255	25.0	23
	Sulfamethoxazole	0.5	5,200	892	1,638	665	24
	Sulfamethoxypyridazine	25.0	25.0	25.0	0.0	25.0	11
	Sulfamonomethoxine	0.5	900	65.2	224	7.9	16
	Sulfanilamide	11.5	1,592	541	910	806	3
	Sulfapyridine	0.4	0.4	0.4	/	0.4	1
	Sulfaquinoxaline (or sulfachinoxalin)	1.5	406	97.4	173	44.3	5
	Sulfathiazole	0.4	6,000	2,501	2,999	4,500	4
	Sulfisoxazole	0.5	0.5	0.5	/	0.5	1

Class	Compound	min	max	average	STD	75th percentile	n. of data
		[ng g^{-1} DM]					#
	Tetracycline	0.8	8675	414	1763	25.0	24
	Tilmicosin	7.0	240	35.4	55.3	34.0	21
	Trimethoprim	0.5	17,000	1,545	4,243	497	31
	Tylosin	0.5	57,570	3,415	10,979	1,128	29
Antiseptics	Methyl triclosan	2.2	2.2	2.2	0.0	2.2	2
	Orthophenylphenol	2.0	250	75.0	74.7	135	23
	Triclosan	1.8	1.8	1.8	0.0	1.8	2
Beta-blockers	Metoprolol	1.7	1.7	1.7	0.0	1.7	2
Hormones	17α-estradiol (E2α or alfatradiol)	2.7	2.7	2.7	/	2.7	1
	17α-estradiol-3-sulfate	3.1	3.1	3.1	/	3.1	1
	17α-ethynylestradiol (EE2 or ethinyl estradiol)	0.2	7.3	3.2	3.1	7.3	9
	17β-estradiol (E2β or estradiol)	0.2	904	88.7	203	75.5	32
	17β-estradiol-17-sulfate	74.3	74.3	74.3	/	74.3	1
	17β-estradiol-3-glucuronide	1.0	1.0	1.0	0.0	1.0	2
	17β-estradiol-3-sulfate	3.4	48.2	30.8	21.5	46.4	5
	Androstenedione	1.6	96.0	37.7	41.3	69.0	6
	Dienestrol	1.3	2.5	1.9	0.8	2.2	2
	Diethylstilbestrol	1.1	1.8	1.5	0.5	1.6	2
	Epiandrosterone (or trans-Androsterone)	1.7	51.0	24.9	22.8	42.8	6
	Estriol (E3)	0.4	20.0	5.8	6.6	10.1	11
	Estrone (E1)	0.1	321	78.3	90.9	61.5	23
	estrone-3-glucuronide	1.0	1.0	1.0	0.0	1.0	2
	estrone-3-sulfate	2.5	40.0	18.6	13.4	28.0	18
	Hexestrol	1.7	2.3	2.0	0.4	2.2	2
	Mestranol	1.0	1.0	1.0	0.0	1.0	2
	Progesterone	1.3	1,310	173	230	292	40
	Testosterone	0.03	670	52.7	138	21.6	27
	α-zearalanol	1.5	1.8	1.7	0.2	1.7	2
Inhibitors	Allupurinol	1.5	1.5	1.5	0.0	1.5	2
Lipid regulators	Clofibric acid	0.9	0.9	0.9	0.0	0.9	2
	Fenofibrate	1.4	1.4	1.4	0.0	1.4	2
	Gemfibrozil	1.0	1.0	1.0	0.0	1.0	2
Parabens	Methylparaben	1.2	20.0	4.7	4.8	6.7	23
	Propylparaben	1.1	1.1	1.1	0.0	1.1	2
Plasticizers	Bisphenol A	0.1	207	30.9	50.0	38.5	26

Table M6. Descriptive statistical analysis of the occurrence of the selected compounds in in treated cattle manure (ng g^{-1} DM). Values below LOD are underlined once, values below LOQ are underlined twice and *values graphically estimated* are written in italics, (min= minimum, max= maximum, STD= standard deviation, n.of data= number of collected data).

Class	Compound	min	max	average	STD	75th percentile	n. of data
		[ng g^{-1} DM]					#
Analgesics and anti-inflammatories	Ibuprofen	5.0	5.0	5.0	/	5.0	1
Anticonvulsants	Carbamazepine	5.0	5.0	5.0	/	5.0	1
Antimicrobials	Anhydrochlortetracycline	25.0	25.0	25.0	/	25.0	1
	Anhydrotetracycline	20.0	80.0	50.0	42.4	65.0	2
	Chloramphenicol	5.0	5.0	5.0	/	5.0	1
	Chlortetracycline	10.2	353	179	114	238	10
	Ciprofloxacin	50.0	50.0	50.0	/	50.0	1
	Enrofloxacin	50.0	50.0	50.0	/	50.0	1
	epi-chlortetracycline	40.1	105	81.7	36.1	103	3
	epi-oxytetracycline	5.0	5.0	5.0	/	5.0	1
	epi-tetracycline	5.0	210	106	83.8	135	4
	iso-chlortetracycline	300	4,000	2,150	2,616	3,075	2
	Lomefloxacin	50.0	50.0	50.0	/	50.0	1
	Norfloxacin	50.0	50.0	50.0	/	50.0	1
	Ofloxacin	50.0	50.0	50.0	/	50.0	1
	Ormetoprim	5.0	5.0	5.0	/	5.0	1
	Oxytetracycline	20.0	250	128	113	240	5
	Pirlimycin	0.1	0.3	0.2	0.1	0.3	4
	Pristinamycin	5.0	5.0	5.0	/	5.0	1
	Roxithromycin	5.0	5.0	5.0	/	5.0	1
	Sarafloxacin	50.0	50.0	50.0	/	50.0	1
	Sulfamethazine	21.8	43.0	32.7	10.2	40.8	5
	Tetracycline	26.7	400	134	148	183	6
	Tylosin	0.9	50.0	21.5	20.2	34.4	7
Hormones	11-Ketotestosterone	0.1	0.1	0.1	/	0.1	1
	17α-estradiol	0.2	100	33.5	57.6	50.1	3
	17α-hydroxyprogesterone	0.1	1.0	0.5	0.5	1.0	5
	17α-trenbolone	0.2	0.2	0.2	/	0.2	1
	17β-estradiol	0.3	50.0	8.8	20.2	0.9	6
	17β-trenbolone	9.8	9.8	9.8	/	9.8	1
	Androstenedione	1.8	12.0	6.5	4.1	9.5	6
	Androsterone	0.3	13.0	4.3	4.6	6.0	7
	Estriol (E3)	1.9	2.0	1.9	0.1	1.9	3
	Estrone (E1)	5.0	850	113	298	8.8	8
	Melengestrol acetate	0.3	0.3	0.3	/	0.3	1
	Progesterone	0.7	8.0	4.1	3.1	7.1	8
	Testosterone	0.3	5.8	2.4	3.0	3.4	3
	α-zearalanol	0.5	6.0	1.6	2.1	1.1	6
	α-zearalenol	0.1	10.0	3.8	3.8	6.5	8
	β-zearalanol	0.6	0.6	0.6	/	0.6	1
	β-zearalenol	0.5	4.5	1.3	1.6	0.9	6

Table M7. Descriptive statistical analysis of the occurrence of the selected compounds in in treated cattle liquid/semi-liquid manure (ng L^{-1}). Values below LOD are underlined once, values below LOQ are underlined twice and *values graphically estimated* are written in italics, (min= minimum, max= maximum, STD= standard deviation, n.of data= number of collected data).

Class	Compound	min	max	average	STD	75th percentile	n. of data
				[ng L^{-1}]			#
Analgesics and anti-inflammatories	Ibuprofen	50.0	50.0	50.0	/	50.0	1
Anticonvulsants	Carbamazepine	5.0	5.0	5.0	/	5.0	1
Antimicrobials	Azithromycin	5.0	5.0	5.0	/	5.0	1
	Chloramphenicol	10.0	10.0	10.0	/	10.0	1
	Chlortetracycline	10.0	1,400,000	111,025	387,444	419	13
	Ciprofloxacin	5.0	5.0	5.0	/	5.0	1
	Doxycycline	10.0	10.0	10.0	/	10.0	1
	Enrofloxacin	5.0	5.0	5.0	/	5.0	1
	epi-chlortetracycline	10.0	2,500,000	1,143,337	1,263,575	1,715,000	3
	epi-iso-chlortetracycline	10.0	1000	226.6	388.9	232.3	8
	epi-oxytetracycline	10.0	1300000	672,503	544,384	955,000	4
	epi-tetracycline	10.0	380	59.4	130	16.3	8
	Erythromycin	8.0	1,266	400	494	710	11
	Erythromycin-H2O	8.0	8.0	8.0	/	8.0	1
	iso-chlortetracycline	10.0	4,600,000	726,266	1,597,254	1,373	10
	Lincomycin	5.0	54.0	24.2	21.2	50.0	18
	Lomefloxacin	5.0	5.0	5.0	/	5.0	1
	Monensin	50.0	307,800	71,176	85,970	117,152	17
	Norfloxacin	5.0	5.0	5.0	/	5.0	1
	Ofloxacin	5.0	5.0	5.0	/	5.0	1
	Ormetoprim	5.0	5.0	5.0	/	5.0	1
	Oxytetracycline	10.0	6,800,000	540,164	1,722,979	220	20
	Pristinamycin	5.0	50.0	27.7	21.4	50.0	11
	Roxithromycin	5.0	5.0	5.0	/	5.0	1
	Sarafloxacin	5.0	5.0	5.0	/	5.0	1
	Sulfachloropyridazine	5.0	431	62.4	124	50.0	11
	Sulfadiazine	50.0	50.0	50.0	/	50.0	1
	Sulfadimethoxine	27.0	11,000	1,517	3,245	580	18
	Sulfamerazine	10.0	50.0	30.0	21.1	50.0	10
	Sulfamethazine	5.0	14,000	1,902	3,916	543	18
	Sulfamethizole	10.0	452	70.2	136	50.0	10
	Sulfamethoxazole	5.0	4,900	363	1,153	50.0	18
	Sulfathiazole	10.0	130	36.4	36.4	50.0	11
	Tetracycline	10.0	1,843	168	438	83.8	18
	Tiamulin	12.0	455	96.7	137	65.8	10
	Trimethoprim	5.0	24.0	7.4	6.7	5.0	8
	Tylosin	5.0	50.0	30.5	20.8	50.0	11
	α-apo-oxytetracycline	140000.0	300,000	220,000	113,137	260,000	2
Hormones	17α-estradiol	0.1	17600	334	438	500	37
	17α-ethynylestradiol	10.0	10.0	10.0	0.0	10.0	7
	17α-trenbolone	22.0	1,531	813	505	1,215	9
	17α-estradiol-3-sulfate	170	170	170	/	170	1
	17β-estradiol	0.1	1,326	97.2	219	94.0	40
	17β-estradiol-3, 17-disulfate	5.0	5.0	5.0	0.0	5.0	5
	17β-estradiol-3, 17-diglucuronide	1.0	1.0	1.0	/	1.0	1
	17β-estradiol-3-glucuronide	1.0	1.0	1.0	/	1.0	1
	17β-estradiol-3-glucuronide-17-sulfate	1.0	1.0	1.0	/	1.0	1
	17β-estradiol-3-sulfate	5.0	42.0	15.2	16.3	23.0	6
	17β-estradiol-3-sulfate-17-glucuronide	1.0	1.0	1.0	/	1.0	1
	17β-estradiol-17-sulfate	1.0	1.0	1.0	/	1.0	1
	17β-estradiol-17-glucuronide	1.0	1.0	1.0	/	1.0	1
	17β-trenbolone	5.0	98.0	52.3	32.1	71.0	9
	Androstenedione	0.1	70.0	35.1	49.4	52.5	2
	Estriol (E3)	0.1	725	163	251	240	26

Class	**Compound**	**min**	**max**	**average**	**STD**	**75th percentile**	**n. of data**
		[ng L^{-1}]					**#**
	estriol-3-glucuronide	1.0	1.0	1.0	/	1.0	1
	estriol-3-sulfate	1.0	1.0	1.0	/	1.0	1
	Estrone (E1)	0.1	3,123	488	811	461	42
	estrone-3-glucuronide	1.0	1.0	1.0	/	1.0	1
	estrone-3-sulfate	5.0	87.0	27.2	35.7	43.3	6
	Medroxyprogesterone	0.1	47.5	21.7	24.3	47.5	7
	Progesterone	0.2	105	81.0	45.7	105	5
	Testosterone	0.1	100	33.5	57.6	50.3	3
	Trendione	7.0	113	55.7	42.2	93.0	9

Table M8. Descriptive statistical analysis of the occurrence of the selected compounds in in treated swine manure (ng g^{-1} DM). Values below LOD are underlined once, values below LOQ are underlined twice and *values graphically estimated* are written in italics, (min= minimum, max= maximum, STD= standard deviation, n.of data= number of collected data).

Class	Compound	min	max	average	STD	75th percentile	n. of data
		[ng g^{-1} DM]					#
Antimicrobials	Anhydrotetracycline	505	505	505	/	505	1
	Bacitracin A	8.6	3,400	935	1,584	1,554	7
	Carbadox	0.6	0.6	0.6	0.0	0.6	4
	Ceftiofur	2.3	4.6	4.2	1.0	4.6	5
	Chloramphenicol	2.3	2.3	2.3	0.0	2.3	4
	Chlortetracycline	10.0	87,900	8,986	21,417	2,530	21
	Ciprofloxacin	1.4	861	329	380	607	6
	Clarithromycin	0.9	0.9	0.9	0.0	0.9	4
	Colistin A	20.2	20.2	20.2	/	20.2	1
	Danofloxacin	0.5	0.5	0.5	0.0	0.5	4
	Difloxacin	0.6	0.6	0.6	0.0	0.6	4
	Doxycycline	4.4	40,800	2,021	8,886	116	21
	Enrofloxacin	4.4	4,040	588	1,522	20.3	7
	epi-anhydro-tetracycline	342.0	342	342	/	342	1
	epi-chlortetracycline	20,500	25,200	22,850	3,323	24,025	2
	epi-tetracycline	2,830	3,070	2,950	170	3,010	2
	Erythromycin	50.0	1,592	276	437	279	12
	Erythromycin-H2O	0.8	37.5	7.0	13.6	3.8	7
	Fleroxacin	0.7	0.7	0.7	0.0	0.7	5
	Florfenicol	2.0	2.0	2.0	0.0	2.0	7
	iso-chlortetracycline	23,300	33,100	28,200	6,930	30,650	2
	Leucomycin A5	0.9	10.1	2.3	3.4	1.2	7
	Lincomycin	2.7	1,650	217	398	302	18
	Lomefloxacin	0.6	112	22.8	49.8	0.6	5
	Marbofloxacin	0.9	0.9	0.9	0.0	0.9	4
	Methacycline	1.4	3.4	2.4	1.2	3.4	4
	Monensin	705	895	787	74.5	837	6
	Norfloxacin	0.8	1080	155	408	1.1	7
	Novobiocin	0.9	5.8	2.1	2.5	2.1	4
	Ofloxacin	20.1	6,600	1,008	2,467	171	7
	Oleandomycin	0.7	0.7	0.7	/	0.7	1
	Ormetoprim	0.5	0.5	0.5	0.0	0.5	5
	Oxytetracycline	3.4	19,000	1,139	4,121	178	21
	Pefloxacin	0.5	530	80.0	199	14.1	7
	Pristinamycin (or Virginiamycin)	10.0	877	225	343	208	12
	Roxithromycin	0.6	0.6	0.6	0.0	0.6	4
	Sarafloxacin	0.7	0.7	0.7	0.0	0.7	4
	Sulfacetamide	2.5	2.5	2.5	0.0	2.5	4
	Sulfachloropyridazine (sulfaclozine)	2.5	443	124	152	283	17
	Sulfadiazine	0.6	15.6	4.7	5.4	9.2	21
	Sulfadimethoxine	0.6	342	84.1	108	131	16
	Sulfadoxine	0.6	0.6	0.6	0.0	0.6	4
	Sulfamerazine	10.0	548	195	206	401	12
	Sulfameter	0.5	0.5	0.5	0.0	0.5	4
	Sulfamethazine (or sulfadimidine)	0.5	1,060	198	279	343	19
	Sulfamethizole (or Sulfamethazole)	10.0	490	194	183	350	12
	Sulfamethoxazole	0.5	342	70.9	103	77.0	19
	Sulfamonomethoxine	1.2	101	28.3	40.1	39.6	6
	Sulfapyridine	0.6	0.6	0.6	0.0	0.6	4
	Sulfaquinoxaline (or sulfachinoxalin)	2.1	2.1	2.1	0.0	2.1	4
	Sulfathiazole	0.8	540	146	194	245	16
	Sulfisoxazole	0.6	0.8	0.6	0.1	0.6	4
	Tetracycline	6.8	5,050	888	1,493	484	21
	Tiamulin	10.0	3,636	665	1,045	794	12
	Trimethoprim	0.9	4.4	1.4	1.3	0.9	7
	Tylosin	0.6	4,913	400.3	1,094	309	20
Hormones	17α-estradiol (E2α or alfatradiol)	250	300	275	35.4	288	2
	17β-estradiol (E2β or estradiol)	0.5	500	100	192	76	8
	Estrone (E1)	1.8	1,100	257	462	247	8

Table M9. Descriptive statistical analysis of the occurrence of the selected compounds in in treated swine liquid/semi-liquid (ng L^{-1}). Values below LOD are underlined once, values below LOQ are underlined twice and *values graphically estimated* are written in italics, (min= minimum, max= maximum, STD= standard deviation, n.of data= number of collected data).

Class	**Compound**	**min**	**max**	**average**	**STD**	**75th percentile**	**n. of data**
				[ng L^{-1}]			**#**
Antimicrobials	Bacitracin A	13.0	13.0	13.0	0.0	13.0	7
	Carbadox	6.7	6.7	6.7	0.0	6.7	3
	Ceftiofur	19.7	19.7	19.7	0.0	19.7	3
	Chloramphenicol	1.6	1.6	1.6	0.0	1.6	3
	Chlortetracycline	230	1,000,000	118,834	246,808	95,000	29
	Ciprofloxacin	1.2	207	56.5	94.6	91.6	7
	Clarithromycin	1.4	1.4	1.4	0.0	1.4	3
	Cloxacillin	18.4	18.4	18.4	0.0	18.4	3
	Danofloxacin	2.3	2.3	2.3	0.0	2.3	3
	Difloxacin	1.8	1.8	1.8	0.0	1.8	3
	Doxycycline	18.5	11,600	3,210	4,526	6,240	8
	Enrofloxacin	2.4	56.5	16.8	24.6	21.2	8
	epi-anhydro-tetracycline	11,000.0	11,000	11,000	/	11,000	1
	epi-chlortetracycline	25,600	26,100	25,850	354	25,975	2
	epi-tetracycline	4,100	4,610	4,355	361	4,483	2
	Erythromycin	0.5	0.5	0.5	0.0	0.5	6
	Erythromycin-H2O	3.0	2,770	1,461	1,251	2,500	15
	Fleroxacin	1.6	6.3	2.8	1.8	2.7	6
	Florfenicol	4.7	323	119	138	196	8
	iso-chlortetracycline	285,000	304,000	294,500	13,435	299,250	2
	Leucomycin A5	2.2	156	48.4	69.8	87.1	8
	Lincomycin	0.5	240,000	53,997	79,486	74,000	27
	Lomefloxacin	1.4	2.4	1.6	0.4	1.4	6
	Marbofloxacin	1.0	4.4	1.8	1.7	1.8	4
	Methacycline	9.2	9.2	9.2	0.0	9.2	5
	Monensin	0.5	17.5	3.8	5.7	2.7	9
	Narasin	1.1	1.1	1.1	0.0	1.1	3
	Norfloxacin	3.1	110	32.3	40.3	44.9	8
	Novobiocin	5.9	5.9	5.9	0.0	5.9	3
	Ofloxacin	50.4	350	139	93.2	155	8
	Ormetoprim	0.9	1.6	1.2	0.3	1.6	6
	Oxytetracycline	7.7	25,360	2,951	6,395	1,410	17
	Pefloxacin	3.8	3.8	3.8	0.0	3.8	7
	Pristinamycin (or Virginiamycin)	0.5	0.5	0.5	0.0	0.5	6
	Roxithromycin	0.9	0.9	0.9	0.0	0.9	3
	Salinomycin	0.8	4.4	1.5	1.6	0.8	5
	Sarafloxacin	5.8	5.8	5.8	0.0	5.8	3
	Sulfacetamide	4.5	4.5	4.5	0.0	4.5	3
	Sulfachloropyridazine (sulfaclozine)	0.5	8.3	4.1	4.1	8.3	11
	Sulfadiazine	3.4	1,260	254	413	187	8
	Sulfadimethoxine	0.5	14,050	1,694	3,223	2,500	19
	Sulfadoxine	1.7	1.7	1.7	0.0	1.7	3
	Sulfamerazine	0.5	0.9	0.6	0.2	0.5	6
	Sulfameter	1.9	1.9	1.9	0.0	1.9	3
	Sulfamethazine (or sulfadimidine)	0.4	400,000	47,502	112,456	8,443	24
	Sulfamethizole (or Sulfamethazole)	0.5	9.0	3.3	4.3	9.0	9
	Sulfamethoxazole	0.5	1,010	117	335	21.0	9
	Sulfamonomethoxine	2.2	46,700	9,495	15,380	8,600	8
	Sulfapyridine	1.9	1.9	1.9	0.0	1.9	3
	Sulfaquinoxaline (or sulfachinoxalin)	3.0	3.0	3.0	0.0	3.0	3
	Sulfathiazole	0.5	9,830	823	2,837	5.7	12
	Sulfisoxazole	2.8	2.8	2.8	0.0	2.8	3
	Tetracycline	27.3	540,000	46,595	116,857	9,405	26
	Tiamulin	1.2	5,080	811	1,749	26.5	9
	Trimethoprim	1.4	2,500	1,248	1,221	2,500	15
	Tylosin	1.3	4,924,868	389,446	1,208,634	13,300	17

Class	Compound	min	max	average	STD	75th percentile	n. of data
				[ng L^{-1}]			#
Hormones	17α-estradiol (E2α or alfatradiol)	0.1	5,189	926	1,686	1,220	9
	17α-ethynylestradiol (EE2 or ethinyl estradiol)	10.0	155	82.5	103	119	2
	17α-estradiol-3-sulfate	1.0	1.0	1.0	/	1.0	1
	17β-estradiol (E2β or estradiol)	0.1	3,000	665	998	827	22
	17β-estradiol-3, 17-disulfate	1.0	1.0	1.0	/	1.0	1
	17β-estradiol-3, 17-diglucuronide	1.0	1.0	1.0	/	1.0	1
	17β-estradiol-3-glucuronide	1.0	1.0	1.0	/	1.0	1
	17β-estradiol-3-glucuronide-17-sulfate	1.0	1.0	1.0	/	1.0	1
	17β-estradiol-3-sulfate	1.0	1.0	1.0	/	1.0	1
	17β-estradiol-3-sulfate-17-glucuronide	1.0	1.0	1.0	/	1.0	1
	17β-estradiol-17-sulfate	79.8	79.8	79.8	/	79.8	1
	17β-estradiol-17-glucuronide	1.0	1.0	1.0	/	1.0	1
	Estriol (E3)	2.1	45,379	5,749	10,396	7,935	19
	estriol-3-glucuronide	1.0	1.0	1.0	/	1.0	1
	estriol-3-sulfate	1.0	1.0	1.0	/	1.0	1
	Estrone (E1)	0.2	74,700	9,192	16,767	9,853	22
	estrone-3-glucuronide	1.0	1.0	1.0	/	1.0	1
	estrone-3-sulfate	1.8	1.8	1.8	/	1.8	1

Table M10. Descriptive statistical analysis of the occurrence of microorganisms in raw manure from different animals (cfu g^{-1} DM). (ND = Not Detected, min= minimum, max= maximum, STD= standard deviation, n.of data= number of collected data).

Microorganism	Animal	min	max	average	STD	75th percentile	n. of data
Aeromonas hydrophila	Poultry	26	3.4E+04	1.2E+04	1.3E+04	2.4E+04	12
Campylobacter coli	Swine	2	1.5E+04	900	2,917	800	26
	Poultry	4200	2.9E+04	1.7E+04	1.8E+04	2.3E+04	2
	Cattle	7600	1.5E+05	7.9E+04	1.0E+05	1.1E+05	2
	Sheep	860	2,100	1,480	877	1,790	2
Cryptosporidium parvum	Swine	300	3,600	1,950	2,333	2,775	2
	Cattle	270	3,500	1,885	2,284	2,693	2
	Sheep	53	250	152	139	201	2
E. coli	Swine	100	7.5E+05	4.7E+04	1.2E+05	5.7E+04	38
	Poultry	2399	2.3E+08	2.2E+07	6.7E+07	1.5E+06	12
	Cattle	2.9E+06	2.6E+08	1.3E+08	1.8E+08	2.0E+08	2
	Sheep	1.1E+04	4.9E+04	3.0E+04	2.7E+04	4.0E+04	2
Fecal coliforms	Poultry	27	8.1E+05	1.5E+05	2.6E+05	1.3E+05	12
	Cattle	8.3E+06	3.7E+07	2.1E+07	1.5E+07	2.7E+07	3
Fecal streptococci	Cattle	1.3E+07	1.7E+08	7.6E+07	8.6E+07	1.1E+08	3
Giardia intestinalis	Swine	5.3E+04	1.6E+05	1.1E+05	7.6E+04	1.3E+05	2
	Cattle	220	5,000	2,610	3,380	3,805	2
	Sheep	380	1,200	790	580	995	2
Heterotrophic bacteria	Poultry	1.2E+09	1.1E+11	2.7E+10	3.8E+10	2.0E+10	12
Listeria	Swine	4.6E+04	9.7E+05	5.1E+05	6.5E+05	7.4E+05	2
	Poultry	3.2E+04	1.9E+05	1.1E+05	1.1E+05	1.5E+05	2
	Cattle	1.5E+04	4.2E+05	2.2E+05	2.9E+05	3.2E+05	2
	Sheep	450	1,700	1075	884	1,388	2
Pseudomonas aeruginosa	Poultry	158	5.0E+04	1.1E+04	1.7E+04	8,128	9
Salmonella	Swine	ND	7.8E+04	3671	1.6E+04	20	24
	Poultry	5,000	2.2E+04	1.4E+04	1.2E+04	1.8E+04	2
	Cattle	3.9E+04	5.8E+05	3.1E+05	3.8E+05	4.4E+05	2
	Sheep	1,100	2,000	1,550	636	1,775	2
Total coliforms	Poultry	5370	3.3E+08	3.3E+07	9.5E+07	6.7E+06	12
	Cattle	9.3E+06	3.7E+07	2.1E+07	1.4E+07	2.7E+07	3
Yersinia enterocolitica	Poultry	1.0E+04	2.1E+06	3.4E+05	6.9E+05	8.9E+04	9

Table M11. Descriptive statistical analysis of the occurrence of microorganisms in treated manure from different animals (cfu g^{-1} DM). (ND = Not Detected, min= minimum, max= maximum, STD= standard deviation, n.of data= number of collected data).

Microorganism	Animal	min	max	average	STD	75th percentile	n. of data
Campylobacter coli	Swine	ND	1.0E+05	1.6E+04	3.5E+04	6515	8
	Poultry	640	870	755	163	813	2
	Cattle	1.1E+04	1.5E+05	8.1E+04	9.8E+04	1.2E+05	2
	Sheep	100	100	100	/	100	1
Cryptosporidium parvum	Swine	140	310	225	120	268	2
	Cattle	66	480	273	293	377	2
E. coli	Swine	0	2.1E+04	2,294	6,157	230	16
	Cattle	8600	7.5E+04	4.2E+04	4.7E+04	5.8E+04	2
	Sheep	2500	5,000	3,750	1,768	4375	2
Faecal coliforms	Swine	3.0E+04	4.9E+04	4.0E+04	1.4E+04	4.4E+04	2
Faecal streptococci	Swine	4350	1.3E+04	8725	6187	1.1E+04	2
Giardia intestinalis	Swine	12	12	12	/	12	1
	Cattle	6	36	21	21	28	2
Listeria	Swine	1.6E+04	1.5E+05	8.3E+04	9.5E+04	1.2E+05	2
	Poultry	560	1300	930	523	1115	2
	Cattle	2.2E+04	9.8E+05	5.0E+05	6.8E+05	7.4E+05	2
	Sheep	2,100	8,100	5,100	4,243	6,600	2
Salmonella	Swine	0	2,000	963	1,002	1,445	2
	Poultry	4,700	8,000	6,350	2,333	7,175	2
	Cattle	1.9E+05	7.2E+06	3.7E+06	5.0E+06	5.4E+06	2
	Sheep	5,800	5,800	5,800	/	5,800	1
Total coliforms	Swine	1.2E+05	1.9E+05	1.6E+05	4.7E+04	1.7E+05	2

Appendix 3

Table of contents

Table R1. Runoff concentration values: minimum, mean, maximum, standard deviation and number of collected data in all the investigations included in this study, literature ranges of concentrations in secondary effluent, in runoff in case of direct reuse of reclaimed water for irrigation needs and surface water. Data from: Atalay et al., 2007; Eldridge et al., 2009; Giudice and Young, 2011; Gray et al., 2017; Healy et al., 2017; Peyton et al., 2016; Sabourin et al., 2009; Topp et al., 2008; Yang et al., 2012.

		Backgound Concentration [ng L^{-1}]		**Measured concentration [ng L^{-1}] This study**				**n# of data**	**Range in secondary effluent**	**Runoff in case of reuse of reclaimed water[f]**	**Surface water**
		min	**max**	**min**	**max**	**mean**	**SD**		**[ng L^{-1}]**	**[ng L^{-1}]**	**[ng L^{-1}]**
Analgesics & Antinlfammatories	Acetaminophen	17,5	17,5	20,3	146,0	81,8	58,2	10	5-6000[a]		
	Ibuprofen	10,5	10,5	79,2	1477,0	706,8	552,7	11	20-2900[a]	11-1300	
	Naproxen	7,0	7,0	18,1	509,0	263,6	347,1	8	2-3200[a]		
Antibiotics	Sulfamethoxazole	7,0	7,0	3,2	93,0	34,7	41,4	10	10-5000[a]		
Antiseptics	Triclocarban	1,0	3,0	3,4	20,0	8,6	5,1	20	100-6000[c]		
	Triclosan	12,5	51,0	51,0	600,0	202,7	157,7	26	15-580[a]		
Beta blockers	Atenolol	4,0	4,0	39,6	70,0	54,8	21,5	8	10-3160[a]		
Fragrances	Galaxolide	/	/	45,0	95,0	66,6	19,1	5			
	Indole	60,0	60,0	33,0	480,0	175,8	209,8	5			
	Menthol	/	/	300,0	340,0	320,0	20,0	3			
	Skatole	/	/	40,0	85,0	56,6	22,9	5			
	Tonalide	35,0	35,0	350,0	750,0	546,0	152,7	6			
Hormones	11-Ketotestosterone	2,0	2,0	2,0	7,5	3,1	1,8	33			
	17-α-estradiol	0,8	0,8	0,6	2,7	1,3	0,6	33			
	17-β-estradiol	0,8	0,8	0,6	5,9	2,1	1,7	33	0.1-64[f]	3	
	Androstenedione	0,8	1,5	7,1	216,1	73,3	65,8	33			
	Cis-androsterone	0,8	0,8	4,7	174,8	52,5	41,1	33			
	Diethylstibestrol	0,8	0,8	0,8	0,8	0,8	0,0	33			
	Dihydrotestosterone	4,0	4,0	1,3	40,8	11,9	9,7	33			
	Epitestosterone	4,0	4,0	2,4	17,0	6,1	3,9	33			
	Equilenin	2,0	2,0	1,3	4,8	2,4	0,9	33			
	Equilin	4,0	4,0	2,2	8,4	4,0	1,0	33			
	Estriol	2,0	2,0	2,0	3,6	2,2	0,4	33	0.72-275[a]		
	Estrone	0,8	2,2	3,1	25,0	12,3	6,2	33	0.5-110[a]	52	
	Ethynyl Estradiol	0,8	10,0	0,8	10,0	1,8	2,9	37	0.4-10[a]		
	Mestranol	0,8	0,8	0,8	0,8	0,8	0,0	33			
	Norethindrone	0,8	0,8	0,8	0,8	0,8	0,0	33			
	Progesterone	8,0	8,0	8,0	98,9	31,9	27,8	33	< 10[f]	3	
	Testosterone	0,8	0,8	0,9	20,8	6,3	5,5	33	< 10[f]	16	
Lipid regulators	Gemfibrozil	8,0	8,0	8,0	597,0	302,5	416,5	8	180-4760[a]	190-790	
Stimulants	Caffeine	5,0	49,2	35,2	35,2	35,2	/	7	0.5-377[b]	14	
	Cotinine	1,5	1,5	14,7	83,0	48,9	48,3	8	0.058[e]		
Psychiatric drugs	Carbamazepine	2,5	2,5	59,4	221,0	140,2	114,3	8	5-1519[a]	320-440	
Microorganisms	Total coliforms [CFU/ml]	0,0	0,0	63,1	79,4	71,3	11,5	2			
	E. coli [CFU/ml]	0,0	0,0	1,8	2,4	2,1	0,4	2			
	Fecal Coliforms [CFU/100ml]	100,0	100,0	5000	36000	16167	17222	4	10^4-10^6[d]		

	Backgound Concentration [ng L^{-1}]		Measured concentration [ng L^{-1}] This study				n# of data	Range in secondary effluent	Runoff in case of reuse of reclaimed water[f]	Surface water
	min	max	min	max	mean	SD		[ng L^{-1}]	[ng L^{-1}]	[ng L^{-1}]
Total coliforms [MPN/100ml]	/	/	15858	980600	344622	280808	12	10^5-10^7[, d]		
E. coli [MPN/100ml]	251189	251189	14,8	31623	5009	10191	15	10^4-10^5[, d]		

Legend: [a]Verlicchi et al., 2012; [b]Ben et al., 2018; [c]Chalew and Halden, 2009; [d]Masotti and Verlicchi, 2005; [e]Metcalfe et al., 2003; [f]Pedersen et al., 2005

Table R2. Tile drainage concentration values: minimum, mean, maximum, standard deviation and number of collected data in all the investigations included in this study, literature ranges of concentrations in secondary effluent, in runoff in case of direct reuse of reclaimed water for irrigation needs and surface water. Data from: Edwards et al., 2009; Giudice and Young, 2011; Gottschall et al., 2013, 2012; Lapen et al., 2008b, 2008a.

		BC [ng L^{-1}]		MEC [ng L^{-1}]				n# of data	Range in secondary effluent
		min	max	min	max	mean	St dev		ng L^{-1}
Analgesics	Acetaminophen	11,0	17,5	13,0	435,0	87,8	114,9	75	5-6000
	Ibuprofen	6,0	11,5	10,5	4117,0	530,8	1333,5	70	20-2900
	Naproxen	4,0	35,0	4,0	1045,0	218,2	409,2	67	2-3200
Antibiotics	4-Epitetracycline	6,0	6,0	6,0	6,0	6,0	/	2	
	Ciprofloxacin	6,0	6,0	6,0	6,0	6,0	/	2	7-5700
	Ofloxacin	1,5	1,5	1,5	1,5	1,5	/	2	20-1650
	Oxytetracycline	6,0	6,0	6,0	6,0	6,0	/	2	3-20
	Sulfamethoxazole	4,0	7,0	7,0	322,8	109,1	145,8	47	5-700
	Sulfapyridine	7,0	7,0	11,0	22,4	13,3	4,7	45	20-1112
	Tetracycline	6,0	6,0	6,0	6,0	6,0	/	2	2-370
Antifungals	Miconazole	1,5	1,5	1,5	1,5	1,5	/	2	
Antihistamines	Diphenhydramine	0,6	0,6	0,6	0,6	0,6	/	2	
Antiseptics	Triclocarban	1,0	2,5	2,3	40,0	4,0	7,2	33	100-6000^{c}
	Triclosan	6,0	95,0	9,5	3676,0	550,8	1231,1	90	15-580 ^{a}
Beta blockers	Atenolol	4,0	135,0	4,0	267,0	67,5	87,7	65	10-730
Hormones	17-α-Estradiol	7,5	7,5	/	/	/	/	1	
	17-β-Estradiol	7,5	7,5	/	/	/	/	1	
	α-Dihydroequilin	7,5	7,5	/	/	/	/	1	
	Androstenedione	18,8	18,8	/	/	/	/	1	
	Androsterone	7,5	7,5	33,7	33,7	33,7	/	2	
	Desogestrel	7,5	7,5	7,5	28,9	18,2	15,1	3	
	Equilenin	7,5	7,5	/	/	/	/	1	
	Equilin	7,5	7,5	/	/	/	/	1	
	Estradiol Benzoate	7,5	7,5	/	/	/	/	1	
	Estriol	7,5	7,5	/	/	/	/	1	
	Estrone	7,5	7,5	3,3	3,3	3,3	/	2	
	Ethynyl Estradiol	7,5	10,0	10,0	10,0	10,0	0,0	5	
	Mestranol	7,5	7,5	/	/	/	/	1	
	Norethindorne	7,5	7,5	/	/	/	/	1	
	Norgestrel	7,5	7,5	/	/	/	/	1	
	Progesterone	18,8	18,8	/	/	/	/	1	
	Testosterone	7,5	7,5	/	/	/	/	1	
Lipid regulators	Gemfibrozil	5,0	8,0	5,0	1040,0	249,7	422,3	49	
Stimulants	Cotinine	1,0	5,0	1,0	301,0	35,2	89,4	85	
Psychiatric drugs	Bupropion	3,0	3,0	3,0	3,0	3,0	/	2	
	Carbamazepine	2,0	2,5	2,5	1136,0	166,5	383,9	80	
	Citalopram	3,0	3,0	24,0	24,0	24,0	/	2	
	Desmethyl citalopram	3,0	3,0	3,0	3,0	3,0	/	2	
	Desmethyl sertraline	3,0	3,0	3,0	3,0	3,0	/	2	
	Desvenlafaxine	3,0	3,0	74,0	74,0	74,0	/	2	

		BC [ng L^{-1}]		**MEC [ng L^{-1}]**				**n# of data**	**Range in secondary effluent**
		min	**max**	**min**	**max**	**mean**	**St dev**		**ng L^{-1}**
	Fluoxetine	3,0	5,5	3,0	5,5	4,3	1,8	4	2-2000
	Norfluoxetine	3,0	3,0	3,0	3,0	3,0	/	2	6
	Norvenlafaxine	3,0	3,0	3,0	3,0	3,0	/	2	
	Paroxetine	3,0	3,0	3,0	3,0	3,0	/	2	7
	Sertraline	3,0	3,0	3,0	3,0	3,0	/	2	
	Venlafaxine	3,0	3,0	33,0	33,0	33,0	/	2	
Microorganisms [cts/100 mL]	*C. perfingens*	4,0	4,0	10,0	185,0	49,7	49,1	46,0	
	E. coli	100,0	100,0	100,0	30000,0	3504,4	6363,4	46,0	
	Fecal coliforms	/	/	100,0	30000,0	3567,8	6962,8	45,0	

Legend: [a]Verlicchi et al., 2012; [f]Pedersen et al., 2005; [g]Bai et al., 2018; [h]Burns et al., 2018; [i]López-Serna et al., 2010; [j]Martín et al., 2011; [k]Pereira et al., 2017; [m]Kasprzyk-Hordern et al., 2009.

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Appendix 4

Excretion factor for the selected compounds for the different types of animals considered.

Class	**Compound**	**Animal type**	**Excretion factor (%)**			
			in faeces	*in urine*	*total*	*References*
Antimicrobials	Amoxicillin	Cattle		50÷60		WHO, 2012
		Poultry			55.8÷70	Peng et al., 2016; Zhang et al., 2015
	Chloramphenicol	Poultry			54.1	Zhang et al., 2015
		Swine	40.6	26.5	67.1	Zhang et al., 2015
	Chlortetracycline	Cattle	75		17÷75	Elmund et al., 1971; Montforts et al., 1999
		Poultry			52.5	Zhang et al., 2015
		Swine	27	56	83	Zhang et al., 2015
	Ciprofloxacin	Cattle	23.2÷50.8			Nouws et al., 1988a
		Poultry			44.9÷53	Peng et al., 2016; Zhang et al., 2015
		Swine	33.7	26.2÷ 53.1	71	Nouws et al., 1988a; Zhang et al., 2015
	Clarithromycin	Poultry			67	Zhang et al., 2015
		Swine	17.5	12.1	29.6	Zhang et al., 2015
	Danofloxacin	Poultry		75÷85		WHO, 2012b
	Difloxacin	Poultry			53	Zhang et al., 2015
	Doxycycline	Poultry			52.5÷ 94.4	Peng et al., 2016; Zhang et al., 2015
		Swine	27	56	83	Zhang et al., 2015
	Enrofloxacin	Cattle			60÷87	Slana & Sollner-Dolenc, 2016
		Poultry			53÷74	Slana et al., 2014; Zhang et al., 2015
	Erythromycin-H2O	Poultry			67	Zhang et al., 2015
		Swine	18.2	12.6	30.8	Zhang et al., 2015
	Fleroxacin	Poultry			53	Zhang et al., 2015
		Swine	25.5	27.8	53.3	Zhang et al., 2015
	Florfenicol	Cattle		64		Riviere & Papich, 2018
		Swine	18.2	12.6÷60	30.8	EMEA, 1999
		Poultry			42	EMEA, 1999
	Lasalocid	Cattle	50			EC, 1990
	Leucomycin A5	Poultry			67	Zhang et al., 2015
		Swine	4.5	3.5	8	Zhang et al., 2015
	Lincomycin	Poultry			53÷83	Hornish et al., 1987
		Swine	15.1	5.5	20.6÷32	Hornish et al., 1987; Kuchta & Cessna, 2009; Zhang et al., 2015
	Lomefloxacin	Poultry			53	Zhang et al., 2015
		Swine	25.5	27.8	53.3	Zhang et al., 2015
	Methacycline	Poultry			53	Zhang et al., 2015
		Swine	27	56	83	Zhang et al., 2015
	Monensin	Cattle			13÷50	Donoho et al., 1978; Spirito et al., 2018
	Norfloxacin	Poultry			53	Zhang et al., 2015
		Swine	27.5	30	57.6	Zhang et al., 2015
	Ofloxacin	Poultry			53	Zhang et al., 2015
		Swine	25.5	27.8	53.3	Zhang et al., 2015
	Ormetoprim	Poultry			28.8	Zhang et al., 2015
		Swine	1.3	34.7	36	Zhang et al., 2015
	Oxytetracycline	Cattle			10÷23	Arikan et al., 2007; Ince et al., 2013
		Poultry			52.5	Zhang et al., 2015

Class	Compound	Animal type	Excretion factor (%)			
			in faeces	*in urine*	*total*	*References*
		Swine	28.1	42÷75		Mevius et al., 1986; Xia et al., 1983; Zhang et al., 2015
	Pefloxacin	Poultry			53	Zhang et al., 2015
		Swine	25.5	27.8	53.3	Zhang et al., 2015
	Pristinamycin (Virginiamycin)	Swine	0÷31			Feinman et al., 1978
	Roxithromycin	Poultry			67	Zhang et al., 2015
		Swine	34.7	24	58.7	Zhang et al., 2015
	Sulfachloropyridazine	Poultry			28.8	Zhang et al., 2015
		Swine	1.4÷23.2	35.3÷43.1	36.7÷66.3	Qiu et al., 2016; Zhang et al., 2015
	Sulfadiazine	Cattle		62÷74		Nouws et al., 1988b
		Poultry			28.8	Zhang et al., 2015
		Swine	1.6	20÷50	44	Lamshöft et al., 2007; Nielsen et al., 1986; Nouws et al., 1989; Zhang et al., 2015
	Sulfadimethoxine	Cattle		5÷18		Bourne et al., 1981
	Sulfadoxine	Cattle		80		Nielsen, 1973
	Sulfaguanidine	Poultry			28.8	Zhang et al., 2015
		Swine	1.4	35.3	36.7	Zhang et al., 2015
	Sulfamerazine	Cattle		24÷25		Nouws et al., 1988b
		Swine	25.6÷26.6	4÷18.8	41.1÷44.3	Nouws et al., 1989; Qiu et al., 2016
	Sulfamethazine (or sulfadimidine)	Cattle		9÷38		Bevill et al., 1977; Nouws et al., 1988a
		Poultry			13.9	Zhang et al., 2015
		Swine	0.9	24.5÷24.7	25.4	Duffee et al., 1984; Feinman et al., 1978; Zhang et al., 2015
	Sulfameter	Swine	1.4	35.3	36.7	Zhang et al., 2015
	Sulfamethizole	Poultry			28.8	Zhang et al., 2015
		Swine	0.9	24.5÷59.7		Aschbacher et al., 1995; Zhang et al., 2015
	Sulfamethoxazole	Cattle		68		Nielsen, 1973
		Poultry			28.8	Zhang et al., 2015
		Swine	1	20	21	Nouws et al., 1991
	Sulfamethoxazole + Trimethoprim	Swine		9.8÷23.7		Nouws et al., 1991
	Sulfamonomethoxine	Swine	0.2	4.6	4.8	Shimoda et al., 1990; Zhang et al., 2015
	Sulfanilamide	Cattle		70		Nielsen, 1973
	Sulfaquinoxaline	Poultry			28.8	Zhang et al., 2015
		Swine	1.4÷17.7	35.3÷69.2	36.7÷86.9	Qiu et al., 2016; Zhang et al., 2015
	Tetracycline	Swine	10÷25	25÷30	35÷72	Feinman et al., 1978
	Tilmicosin	Swine	80	15	95	WHO, 2012b
	Trimethoprim	Cattle		3		Nielsen, 1973
		Poultry			28.8	Zhang et al., 2015
		Swine		15÷78		Gyrd-Hansen et al., 2009; Poul Nielsen & Rasmussen, 2009a, 2009b
	Tylosin	Poultry			7÷76	Feinman et al., 1978; Zhang et al., 2015
		Swine	38.6÷67	0.4	67	EMEA, 1997; Feinman et al., 1978
Hormones	17α-estradiol (E2α or alfatradiol)	Cattle		59÷88		Erb et al., 1977
	17β-estradiol (E2β or estradiol)	Cattle		3÷18		Erb et al., 1977

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Appendix 5

Half-life time, DT90 (time at which 90% of the compound concentration has been reduced) for different compounds in different types of manure; kinetic models, with the specific parameters (see equations mentioned in Chapter 6, reported here in the footnote) adopted for the assessment of the half-life time and the corresponding references.

Class	Compound	Animal Type	Persistence									
			In manure									
			Half-life (d)	*DT_{90} (d)*	*Kinetic model*[1]	*Parameters (see equations in the footnote)*						*References*
						K_1	*K_2*	*g*[2]	*t_b*[2]	*k''*	*a*	
Antimicrobials	Chlortetracycline	Cattle	$1.8 \div 58^{a}$	$118^{b} \div 193^{a}$	SFO	$0.0119^{a} \div 0.393$						Berendsen et al., 2018; Sura et al., 2014
		Poultry	18	61	SFO	0.0378						Berendsen et al., 2018
		Swine	$1 \div 23.1$	62	SFO	$0.030 \div 0.6$						Berendsen et al., 2018; Joy et al., 2014; Zhang & Zhang, 2010
	Ciprofloxacin	Cattle	$1.3^{a} \div 61^{b}$	$58^{a} \div 277^{b}$	HS/ DFOP	$0.1967^{b} \div 1.6204^{c}$	$0.0077^{b} \div 0.025^{a}$	[3]	[3]			Berendsen et al., 2018
		Poultry	23	221	DFOP	0.9724	0.008	[3]				Berendsen et al., 2018
		Swine	6	85	DFOP	0.9556	0.02	[3]				Berendsen et al., 2018
	Danofloxacin	Cattle	$54^{a} \div 372^{c}$	$180^{a} \div 1236^{c}$	SFO	$0.0019^{c} \div 0.0128^{a}$						Berendsen et al., 2018
		Poultry	58	192	SFO	0.012						Berendsen et al., 2018
		Swine	7	78	DFOP	0.8777	0.022	0.469				Berendsen et al., 2018
	Difloxacin	Cattle	$68^{a} \div 374^{c}$	$226^{a} \div 1243^{c}$	SFO	$0.0019^{c} \div 0.0102^{a}$						Berendsen et al., 2018
		Poultry	41	194	DFOP	0.5063	0.01	0.246				Berendsen et al., 2018
		Swine	11	99	DFOP	0.9917	0.018	0.411				Berendsen et al., 2018
	Doxycycline	Cattle	$26^{a} \div 127^{c}$	$117^{a} \div 422^{c}$	SFO/ DFOP	$0.0055^{c} \div 0.762^{a}$	0.017	0.258				Berendsen et al., 2018
		Poultry	20	268	DFOP	0.5353	0.006	0.4479				Berendsen et al., 2018
		Swine	$10 \div 53$	98	SFO/ DFOP	$0.013 \div 0.8611$	0.018	0.4454				Berendsen et al., 2018
	Enrofloxacin	Cattle	$49^{a} \div 1751^{c}$	164^{a}	SFO	$0.0004^{c} \div 0.014^{a}$						Berendsen et al., 2018
		Poultry	103	343	SFO	0.0067						Berendsen et al., 2018
		Swine	6	83	DFOP	1.1401	0.02	0.448				Berendsen et al., 2018
	Erythromycin	Cattle	$5.8^{a} \div 32^{b}$	$19^{a} \div 106^{b}$	SFO	$0.0217^{b} \div 0.119^{a}$						Berendsen et al., 2018
		Poultry	17	56	SFO	0.0412						Berendsen et al., 2018
		Swine	$41 \div 52$	172	SFO	$0.0134 \div 0.017$						Berendsen et al., 2018; Schlüsener et al., 2006
	Flumequine	Cattle	$75^{a} \div 569^{c}$	$247^{a} \div 1890^{c}$	SFO	$0.0012^{c} \div 0.0093^{a}$						Berendsen et al., 2018
		Poultry	197	655	SFO	0.0035						Berendsen et al., 2018
		Swine	44	146	SFO	0.0158						Berendsen et al., 2018
	Josamycin	Cattle	$16^{a} \div 27^{b}$	$67^{a} \div 89^{b}$	SFO/ DFOP	$0.0259^{b} \div 0.7347^{a}$	0.031	0.194				Berendsen et al., 2018
		Poultry	43	141	SFO	0.0163						Berendsen et al., 2018
		Swine	231	769	SFO	0.003						Berendsen et al., 2018
	Lasolacid	Poultry	61.8		FOMC	[3]	[3]					Žižek et al., 2015
	Lincomycin	Cattle	$95^{a} \div 214^{c}$	$315^{a} \div 711^{c}$	SFO	$0.0032^{c} \div 0.0073^{a}$						Berendsen et al., 2018
		Swine	269	892	SFO	0.0026						Berendsen et al., 2018
	Marbofloxacin	Cattle	$77^{a} \div 205^{c}$	$346^{a} \div 683^{c}$	SFO/HS	$0.0034^{c} \div 0.0962^{a}$	0.006		2.432			Berendsen et al., 2018
		Poultry	300	388	SFO	0.0077						Berendsen et al., 2018
		Swine	91	70	DFOP	0.943	0.018	0.468				Berendsen et al., 2018
	Nalidixic acid	Cattle	$87^{a} \div 1410^{c}$	$290^{a} \div 4683^{c}$	SFO	$0.0005^{c} \div 0.0079^{a}$						Berendsen et al., 2018
		Poultry	388	1290	SFO	0.0018						Berendsen et al., 2018
		Swine	70	295	HS	0.092	0.007		2.344			Berendsen et al., 2018
	Norfloxacin	Cattle	$1.6^{a} \div 60^{b}$	$67^{a} \div 254^{b}$	DFOP/ HS	$0.0876^{b} \div 1.3243^{a}$	$0.008^{b} \div 0.022^{a}$	$0.273^{c} \div 0.568^{a}$	2.7456			Berendsen et al., 2018
		Poultry	18	179	DFOP	1.0012	0.010	0.421				Berendsen et al., 2018
		Swine	5	79	DFOP	0.953	0.021	0.456				Berendsen et al., 2018
	Oxolinic acid	Cattle	$58^{a} \div 348^{c}$	$193^{a} \div 1158^{c}$	SFO	$0.002^{c} \div 0.0119^{a}$						Berendsen et al., 2018
		Poultry	116	387	SFO	0.006						Berendsen et al., 2018
		Swine	36	181	HS	0.1335	0.011		2.6406			Berendsen et al., 2018
	Oxytetracycline	Cattle	$8 \div 98^{b}$	$103^{a} \div 327^{c}$	SFO/ DFOP/ AAFO	$0.0071^{c} \div 0.096$	0.023	0.919		$0.095 \div 0.102$	$0.0339 \div 0.0545$	Berendsen et al., 2018; De Liguoro et al., 2003; Wang & Yates, 2008

Class	Compound	Animal Type	Persistence									References
			In manure									
			Half-life (d)	DT_{90} (d)	Kinetic model[1]	Parameters (see equations in the footnote)						
						K_1	K_2	g^2	t_b^2	k''	a	
		Poultry	30	221	DFOP	0.5373	0.008	0.3743				Berendsen et al., 2018
		Swine	16÷79	171	DFOP/SFO	0.0088÷0.6889	0.010	0.4353				Berendsen et al., 2018; Blackwell et al., 2005
	Pirlimycin	Cattle	$19^{a}\div699^{c}$	$135^{a}\div2320^{c}$	SFO/DFOP	$0.001^{c}\div0.2653^{a}$	0.013	0.348				Berendsen et al., 2018
		Poultry	443	1473	SFO	0.5373						Berendsen et al., 2018
		Swine	125	414	SFO	0.6889						Berendsen et al., 2018
	Roxithromycin	Swine	130		SFO	0.005						Schlüsener et al., 2006
	Sarafloxacin	Cattle	398^{b}	1152^{a}	SFO	$0.0017^{b}\div0^{c}$						Berendsen et al., 2018
		Poultry	176	585	SFO	0.0039						Berendsen et al., 2018
		Swine	562	1867	SFO	0.0012						Berendsen et al., 2018
	Salinomycin	Poultry	4		SFO	0.1731						Ramaswamy et al., 2010
		Swine	5.1		SFO	0.135						Schlüsener et al., 2006
	Sulfacetamide	Cattle	$4.4^{c}\div39^{a}$	$36^{b}\div131^{a}$	SFO/FOMC	$0.0176^{a}\div1.065^{c}$	4.826					Berendsen et al., 2018
		Poultry	4.9	100	DFOP	0.5373	0.010	0.707				Berendsen et al., 2018
		Swine	1.5	8	FOMC	0.6889	3.198					Berendsen et al., 2018
	Sulfachloropyridazine	Cattle	$1^{c}\div12^{a}$	$8^{c}\div60^{a}$	FOMC/DFOP	$0.474^{a}\div1.186^{c}$	$0.033^{a}\div1.3^{c}$	0.282÷0.458				Berendsen et al., 2018
		Poultry	2.9	15	DFOP	0.389	0.028	0.703				Berendsen et al., 2018
		Swine	1.6÷127	38	FOMC/SFO	0.0055÷0.979	1.563					Berendsen et al., 2018; Blackwell et al., 2005
	Sulfadiazine	Cattle	$1.5^{c}\div25^{a}$	$11^{c}\div84^{a}$	SFO/FOMC/DFOP	$0.0176^{a}\div1.065^{c}$	$0.055^{b}\div2.004^{c}$	0.383				Berendsen et al., 2018
		Poultry	4.4	83	FOMC	0.1657	0.032	0.822				Berendsen et al., 2018
		Swine	2.2	18	DFOP	1.089	2.431					Berendsen et al., 2018
	Sulfadimethoxine	Cattle	$1\div39^{a}$	$14^{c}\div59^{a}$	FOMC/DFOP/AAFO	$0.444^{a}\div1.155^{b}$	$0.033^{a}\div1.63^{c}$	$0.272^{a}\div0.363^{b}$		0.08÷0.699	0.033÷0.495	Berendsen et al., 2018; Wang et al., 2006
		Poultry	3.4	37	DFOP	0.426	0.037	0.585				Berendsen et al., 2018
		Swine	3.2	21	DFOP	1.1242	0.089	0.348				Berendsen et al., 2018
	Sulfadoxine	Cattle	$2.1^{c}\div29^{a}$	$14^{c}\div59^{a}$	SFO/FOMC/DFOP	$0.0169^{a}\div1.128^{c}$	$0.046^{b}\div2.419^{c}$	0.311				Berendsen et al., 2018
		Poultry	5	89	DFOP	0.2976	0.014	0.838				Berendsen et al., 2018
		Swine	3	25	FOMC	1.074	3.256					Berendsen et al., 2018
	Sulfamerazine	Cattle	$1.3^{c}\div19^{a}$	$9^{c}\div62^{a}$	SFO/FOMC/DFOP	$0.0369^{a}\div1.259^{c}$	$0.062^{b}\div1.709^{c}$	0.414				Berendsen et al., 2018
		Poultry	3.7	45	DFOP	0.3644	0.029	0.605				Berendsen et al., 2018
		Swine	1.8	17	FOMC	1.009	1.871					Berendsen et al., 2018
	Sulfamethazine (or sulfadimidine)	Cattle	20.8		SFO	0.033						Sura et al., 2014
	Sulfamethizole	Cattle	$1^{c}\div13^{a}$	$7^{c}\div60^{a}$	FOMC/DFOP	$0.1068^{a}\div1.37^{c}$	$0.034^{a}\div1.53^{c}$	$0.489^{b}\div0.535^{a}$				Berendsen et al., 2018
		Poultry	2.4	23	DFOP	0.3783	0.027	0.803				Berendsen et al., 2018
		Swine	1	9	FOMC	1.050	1.080					Berendsen et al., 2018
	Sulfamethoxazole	Cattle	$1.6^{c}\div5.7^{a}$	$12^{c}\div21^{b}$	SFO/FOMC/DFOP	$0.122^{a}\div1.197^{c}$	$0.087^{b}\div2.03^{c}$	0.395				Berendsen et al., 2018
		Poultry	2.5	53	DFOP	0.3783	0.027	0.744				Berendsen et al., 2018
		Swine	2.6	22	FOMC	1.050	1.080					Berendsen et al., 2018
	Sulfamethoxypyridazine	Cattle	$0.9^{c}\div9^{a}$	$7^{c}\div48^{a}$	FOMC/DFOP	$0.6176^{a}\div1.298^{b}$	$0.041^{a}\div1.2^{c}$	$0.286^{b}\div0.513^{a}$				Berendsen et al., 2018
		Poultry	2.5	29	DFOP	0.5048	0.044	0.645				Berendsen et al., 2018
		Swine	1.6	14	FOMC	1.013	1.614					Berendsen et al., 2018
Antimicrobials	Sulfamonomethoxine	Cattle	$1.4^{c}\div13^{a}$	$9^{c}\div64^{a}$	FOMC/DFOP	$0.5013^{a}\div1.323^{c}$	$0.031^{a}\div1.973^{c}$	$0.264^{a}\div0.386^{b}$				Berendsen et al., 2018
		Poultry	3.3	44	DFOP	0.4037	0.029	0.629				Berendsen et al., 2018
		Swine	2.1	19	FOMC	0.990	2.047					Berendsen et al., 2018
	Sulfaphenazole	Cattle	$0.9^{c}\div4.9^{a}$	$6^{c}\div22^{a}$	FOMC/DFOP	$0.7262^{a}\div1.284^{b}$	$0.089^{b}\div1.198^{c}$	$0.211^{b}\div0.478^{a}$				Berendsen et al., 2018
		Poultry	0.7	3.2	FOMC	2.811	2.517					Berendsen et al., 2018
		Swine	1.6	13	FOMC	1.150	1.980					Berendsen et al., 2018
	Sulfapyridine	Cattle	$0.8^{c}\div6^{a}$	$6^{c}\div41^{a}$	FOMC/DFOP	$0.504^{a}\div1.4533^{b}$	$0.044^{b}\div1.014^{c}$	$0.376^{a}\div0.510^{b}$				Berendsen et al., 2018
		Poultry	3.2	41	DFOP	0.3803	0.028	0.6674				Berendsen et al., 2018
		Swine	1.4	13	FOMC	0.992	1.432					Berendsen et al., 2018
	Sulfaquinoxaline	Cattle	$0.8^{c}\div4.2^{a}$	$7^{c}\div37^{a}$	FOMC/DFOP	$0.4748^{a}\div1.351^{b}$	$0.045^{a}\div0.777^{c}$	$0.461^{a}\div0.543^{b}$				Berendsen et al., 2018
		Poultry	2.2	30	DFOP	0.6456	0.044	0.615				Berendsen et al., 2018
		Swine	3.8	13	SFO	0.182						Berendsen et al., 2018
	Sulfathiazole	Cattle	$0.6^{c}\div3.7^{a}$	$4^{c}\div24^{a}$	FOMC/DFOP	$0.5202^{a}\div1.4182^{b}$	$0.072^{a}\div0.832^{c}$	$0.405^{a}\div0.606^{b}$				Berendsen et al., 2018

Class	Compound	Animal Type	Persistence									
			In manure									
			Half-life (d)	DT_{90} (d)	Kinetic model[1]	Parameters (see equations in the footnote)						References
						K_1	K_2	g^2	$t_b{}^2$	k''	a	
		Poultry	2	18	FOMC	1.015	2.036					Berendsen et al., 2018
		Swine	1.2	11	FOMC	1.007	1.197					Berendsen et al., 2018
	Sulfisoxazole	Cattle	1.1^{a}÷2^{b}	3.7^{a}÷ 10^{b-c}	SFO/ FOMC/ DFOP	0.34^{b}÷ 1.201^{c}	0.095^{b}÷ 1.666^{c}	0.9112				Berendsen et al., 2018
		Poultry	0.7	10	HS	0.9919	0.064		2.112			Berendsen et al., 2018
		Swine	1.3	10	FOMC	1.177	1.578					Berendsen et al., 2018
	Tetracycline	Cattle	12^{a}÷55^{c}	86^{a}÷ 181^{c}	SFO	0.0127^{c}÷ 0.7218^{a}	0.021	0.3939				Berendsen et al., 2018
		Poultry	62	330	DFOP	0.5373	0.008	0.3089				Berendsen et al., 2018
		Swine	12÷105	111	DFOP/ SFO	0.0066÷ 0.6889	0.01	0.3886				Berendsen et al., 2018; Winckler & Grafe, 2001
	Tiamulin	Cattle	43^{a}	144^{a}	SFO	0.0021^{a} ÷0^{c}						Berendsen et al., 2018
		Poultry	280	930	SFO	0.0025						Berendsen et al., 2018
		Swine	101	335	SFO	0.0069						Berendsen et al., 2018
	Tilmicosin	Cattle	11^{a}÷ 104^{b}	38a÷ 346b	SFO	0.0067^{b} ÷ 0.0614^{a}						Berendsen et al., 2018
		Poultry	71	235	SFO	0.0098						Berendsen et al., 2018
		Swine	47	220	HS	0.1211	0.009		2.748			Berendsen et al., 2018
	Tylosin	Cattle	4÷6		SFO/ PFO	0.189						De Liguoro et al., 2003; Sura et al., 2014; Teeter & Meyerhoff, 2003
		Poultry	<8		SFO	0.091						Teeter & Meyerhoff, 2003
		Swine	7.6÷42	179	HS/SFO	0.091÷ 0.1813	0.011		1.642			Berendsen et al., 2018; Joy et al., 2014; Teeter & Meyerhoff, 2003
Hormones	17α-trombolone	Cattle	267		SFO	0.0026						Schiffer et al., 2001
	17β-trombolone	Cattle	257		SFO	0.0027						Schiffer et al., 2001

[1] AAFO (Availability-adjusted first-order) $C=C_0 \exp(-k''/a\,(1-\exp(-at))$; DFOP (Double First Order in Parallel or Biexponential) $C=C_0\, g\, e^{-k1t} + C_0\,(1-g)\, e^{-k2t}$; FOMC (First Order Multi-Compartment) $C=C_0(t/k_2+1)-k_1$; HS (Hockey Stick, Bi-phasic first order) $C=C_0\, e^{-k1tb}\, e^{-k2\,(t-tb)}$; PFO (Pseudo-First Order) $C=C_0-C_0\, e^{-kt}$; SFO (Single First Order) $C=C_0\, e^{-kt}$

[2] Find with Solver of Excel

[3] Not enough data for find the parameter with Solver of Excel

[a] Cattle liquid (Three difference types of manure are selected according to observation of large differences in specifically the consistency of manure by Berendsen et al., 2018)

[b] Cattle semi-solid (Three difference types of manure are selected according to observation of large differences in specifically the consistency of manure by Berendsen et al., 2018)

[c] Cattle solid (Three difference types of manure are selected according to observation of large differences in specifically the consistency of manure by Berendsen et al., 2018)

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Appendix 6

Table of contents

Table II. Dataset used in the cluster analysis. All the measures are expressed in ratios between each PhAC mean concentration and total concentration of the related sampling campaign.

	Country	Sampling area	Amitriptyline	Artemisinin	Atenolol	Caffeine	Carbamazepine	Ceterizine	Cimetidine	Ciprofloxacin	Citalopram	Clarithromycin	Codeine	Cotinine	Desvenlafaxine	Diazepam	Diltiazem	Enrofloxacin	Erythromycin	Fexofenadine	Fluconazole	Gabapentin	Hydrocodone	Ketotifen	Lidocaine	Lincomyacin	Loratadine	Metformin	Metronidazole	Naproxen	Nevirapine	Nicotine	Noreistherone	Paracetamol	Pregabalin	Propranolol	Ranitidine	Salbutamol	Sitagliptin	Sulfadiazine	Sulfamethoxazole	Temazepam	Thiabendazole	Tramadol	Triamterene	Trimethoprim	Tylosin	Venlafaxine	Verapamil
Africa	Angola	Luanda	0%	0%	0%	9%	2%	0%	0%	6%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	19%	6%	0%	1%	2%	0%	41%	0%	0%	1%	0%	1%	0%	7%	0%	0%	0%	0%	1%	0%	0%	0%
	Burkina Faso	Ouagadougou	0%	0%	1%	57%	0%	0%	1%	0%	0%	0%	0%	5%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	10%	16%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	8%	0%	0%	0%	0%	2%	0%	0%	0%
	Cameroon	Yaounde	0%	0%	0%	7%	1%	0%	2%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	5%	0%	0%	0%	29%	0%	35%	0%	0%	0%	0%	0%	0%	12%	0%	0%	7%	0%	1%	0%	0%	0%
	Democratic Republic of the Congo	Bukavu	0%	0%	0%	5%	0%	0%	1%	0%	0%	0%	0%	1%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	12%	7%	0%	1%	2%	0%	44%	0%	0%	0%	0%	0%	0%	22%	0%	0%	0%	0%	3%	0%	0%	0%
	Democratic Republic of the Congo	Kinshasa	0%	0%	0%	14%	0%	0%	0%	1%	0%	0%	0%	4%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	6%	0%	4%	0%	0%	0%	6%	0%	45%	0%	0%	0%	0%	4%	0%	14%	0%	0%	0%	0%	1%	0%	0%	0%
	Ethiopia	Addis Ababa	0%	0%	0%	68%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	13%	2%	0%	0%	1%	0%	10%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	1%	0%	0%	0%
	Ghana	Accra	0%	0%	0%	11%	0%	1%	0%	1%	0%	0%	0%	1%	0%	1%	0%	0%	7%	0%	0%	1%	0%	0%	0%	0%	0%	12%	41%	3%	0%	1%	0%	1%	0%	0%	0%	0%	0%	0%	18%	0%	0%	0%	0%	2%	0%	0%	0%
	Kenya	Nairobi	0%	0%	0%	0%	1%	1%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	2%	48%	2%	1%	0%	0%	0%	0%	0%	19%	2%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	16%	0%	0%	0%	0%	5%	0%	0%	0%
	Lesotho	Maseru	0%	0%	1%	8%	1%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	52%	0%	0%	4%	4%	0%	5%	0%	0%	0%	0%	0%	0%	21%	0%	0%	0%	0%	1%	0%	0%	0%
	Liberia	Monrovia	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	10%	0%	0%	0%	18%	0%	54%	0%	0%	0%	0%	0%	0%	11%	0%	0%	0%	0%	2%	0%	0%	0%
	Mali	Bamako	0%	0%	1%	54%	0%	0%	1%	0%	0%	0%	1%	3%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	10%	0%	0%	0%	8%	0%	7%	0%	0%	0%	0%	0%	0%	8%	0%	0%	2%	0%	2%	0%	0%	0%
	Morocco	Tangier - Tetuan	0%	0%	0%	4%	3%	2%	0%	0%	0%	0%	0%	4%	0%	0%	0%	0%	0%	1%	0%	8%	0%	0%	0%	0%	0%	71%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	6%	0%	0%	0%	0%	1%	0%	0%	0%
	Nigeria	Lagos	0%	3%	0%	12%	0%	0%	1%	1%	0%	0%	0%	1%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	1%	0%	8%	0%	1%	1%	0%	0%	63%	0%	0%	0%	0%	0%	0%	5%	0%	0%	0%	0%	1%	0%	0%	0%
	Pretoria	Pretoria	0%	0%	2%	40%	1%	1%	0%	0%	0%	0%	3%	1%	1%	0%	0%	0%	0%	1%	0%	2%	0%	0%	0%	0%	0%	25%	0%	0%	0%	1%	0%	12%	0%	0%	0%	0%	0%	0%	7%	0%	0%	0%	0%	2%	0%	0%	0%
	Republic of the Congo	Brazzaville	0%	0%	0%	11%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	1%	0%	4%	22%	0%	1%	9%	0%	43%	0%	0%	0%	0%	0%	0%	5%	0%	0%	0%	0%	1%	0%	0%	0%
	Rwanda	Kigali	0%	0%	0%	66%	0%	0%	1%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	6%	0%	0%	0%	0%	0%	10%	0%	0%	0%	0%	0%	0%	13%	0%	0%	0%	0%	2%	0%	0%	0%
	South Sudan	Juba	0%	0%	0%	61%	1%	0%	1%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	4%	0%	0%	0%	0%	0%	11%	0%	0%	0%	0%	0%	0%	17%	0%	0%	0%	4%	0%	0%	0%	0%
	Tanzania	Dar es Salaam	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	2%	0%	87%	0%	0%	0%	0%	0%	0%	5%	0%	0%	0%	0%	2%	0%	0%	0%
	Tanzania	Arusha	0%	0%	0%	0%	2%	7%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	6%	0%	0%	0%	0%	0%	25%	0%	0%	33%	0%	0%	0%	0%	0%	0%	0%	0%	0%	25%	0%	0%	0%	0%	0%	0%	0%	0%
	Tunisia	Tunis	0%	0%	1%	13%	0%	1%	0%	0%	0%	1%	0%	2%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	25%	0%	2%	0%	3%	0%	47%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Asia	Armenia	Yerevan	0%	0%	0%	56%	0%	0%	0%	0%	0%	1%	0%	2%	0%	0%	0%	0%	0%	0%	0%	3%	0%	0%	0%	0%	0%	21%	0%	1%	0%	10%	0%	5%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Bangladesh	Barisal	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	9%	73%	3%	0%	0%	0%	0%	0%	0%	10%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	China	Panyu	0%	0%	0%	57%	0%	0%	3%	0%	0%	0%	0%	10%	0%	0%	0%	0%	3%	0%	0%	0%	0%	0%	0%	0%	0%	8%	0%	0%	0%	8%	0%	9%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%
	China	Guangzhou	0%	0%	0%	21%	0%	0%	1%	0%	0%	0%	0%	6%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	66%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	1%	0%	1%	0%	0%	0%	0%	1%	0%	0%	0%
	Georgia	Tbilisi	0%	0%	0%	43%	0%	0%	0%	1%	0%	2%	0%	1%	0%	0%	0%	0%	0%	0%	0%	3%	0%	0%	0%	0%	0%	10%	17%	0%	0%	12%	0%	2%	0%	0%	0%	0%	9%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Hong Kong	Lam Tsuen River	0%	0%	1%	31%	0%	3%	0%	0%	0%	1%	0%	0%	1%	0%	0%	0%	0%	3%	0%	3%	0%	0%	0%	0%	0%	27%	0%	0%	0%	3%	0%	21%	0%	0%	0%	0%	3%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Hong Kong	Kai Tak River	0%	0%	2%	7%	2%	4%	11%	4%	1%	4%	0%	0%	4%	0%	0%	0%	1%	3%	0%	14%	1%	0%	1%	0%	1%	19%	0%	2%	0%	0%	0%	0%	0%	0%	1%	0%	9%	0%	1%	0%	2%	3%	0%	0%	0%	0%	0%
	India	Delhi	0%	0%	1%	31%	0%	1%	0%	0%	0%	0%	0%	6%	0%	0%	0%	0%	0%	1%	0%	1%	0%	0%	0%	0%	0%	39%	0%	1%	0%	8%	0%	4%	2%	0%	0%	0%	1%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%
	Iran	Rudbar	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	6%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	70%	0%	0%	0%	21%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Iraq	Baghdad	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	44%	0%	1%	0%	0%	0%	0%	0%	28%	1%	0%	0%	2%	0%	20%	0%	0%	0%	0%	0%	0%	1%	0%	0%	1%	0%	0%	0%	0%	0%
	Israel	Alexander River (whole country)	0%	0%	5%	0%	2%	0%	0%	1%	0%	1%	0%	11%	0%	0%	0%	0%	0%	3%	0%	8%	0%	0%	1%	0%	0%	0%	0%	7%	0%	5%	0%	42%	4%	0%	3%	0%	1%	0%	2%	0%	0%	0%	0%	0%	2%	0%	0%
	Kazakhstan	Astana	0%	0%	0%	34%	1%	0%	0%	0%	0%	0%	0%	3%	0%	0%	0%	0%	0%	0%	0%	4%	0%	0%	0%	0%	0%	39%	0%	0%	0%	8%	0%	5%	0%	0%	0%	0%	0%	0%	5%	0%	0%	0%	0%	0%	0%	0%	0%
	Malaysia	Kajang	0%	0%	1%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	69%	0%	1%	0%	0%	0%	0%	0%	23%	0%	1%	0%	2%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Pakistan	Lahore	0%	0%	1%	19%	0%	0%	1%	0%	0%	1%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	23%	0%	3%	0%	4%	0%	40%	1%	0%	0%	0%	1%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%
	Palestine	West Bank	0%	0%	1%	33%	6%	0%	0%	1%	0%	0%	0%	4%	0%	0%	0%	2%	0%	2%	0%	4%	0%	0%	1%	1%	0%	23%	0%	4%	0%	4%	0%	10%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Philippines	Manila	0%	0%	1%	50%	0%	0%	0%	0%	0%	0%	0%	6%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	13%	0%	0%	0%	12%	0%	13%	0%	0%	0%	0%	3%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%
	South Korea	Seoul	0%	0%	1%	3%	1%	2%	2%	0%	0%	2%	0%	3%	1%	0%	0%	0%	0%	2%	0%	20%	0%	0%	1%	0%	0%	49%	0%	1%	0%	0%	0%	0%	3%	0%	0%	0%	4%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%
	Sri Lanka	Colombo	0%	0%	1%	0%	1%	4%	4%	0%	0%	1%	0%	1%	1%	0%	0%	0%	0%	2%	0%	17%	0%	0%	2%	1%	0%	54%	0%	0%	0%	0%	0%	2%	0%	0%	1%	0%	7%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Taiwan	Taoyuan	0%	0%	1%	38%	1%	0%	10%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	4%	0%	0%	0%	0%	1%	2%	0%	37%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	3%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%
	Thailand	Bangkok	0%	0%	2%	9%	1%	2%	1%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	1%	0%	4%	0%	0%	0%	0%	0%	15%	0%	0%	0%	8%	0%	54%	0%	0%	0%	0%	2%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%
	Turkey	Saray	0%	0%	0%	27%	1%	1%	0%	0%	0%	0%	0%	3%	2%	0%	0%	0%	0%	0%	0%	11%	0%	0%	1%	0%	0%	25%	0%	6%	0%	2%	0%	10%	6%	0%	0%	0%	1%	0%	1%	0%	0%	2%	0%	0%	0%	1%	0%

	Country	Sampling area	Amitriptyline	Artemisinin	Atenolol	Caffeine	Carbamazepine	Ceterizine	Cimetidine	Ciprofloxacin	Citalopram	Clarithromycin	Codeine	Cotinine	Desvenlafaxine	Diazepam	Diltiazem	Enrofloxacin	Erythromycin	Fexofenadine	Fluconazole	Gabapentin	Hydrocodone	Ketotifen	Lidocaine	Lincomycin	Loratadine	Metformin	Metronidazole	Naproxen	Nevirapine	Nicotine	Noreisthrone	Paracetamol	Pregabalin	Propranolol	Ranitidine	Salbutamol	Sitagliptin	Sulfadiazine	Sulfamethoxazole	Temazepam	Thiabendazole	Tramadol	Triamterene	Trimethoprim	Tylosin	Venlafaxine	Verapamil
Europe	Austria	Vienna	0%	0%	4%	4%	1%	2%	0%	1%	1%	6%	1%	1%	3%	0%	0%	0%	1%	2%	0%	12%	0%	0%	1%	0%	0%	8%	0%	11%	0%	1%	0%	0%	20%	0%	2%	0%	0%	4%	0%	0%	0%	0%	7%	0%	2%	3%	0%
	Belgium	Brussels	0%	0%	1%	2%	19%	20%	0%	0%	1%	1%	0%	1%	4%	0%	0%	0%	0%	0%	0%	7%	0%	0%	0%	0%	0%	22%	0%	2%	0%	1%	0%	0%	4%	1%	3%	0%	0%	1%	0%	1%	1%	0%	4%	0%	0%	1%	0%
	Belgium	Antwerp	1%	0%	3%	0%	3%	7%	0%	2%	1%	0%	1%	0%	8%	0%	0%	0%	1%	1%	0%	12%	0%	0%	1%	0%	0%	11%	0%	5%	0%	0%	0%	2%	6%	3%	1%	0%	0%	3%	0%	1%	0%	0%	27%	0%	1%	1%	0%
	Bulgaria	Sofia	0%	0%	0%	25%	0%	0%	0%	0%	0%	1%	0%	2%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	62%	0%	1%	0%	2%	0%	3%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Croatia	Zagreb	0%	0%	0%	20%	1%	5%	0%	0%	0%	0%	0%	3%	1%	0%	0%	0%	0%	1%	0%	3%	0%	0%	0%	0%	0%	43%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	22%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Czech Republic	Prague	0%	0%	0%	0%	2%	2%	0%	2%	0%	3%	0%	0%	3%	0%	0%	0%	0%	0%	1%	14%	0%	0%	0%	0%	0%	66%	0%	0%	0%	2%	0%	1%	0%	0%	0%	0%	2%	0%	1%	0%	0%	0%	0%	0%	1%	1%	0%
	Denmark	Odense	0%	0%	0%	3%	2%	10%	0%	0%	1%	0%	1%	1%	7%	0%	0%	0%	0%	18%	2%	20%	0%	0%	2%	0%	0%	17%	0%	0%	0%	7%	0%	0%	0%	1%	0%	0%	5%	0%	1%	0%	0%	0%	0%	0%	0%	3%	0%
	Finland	Helsinki	0%	0%	0%	0%	2%	0%	0%	3%	0%	0%	0%	5%	7%	0%	0%	0%	0%	3%	0%	29%	0%	0%	0%	0%	0%	35%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	11%	0%	0%	0%	0%	0%	0%	1%	3%	0%
	France	Beaujeu	0%	0%	1%	22%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	4%	0%	0%	0%	3%	0%	0%	0%	16%	0%	16%	0%	0%	0%	3%	0%	34%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	France	Paris	0%	0%	0%	0%	3%	0%	0%	0%	0%	0%	0%	0%	8%	0%	1%	0%	0%	0%	0%	15%	0%	0%	2%	0%	0%	38%	0%	0%	0%	10%	0%	8%	0%	1%	0%	0%	9%	0%	3%	0%	0%	0%	0%	0%	0%	2%	0%
	Germany	Berlin	0%	0%	1%	0%	6%	4%	0%	1%	2%	0%	0%	0%	9%	0%	0%	0%	0%	3%	0%	13%	0%	0%	2%	0%	0%	12%	0%	8%	0%	3%	0%	0%	0%	0%	0%	0%	17%	0%	3%	0%	0%	13%	0%	1%	0%	3%	0%
	Germany	Frankfurt	0%	0%	0%	18%	4%	5%	0%	0%	0%	0%	0%	1%	5%	0%	0%	0%	0%	1%	0%	20%	0%	0%	1%	0%	0%	30%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	10%	0%	1%	1%	0%	0%	0%	0%	0%	2%	0%
	Germany	Tubingen	4%	0%	3%	11%	3%	4%	2%	1%	5%	2%	6%	0%	2%	3%	4%	0%	0%	2%	0%	3%	0%	4%	0%	0%	0%	4%	3%	0%	5%	5%	0%	0%	0%	3%	2%	8%	2%	0%	2%	0%	0%	2%	5%	1%	0%	0%	0%
	Greece	Oinofyta	0%	0%	0%	25%	1%	1%	0%	0%	0%	0%	0%	1%	2%	0%	0%	0%	0%	0%	0%	4%	0%	0%	2%	0%	0%	40%	0%	12%	0%	0%	0%	8%	0%	0%	0%	0%	3%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Hungary	Budapest	0%	0%	1%	11%	6%	2%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	3%	0%	17%	0%	0%	2%	0%	0%	34%	0%	0%	0%	5%	0%	6%	5%	0%	1%	0%	4%	0%	1%	0%	0%	2%	0%	0%	0%	0%	0%
	Ireland	Dublin	0%	0%	0%	0%	17%	11%	0%	0%	0%	0%	0%	0%	10%	0%	0%	0%	0%	5%	0%	42%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	13%	0%	0%	0%	0%	2%	0%
	Italy	Rome	0%	0%	2%	0%	1%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	1%	0%	7%	0%	0%	0%	0%	0%	72%	0%	0%	0%	0%	0%	13%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%
	Luxembourg	City	0%	0%	0%	12%	1%	1%	0%	0%	0%	1%	1%	4%	2%	0%	0%	0%	0%	0%	0%	9%	0%	0%	0%	0%	0%	31%	0%	4%	0%	7%	0%	17%	4%	0%	0%	0%	4%	0%	0%	0%	0%	2%	0%	0%	0%	1%	0%
	Netherlands	Utrecht	0%	0%	1%	7%	2%	1%	0%	0%	0%	0%	0%	1%	4%	0%	0%	0%	0%	3%	0%	11%	0%	0%	0%	0%	0%	19%	0%	10%	0%	0%	0%	4%	10%	0%	0%	0%	2%	0%	1%	17%	0%	4%	0%	1%	0%	1%	0%
	Northern Ireland	Belfast	0%	0%	0%	34%	0%	0%	0%	0%	0%	0%	1%	0%	1%	0%	0%	0%	0%	1%	0%	8%	0%	0%	0%	0%	0%	16%	0%	2%	0%	1%	1%	29%	4%	0%	1%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%
	Poland	Suwalki	0%	0%	0%	0%	8%	17%	0%	14%	0%	0%	0%	0%	15%	0%	0%	0%	0%	16%	0%	8%	0%	0%	2%	0%	0%	0%	0%	6%	0%	0%	0%	0%	0%	0%	6%	0%	0%	0%	5%	0%	0%	0%	0%	0%	0%	4%	0%
	Portugal	Lisbon	0%	0%	0%	15%	1%	2%	0%	4%	0%	0%	0%	1%	2%	0%	0%	2%	0%	1%	0%	2%	0%	0%	0%	0%	0%	59%	0%	3%	0%	1%	0%	8%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Romania	Bucharest	0%	0%	0%	55%	1%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	5%	0%	0%	0%	0%	0%	37%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%
	Russia	Moscow	0%	0%	0%	35%	1%	0%	0%	0%	0%	0%	0%	6%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	16%	0%	0%	0%	36%	0%	6%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Serbia	Belgrade	0%	0%	0%	41%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	44%	0%	1%	0%	2%	0%	6%	0%	0%	1%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Slovakia	Bratislava	0%	0%	0%	25%	3%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	27%	0%	0%	0%	0%	0%	30%	0%	0%	0%	7%	0%	0%	0%	0%	0%	0%	3%	0%	2%	0%	0%	0%	0%	1%	0%	0%	0%
	Slovenia	Ljubljana	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	7%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	25%	0%	65%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%
	Spain	Madrid	0%	0%	2%	12%	0%	1%	0%	0%	0%	0%	1%	0%	5%	0%	0%	0%	0%	0%	0%	16%	0%	0%	1%	0%	0%	16%	1%	7%	0%	1%	0%	1%	18%	0%	1%	0%	6%	0%	1%	0%	0%	7%	0%	1%	0%	1%	0%
	Sweden	Uppsala	0%	0%	2%	26%	2%	3%	0%	0%	1%	0%	3%	0%	7%	0%	0%	0%	0%	4%	0%	19%	0%	0%	1%	0%	0%	23%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	4%	0%	0%	0%	0%	0%	0%	1%	0%	4%	0%
	Switzerland	Basel	0%	0%	1%	21%	1%	0%	0%	0%	0%	0%	0%	0%	3%	0%	0%	0%	0%	1%	0%	11%	0%	0%	0%	0%	0%	56%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	4%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%
	UK	London	14%	0%	0%	45%	2%	0%	0%	2%	0%	1%	1%	0%	2%	0%	0%	0%	1%	7%	0%	8%	0%	0%	0%	0%	0%	11%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	1%	0%
	UK	York	0%	0%	3%	0%	0%	0%	0%	0%	0%	0%	1%	0%	1%	0%	1%	0%	0%	2%	0%	19%	0%	0%	1%	0%	0%	62%	0%	0%	0%	0%	0%	0%	0%	7%	1%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	UK	Glasgow	0%	0%	1%	6%	1%	1%	0%	0%	0%	1%	2%	0%	2%	0%	0%	0%	2%	2%	0%	8%	1%	0%	1%	0%	0%	24%	0%	0%	0%	0%	0%	16%	10%	1%	1%	0%	2%	0%	0%	6%	0%	9%	0%	1%	0%	1%	0%
	UK	Wales - River Dee	0%	0%	1%	19%	2%	3%	0%	0%	0%	6%	2%	1%	3%	0%	0%	0%	0%	6%	0%	15%	1%	0%	0%	0%	0%	16%	0%	0%	0%	0%	0%	20%	0%	0%	1%	0%	2%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%
	UK	Leeds	0%	0%	1%	10%	4%	3%	0%	0%	0%	0%	1%	0%	4%	0%	0%	0%	0%	9%	0%	17%	2%	0%	0%	0%	0%	32%	0%	0%	0%	0%	0%	4%	0%	1%	2%	0%	4%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%
	Ukraine	Kharkiv	1%	0%	3%	0%	8%	8%	0%	0%	0%	0%	5%	0%	0%	0%	0%	0%	0%	3%	0%	15%	2%	0%	13%	2%	0%	28%	0%	0%	0%	0%	0%	7%	0%	1%	3%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%
North America	Canada	Calgary	0%	0%	0%	79%	0%	1%	0%	0%	0%	0%	0%	1%	1%	0%	0%	0%	0%	0%	0%	1%	0%	0%	1%	0%	0%	0%	0%	7%	0%	4%	0%	3%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Canada	Toronto	0%	0%	1%	27%	1%	1%	0%	0%	0%	0%	2%	2%	0%	0%	0%	0%	0%	0%	0%	13%	0%	0%	1%	0%	0%	16%	0%	0%	0%	11%	0%	13%	0%	0%	0%	0%	4%	0%	1%	0%	0%	4%	0%	0%	0%	1%	0%
	Costa Rica	San Jose	0%	0%	2%	35%	0%	0%	0%	0%	0%	3%	0%	1%	1%	0%	0%	0%	0%	1%	0%	7%	0%	0%	0%	0%	0%	39%	0%	5%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	1%	0%	0%	0%
	Mexico	P. Vallarta	0%	0%	0%	0%	1%	0%	0%	1%	0%	0%	0%	2%	1%	0%	0%	0%	0%	1%	0%	23%	0%	0%	2%	0%	0%	28%	0%	0%	0%	14%	0%	20%	0%	0%	0%	0%	1%	0%	6%	0%	0%	0%	0%	0%	0%	0%	0%
	Panama	Panama City	0%	0%	1%	36%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	1%	0%	6%	0%	0%	0%	0%	0%	34%	0%	0%	0%	2%	0%	17%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%
	USA	Denver	0%	0%	1%	7%	1%	6%	0%	0%	1%	0%	0%	1%	7%	0%	0%	0%	0%	15%	0%	8%	0%	0%	2%	0%	0%	17%	0%	5%	0%	2%	0%	1%	0%	1%	0%	0%	2%	0%	2%	6%	0%	9%	1%	1%	0%	2%	0%
	USA	Iowa City	0%	0%	2%	0%	2%	14%	1%	2%	1%	0%	0%	0%	16%	0%	0%	2%	0%	15%	1%	7%	0%	0%	2%	0%	0%	12%	1%	2%	0%	0%	0%	0%	0%	1%	3%	0%	1%	0%	4%	0%	0%	4%	1%	0%	0%	6%	0%
	USA	Las Vegas	0%	0%	2%	0%	2%	13%	0%	1%	1%	0%	1%	0%	9%	0%	1%	0%	0%	0%	4%	6%	1%	0%	6%	0%	0%	27%	1%	4%	0%	0%	0%	0%	0%	0%	4%	0%	9%	0%	3%	2%	0%	0%	1%	1%	0%	2%	0%
	USA	Dallas	0%	0%	1%	0%	2%	11%	0%	0%	0%	0%	2%	0%	5%	0%	0%	0%	0%	26%	0%	9%	0%	0%	5%	0%	0%	15%	0%	6%	0%	0%	0%	0%	0%	0%	0%	0%	6%	0%	1%	1%	0%	5%	1%	2%	0%	1%	0%
	USA	Missouri - St Louis	0%	0%	0%	36%	0%	0%	0%	0%	0%	0%	12%	0%	0%	0%	0%	0%	0%	5%	0%	43%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	3%	0%	0%	0%	0%	0%	0%	0%	0%
	USA	Miami	0%	0%	0%	46%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	20%	0%	0%	1%	0%	0%	11%	0%	0%	0%	0%	0%	20%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%
	USA	New York City	0%	0%	1%	39%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	2%	0%	9%	0%	0%	1%	0%	0%	35%	0%	0%	0%	3%	0%	4%	0%	0%	0%	0%	3%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%
O	Australia	Sydney	0%	0%	0%	21%	7%	0%	0%	0%	0%	0%	0%	3%	2%	0%	0%	0%	0%	6%	0%	4%	0%	0%	1%	0%	0%	0%	0%	4%	0%	0%	0%	7%	0%	0%	0%	0%	0%	0%	0%	43%	0%	0%	0%	0%	0%	3%	0%

	Country	Sampling area	Amitriptyline	Artemisinin	Atenolol	Caffeine	Carbamazepine	Ceterizine	Cimetidine	Ciprofloxacin	Citalopram	Clarithromycin	Codeine	Cotinine	Desvenlafaxine	Diazepam	Diltiazem	Enrofloxacin	Erythromycin	Fexofenadine	Fluconazole	Gabapentin	Hydrocodone	Ketotifen	Lidocaine	Lincomyacin	Loratadine	Metformin	Metronidazole	Naproxen	Nevirapine	Nicotine	Noreistherone	Paracetamol	Pregabalin	Propranolol	Ranitidine	Salbutamol	Sitagliptin	Sulfadiazine	Sulfamethoxazole	Temazepam	Thiabendazole	Tramadol	Triamterene	Trimethoprim	Tylosin	Venlafaxine	Verapamil
	Australia	Melbourne	0%	0%	0%	0%	5%	0%	0%	0%	0%	0%	0%	0%	25%	0%	0%	0%	0%	15%	0%	0%	0%	0%	0%	0%	0%	13%	0%	0%	0%	38%	0%	0%	0%	0%	0%	0%	5%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Indonesia	Bandung	0%	0%	0%	64%	0%	0%	0%	0%	0%	0%	0%	4%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	15%	0%	0%	0%	3%	0%	11%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
South America	Antigua	St John's	0%	0%	1%	32%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	4%	0%	0%	0%	0%	0%	17%	0%	3%	1%	2%	0%	33%	2%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Argentina	Buenos Aires	0%	0%	1%	30%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	1%	0%	1%	0%	0%	0%	0%	0%	0%	0%	34%	0%	1%	0%	2%	0%	23%	2%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%
	Bolivia	La Paz	0%	0%	0%	5%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	1%	0%	23%	2%	1%	0%	0%	0%	59%	0%	0%	1%	0%	0%	0%	3%	0%	0%	0%	0%	1%	0%	0%	0%
	Brazil	Natal	0%	0%	1%	0%	2%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	89%	1%	1%	0%	0%	0%	0%	0%	0%	0%	2%	2%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Brazil	Americana	0%	0%	2%	46%	1%	0%	0%	0%	0%	0%	0%	1%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	38%	0%	0%	0%	6%	0%	3%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Chile	Santiago	0%	0%	3%	14%	6%	2%	0%	0%	0%	1%	0%	4%	0%	0%	0%	0%	0%	4%	0%	3%	0%	0%	2%	0%	0%	41%	4%	2%	0%	0%	0%	0%	7%	0%	0%	0%	1%	0%	1%	0%	0%	3%	0%	1%	0%	1%	0%
	Colombia	Cali	0%	0%	0%	26%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	27%	0%	8%	0%	1%	0%	35%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%
	Ecuador	Guayaquil	0%	0%	1%	19%	1%	1%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	3%	0%	0%	1%	0%	0%	30%	1%	3%	0%	1%	0%	30%	3%	0%	0%	0%	0%	0%	4%	0%	0%	0%	0%	1%	0%	0%	0%
	Peru	Huancayo	0%	0%	1%	45%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	3%	0%	0%	0%	0%	0%	21%	0%	0%	0%	2%	0%	26%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Uruguay	Montevideo	0%	0%	1%	32%	0%	0%	0%	0%	0%	3%	0%	1%	0%	0%	0%	0%	0%	1%	0%	2%	0%	0%	0%	0%	0%	33%	0%	0%	0%	3%	0%	21%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Table I2. Dataset used in the cluster analysis. All the measures are expressed PhAC concentration.

	Country	Sampling area	Total concentration	Amitriptyline	Artemisinin	Atenolol	Caffeine	Carbamazepine	Ceterizine	Cimetidine	Ciprofloxacin	Citalopram	Clarithromycin	Codeine	Cotinine	Desvenlafaxine	Diazepam	Diltiazem	Enrofloxacin	Erythromycin	Fexofenadine	Fluconazole	Gabapentin	Hydrocodone	Ketotifen	Lidocaine	Lincomyacin	Loratadine	Metformin	Metronidazole	Naproxen	Nevirapine	Nicotine	Noreistherone	Paracetamol	Pregabalin	Propranolol	Ranitidine	Salbutamol	Sitagliptin	Sulfadiazine	Sulfamethoxazole	Temazepam	Thiabendazole	Tramadol	Triamterene	Trimethoprim	Tylosin	Venlafaxine	Verapamil
Africa	Angola	Luanda	27184	0	0	128	2405	502	0	126	1568	5	0	0	453	0	0	3	0	0	0	0	368	0	0	46	0	0	5100	1733	0	187	463	0	11208	0	0	271	0	344	0	1991	0	0	0	0	285	0	0	0
	Burkina Faso	Ouagadougou	2571	0	0	27	1459	9	0	14	0	0	0	0	132	0	0	0	0	0	0	0	0	0	0	0	0	0	246	412	0	0	0	0	0	0	0	0	0	0	0	217	0	0	0	0	55	0	0	0
	Cameroon	Yaounde	12797	0	0	0	923	122	0	239	0	0	0	0	240	0	0	0	0	0	0	0	0	0	0	78	0	0	577	0	0	0	3766	0	4421	0	0	0	0	0	0	1483	0	0	877	0	72	0	0	0
	Democratic Republic of the Congo	Bukavu	47150	0	0	38	2442	73	0	425	0	0	0	0	338	0	0	0	0	684	0	0	0	0	0	26	0	0	5761	3150	0	301	1051	0	20953	0	0	0	0	0	0	10510	0	0	134	0	1265	0	0	0
	Democratic Republic of the Congo	Kinshasa	20891	0	0	0	2924	65	0	16	149	0	0	0	913	0	8	0	0	0	0	0	0	0	0	23	1286	0	792	0	0	0	1310	0	9417	0	0	34	0	871	0	2854	0	0	0	0	230	0	0	0
	Ethiopia	Addis Ababa	52364	0	0	125	35801	68	0	151	96	0	115	0	365	0	0	0	0	0	24	0	572	0	0	16	0	0	6921	830	0	240	710	0	5276	0	0	7	0	44	0	573	0	0	0	0	429	0	0	0
	Ghana	Accra	21564	0	0	70	2278	63	165	0	122	0	0	60	243	0	170	0	0	1594	11	0	156	0	0	51	0	0	2574	8835	639	0	151	0	182	0	0	0	0	0	0	3854	0	0	0	0	346	0	0	0
	Kenya	Nairobi	40317	102	0	127	0	244	339	51	78	0	278	91	0	0	29	0	0	772	19225	619	424	0	0	27	0	0	7626	734	84	0	21	0	0	0	918	28	54	78	0	6351	0	0	112	0	1904	0	0	0
	Lesotho	Maseru	3331	0	0	46	250	40	0	12	0	0	0	0	52	0	0	0	0	0	13	0	0	0	0	0	0	0	1732	0	0	136	142	0	181	0	0	0	0	0	0	691	0	0	0	0	37	0	0	0
	Liberia	Monrovia	754	0	0	0	0	2	0	15	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	78	0	0	0	134	0	407	0	0	0	0	0	0	81	0	0	0	0	18	0	0	0
	Mali	Bamako	5469	0	0	28	2954	16	0	66	0	0	0	31	172	0	0	0	0	0	0	0	0	0	0	20	98	0	558	0	0	0	424	0	404	0	0	0	0	25	0	439	0	0	121	0	113	0	0	0
	Morocco	Tangier - Tetuan	1050	0	0	0	44	28	20	0	0	0	0	0	45	0	0	0	0	0	7	0	84	0	0	5	0	0	744	0	0	0	0	0	0	0	0	0	0	0	0	65	0	0	0	0	8	0	0	0
	Nigeria	Lagos	25627	16	832	71	3173	102	23	234	145	0	50	67	167	0	6	0	0	236	18	0	0	0	0	27	191	0	2059	35	149	300	110	0	16243	0	0	0	0	29	0	1192	0	0	0	0	149	0	4	0
	Pretoria	Pretoria	12272	0	0	256	4932	180	79	30	0	29	0	341	100	127	0	0	0	0	136	0	247	24	0	16	0	0	3120	0	0	40	91	0	1509	0	0	0	0	0	0	830	0	0	0	0	185	0	0	0
	Republic of the Congo	Brazzaville	12797	0	0	22	1426	19	28	21	0	0	0	17	170	0	0	0	0	82	0	0	0	0	0	8	83	0	486	2814	0	173	1151	0	5472	0	0	0	0	0	0	643	0	0	0	0	181	0	0	0
	Rwanda	Kigali	783	0	0	0	513	0	0	5	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	80	0	0	0	0	0	0	103	0	0	0	0	13	0	0	0
	South Sudan	Juba	3247	0	0	0	1968	30	0	16	0	0	0	0	54	0	0	0	0	0	13	0	0	0	0	5	0	0	129	0	0	0	0	0	349	0	0	0	0	0	0	556	0	0	0	127	0	0	0	0
	Tanzania	Dar es Salaam	9762	0	0	10	0	13	65	5	48	0	0	0	0	0	0	0	0	0	0	238	0	0	0	0	0	0	0	73	0	0	165	0	8470	0	0	0	0	0	0	512	0	0	0	0	162	0	0	0
	Tanzania	Arusha	360	0	0	0	0	6	24	0	0	0	0	0	0	0	0	0	0	7	0	0	21	0	0	0	0	0	92	0	0	119	0	0	0	0	0	0	0	0	0	90	0	0	0	0	0	0	0	0
	Tunisia	Tunis	66593	18	0	430	8867	244	395	15	0	0	521	228	1294	205	0	43	0	97	29	0	476	0	0	28	0	0	16604	117	1455	0	2216	0	31247	1250	30	0	34	209	0	303	0	0	149	0	63	0	24	3
A	Armenia	Yerevan	36378	5	0	109	20524	33	48	0	0	0	190	0	678	23	0	3	0	0	20	0	995	0	0	58	0	0	7597	0	459	0	3507	0	1724	0	0	76	0	133	0	149	0	0	0	0	50	0	0	0

	Country	Sampling area	Total concentration	Amitriptyline	Artemisinin	Atenolol	Caffeine	Carbamazepine	Ceterizine	Cimetidine	Ciprofloxacin	Citalopram	Clarithromycin	Codeine	Cotinine	Desvenlafaxine	Diazepam	Diltiazem	Enrofloxacin	Erythromycin	Fexofenadine	Fluconazole	Gabapentin	Hydrocodone	Ketotifen	Lidocaine	Lincomyacin	Loratadine	Metformin	Metronidazole	Naproxen	Nevirapine	Nicotine	Noreistherone	Paracetamol	Pregabalin	Propranolol	Ranitidine	Salbutamol	Sitagliptin	Sulfadiazine	Sulfamethoxazole	Temazepam	Thiabendazole	Tramadol	Triamterene	Trimethoprim	Tylosin	Venlafaxine	Verapamil
	Bangladesh	Barisal	10052	7	0	0	0	15	68	0	30	0	0	0	0	0	26	0	0	0	0	37	0	0	0	146	0	0	950	7375	325	0	23	0	0	0	22	1029	0	0	0	0	0	0	0	0	0	0	0	0
	China	Panyu	15697	0	0	0	8910	9	0	408	0	0	0	0	1535	58	0	0	0	469	0	0	0	0	0	12	0	0	1292	26	0	0	1253	0	1473	0	0	67	0	59	0	106	0	0	0	0	21	0	0	0
	China	Guangzhou	2801	0	0	0	581	9	0	24	0	0	0	0	160	0	0	0	0	0	0	0	0	0	0	0	42	0	1857	0	0	0	56	0	0	0	0	0	0	30	0	23	0	0	0	0	19	0	0	0
	Georgia	Tbilisi	9088	0	0	16	3942	14	0	0	62	0	163	0	134	0	0	0	0	0	14	0	243	0	0	0	0	0	870	1553	0	0	1074	0	191	0	0	0	0	811	0	0	0	0	0	0	0	0	0	0
	Hong Kong	Lam Tsuen River	4231	0	0	46	1308	14	138	15	0	9	46	0	0	45	0	4	0	0	107	0	138	0	0	13	0	0	1157	0	0	0	144	0	881	0	0	0	0	147	0	0	0	0	0	0	3	0	17	0
	Hong Kong	Kai Tak River	7965	14	0	132	567	169	340	890	330	57	318	35	16	305	0	31	0	57	267	0	1088	92	0	90	0	41	1477	8	162	0	36	0	26	0	30	91	9	699	0	77	0	177	248	9	37	0	39	0
	India	Delhi	52173	0	0	589	16347	127	701	0	0	0	0	73	2957	66	0	0	0	68	439	0	774	0	0	133	0	0	20565	0	585	0	4016	0	2343	1095	16	79	88	434	0	586	0	0	0	0	93	0	0	0
	Iran	Rudbar	252	0	0	0	0	2	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	177	0	0	0	53	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Iraq	Baghdad	11157	0	0	40	0	18	0	5	125	0	36	0	0	0	0	0	0	0	4878	19	132	0	0	20	0	0	3086	77	0	0	216	0	2281	0	0	27	15	40	0	58	0	0	63	0	21	0	0	0
	Israel	Alexander River (whole country)	33127	12	0	1802	0	750	90	0	397	0	344	0	3796	33	0	6	0	0	935	0	2610	0	0	197	0	0	0	61	2294	0	1745	0	13972	1341	12	1085	0	300	0	532	0	0	0	0	162	644	0	4
	Kazakhstan	Astana	1356	0	0	0	467	8	0	0	0	0	0	0	44	0	0	0	0	0	0	0	56	0	0	0	0	0	535	0	0	0	108	0	73	0	0	0	0	0	0	62	0	0	0	0	4	0	0	0
	Malaysia	Kajang	14176	2	0	100	0	15	84	44	53	0	35	0	0	0	0	0	116	0	9746	26	152	0	0	9	0	0	3207	0	113	0	247	0	154	0	0	10	0	43	0	11	0	0	0	0	5	0	0	0
	Pakistan	Lahore	71501	9	0	629	13520	74	333	386	52	6	476	114	1453	32	5	6	14	97	215	0	335	0	0	237	869	0	16418	278	1816	0	2683	0	28571	599	27	0	67	534	0	1356	0	0	0	0	289	0	0	0
	Palestine	West Bank	23350	0	0	167	7649	1419	81	0	319	5	0	0	904	86	0	0	411	0	555	0	968	0	0	252	180	38	5442	0	998	0	866	0	2411	0	22	100	0	343	0	77	0	0	0	0	57	0	0	0
	Philippines	Manila	6908	0	0	68	3479	7	0	0	0	0	0	0	412	0	0	0	0	0	37	0	0	0	0	14	0	0	897	0	0	0	813	0	882	0	0	0	0	200	0	88	0	0	0	0	11	0	0	0
	South Korea	Seoul	4607	0	0	36	156	35	89	85	0	3	108	0	138	25	0	5	0	0	106	0	923	5	0	60	0	0	2280	0	66	12	0	0	0	145	0	13	0	194	0	17	0	0	95	0	11	0	0	0
	Sri Lanka	Colombo	4688	0	0	61	0	46	185	180	0	5	57	6	46	39	0	7	0	0	103	0	788	6	0	103	37	0	2511	0	0	0	0	0	77	0	11	53	0	332	0	11	0	0	0	0	17	0	9	0
	Taiwan	Taoyuan	1479	0	0	21	562	10	0	142	0	0	0	0	0	28	0	0	0	0	59	0	0	0	0	16	30	0	542	0	0	0	0	0	0	0	0	0	0	48	0	20	0	0	0	0	0	0	0	0
	Thailand	Bangkok	2992	0	0	49	265	32	49	22	0	0	0	0	56	0	4	0	0	0	25	0	121	0	0	0	0	0	441	0	0	0	233	0	1623	0	0	0	0	54	0	0	0	0	17	0	0	0	0	0
	Turkey	Saray	6615	0	0	8	1771	79	74	0	0	14	0	0	216	113	0	3	0	0	12	0	725	0	0	83	28	0	1630	0	421	0	103	0	657	404	0	0	0	58	0	52	0	0	112	0	5	0	47	0
Europe	Austria	Vienna	9048	10	0	323	366	134	193	45	59	126	512	101	77	284	0	12	0	130	142	0	1066	17	0	112	0	0	688	12	1006	0	113	0	0	1810	35	204	0	0	343	0	20	0	0	635	0	175	281	18
	Belgium	Brussels	11707	21	53	81	208	2277	2365	0	52	61	111	52	103	514	3	17	0	53	42	0	848	0	0	53	0	0	2546	0	251	0	164	0	0	428	112	326	0	0	142	0	87	126	0	458	0	19	136	0
	Belgium	Antwerp	4107	39	0	117	0	104	289	4	82	37	0	28	0	325	0	13	0	27	57	0	481	13	0	37	0	0	435	0	203	0	0	0	77	239	103	38	0	0	113	0	59	0	0	1113	0	21	54	0
	Bulgaria	Sofia	12822	0	0	14	3215	37	42	0	0	0	113	15	239	29	0	0	0	0	15	0	266	0	0	8	0	0	7956	0	68	0	279	0	383	0	0	61	0	17	0	42	0	0	0	0	23	0	0	0
	Croatia	Zagreb	1075	0	0	0	211	13	59	0	0	0	0	0	30	12	0	0	0	0	14	0	32	0	0	0	0	0	464	0	0	0	0	0	0	0	0	0	0	241	0	0	0	0	0	0	0	0	0	0
	Czech Republic	Prague	1503	1	3	7	0	28	37	0	24	0	40	0	0	41	0	0	0	0	0	11	214	0	0	4	0	0	991	0	0	0	25	0	20	0	0	0	0	23	0	13	0	0	0	0	0	11	12	0
	Denmark	Odense	1964	0	0	0	64	30	199	0	0	11	0	17	15	128	0	0	0	0	363	47	387	0	0	39	0	0	335	0	0	0	138	0	0	0	17	0	0	98	0	15	0	0	0	0	0	0	60	0
	Finland	Helsinki	1241	0	0	0	0	26	0	0	41	0	0	0	58	89	0	0	0	0	35	0	363	0	0	5	0	0	440	0	0	0	0	0	0	0	0	0	0	0	142	0	0	0	0	0	0	7	34	0
	France	Beaujeu	1337	0	0	14	293	2	0	0	0	0	0	0	0	0	0	0	59	0	0	0	36	0	0	0	216	0	216	0	0	0	43	0	458	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	France	Paris	970	0	0	0	0	25	0	0	0	0	0	0	0	82	0	6	0	0	0	0	149	0	0	15	0	0	373	0	0	0	99	0	76	0	11	0	0	88	0	27	0	0	0	0	0	0	18	0
	Germany	Berlin	1635	0	0	9	0	94	62	0	23	25	0	0	0	148	0	0	0	0	45	0	214	0	0	32	0	0	191	0	126	0	57	0	0	0	0	0	0	278	0	50	0	0	212	0	22	0	48	0
	Germany	Frankfurt	1898	0	0	0	339	84	100	0	0	0	0	0	22	89	0	0	0	0	24	0	380	0	0	11	0	0	565	0	0	0	0	0	0	0	0	0	0	186	0	28	21	0	0	0	3	0	46	0
	Germany	Tubingen	16257	724	0	525	1780	424	683	327	164	755	270	998	0	278	472	643	0	0	371	0	542	0	581	23	0	0	688	548	0	805	749	0	0	0	476	333	1259	251	0	269	0	0	269	807	242	0	0	0
	Greece	Oinofyta	847	0	0	0	211	7	9	0	0	0	0	0	11	20	0	0	0	0	0	0	33	0	0	21	0	0	343	0	104	0	0	0	66	0	0	0	0	21	0	0	0	0	0	0	0	0	0	0
	Hungary	Budapest	2020	0	0	11	217	116	38	0	0	5	0	0	21	0	0	0	0	0	52	0	348	0	0	36	0	0	686	0	0	0	95	0	122	97	0	17	0	78	0	28	0	0	43	0	10	0	0	0
	Ireland	Dublin	694	0	0	0	0	117	76	0	0	0	0	0	0	69	0	0	0	0	34	0	293	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	94	0	0	0	0	12	0
	Italy	Rome	691	0	0	15	0	10	0	0	0	0	0	0	0	10	0	0	0	0	10	0	51	0	0	0	0	0	500	0	0	0	0	0	92	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0
	Luxembourg	City	11709	0	0	49	1363	88	76	0	0	11	160	105	429	186	0	14	0	0	48	0	1022	0	0	24	0	0	3585	0	451	0	767	0	1944	516	23	29	23	447	0	29	0	0	201	0	15	0	103	0
	Netherlands	Utrecht	4238	19	0	56	285	88	61	0	0	20	0	0	52	156	0	5	0	0	140	0	445	0	0	0	0	0	818	0	405	0	0	0	154	407	16	0	0	89	0	23	733	0	180	0	28	0	56	0
	Northern Ireland	Belfast	5184	0	0	10	1745	16	18	0	0	3	0	38	23	40	0	2	0	0	40	0	427	0	0	3	0	0	822	0	102	0	35	36	1517	189	0	34	0	12	0	5	0	0	55	0	12	0	0	0
	Poland	Suwalki	1421	0	0	0	0	114	240	0	193	0	0	0	0	208	0	0	0	0	233	0	107	0	0	29	0	0	0	0	86	0	0	0	0	0	0	83	0	0	0	76	0	0	0	0	0	0	53	0
	Portugal	Lisbon	2823	0	7	9	427	15	45	0	99	0	0	0	23	45	0	0	56	0	15	0	53	0	0	0	0	0	1655	0	82	0	15	0	233	0	0	0	0	38	0	0	0	0	0	0	5	0	0	0
	Romania	Bucharest	2887	0	0	0	1578	18	0	0	0	0	0	0	40	0	0	0	0	0	0	0	152	0	0	5	0	0	1071	0	0	0	7	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0	0
	Russia	Moscow	1136	0	0	0	400	11	0	0	0	0	0	0	65	0	0	0	0	0	0	0	0	0	0	0	0	0	179	0	0	0	409	0	72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Serbia	Belgrade	2854	0	0	0	1176	11	11	0	0	0	0	0	34	14	0	0	0	0	0	0	54	0	0	0	0	0	1250	0	24	0	64	0	159	0	0	18	0	20	0	14	0	0	0	0	4	0	0	0
	Slovakia	Bratislava	946	0	0	0	239	30	10	0	0	3	0	0	0	0	0	0	0	0	0	0	256	0	0	0	0	0	284	0	0	0	67	0	0	0	0	0	0	33	0	19	0	0	0	0	5	0	0	0
	Slovenia	Ljubljana	237	0	0	0	0	3	0	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60	0	154	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
	Spain	Madrid	23846	18	0	549	2815	91	216	0	0	61	0	275	73	1278	6	56	0	0	84	0	3782	0	0	145	0	0	3828	207	1604	0	134	0	320	4291	34	258	0	1331	0	259	0	0	1775	0	131	0	212	13

	Country	Sampling area	Total concentration	Amitriptyline	Artemisinin	Atenolol	Caffeine	Carbamazepine	Ceterizine	Cimetidine	Ciprofloxacin	Citalopram	Clarithromycin	Codeine	Cotinine	Desvenlafaxine	Diazepam	Diltiazem	Enrofloxacin	Erythromycin	Fexofenadine	Fluconazole	Gabapentin	Hydrocodone	Ketotifen	Lidocaine	Lincomycin	Loratadine	Metformin	Metronidazole	Naproxen	Nevirapine	Nicotine	Noreistherone	Paracetamol	Pregabalin	Propranolol	Ranitidine	Salbutamol	Sitagliptin	Sulfadiazine	Sulfamethoxazole	Temazepam	Thiabendazole	Tramadol	Triamterene	Trimethoprim	Tylosin	Venlafaxine	Verapamil
	Sweden	Uppsala	533	0	0	12	138	9	15	0	0	4	0	14	0	39	0	0	0	0	23	0	101	0	0	6	0	0	122	0	0	0	0	0	0	0	0	0	0	22	0	0	0	0	0	0	5	0	23	0
	Switzerland	Basel	1076	0	0	11	227	13	0	0	0	0	0	0	0	28	0	0	0	0	14	0	119	0	0	0	0	0	603	0	0	0	0	0	0	0	0	0	0	46	0	0	0	0	0	0	4	0	12	0
	UK	London	5572	763	20	20	2506	99	0	0	113	13	39	55	17	137	0	5	0	28	367	0	438	16	0	21	0	0	586	0	139	0	0	0	0	0	13	19	0	0	97	0	0	0	0	0	0	18	40	0
	UK	York	1478	6	0	40	0	7	0	0	0	7	0	18	0	15	0	9	0	0	24	0	282	5	0	8	0	0	923	0	0	0	0	0	0	0	101	13	0	17	0	0	0	0	0	0	0	4	0	0
	UK	Glasgow	14134	15	0	195	900	102	170	44	0	30	84	296	54	228	5	15	0	305	317	0	1187	133	0	76	0	0	3428	33	0	0	0	0	2271	1421	74	85	42	213	0	55	867	0	1332	0	80	0	79	0
	UK	Wales - River Dee	1265	0	0	7	245	22	35	0	0	3	72	31	10	36	0	3	0	0	75	0	191	7	0	4	0	0	199	0	0	0	0	0	247	0	0	14	0	25	0	3	0	0	30	0	5	0	0	0
	UK	Leeds	2800	10	0	24	293	112	95	6	0	13	0	36	0	115	0	13	0	0	262	0	481	43	0	12	0	0	907	0	0	0	0	0	115	0	26	65	0	118	0	8	0	0	0	0	45	0	0	0
	Ukraine	Kharkiv	1510	16	0	45	0	116	121	0	0	0	0	81	0	0	0	0	0	0	41	0	231	34	0	191	27	0	420	0	0	0	0	0	101	0	12	40	0	0	0	0	0	0	0	0	34	0	0	0
North America	Canada	Calgary	6931	0	0	0	5483	20	42	0	0	4	0	30	77	66	4	3	0	0	11	0	90	0	0	43	0	0	0	0	487	0	289	26	177	0	0	0	0	51	0	0	0	0	0	0	9	0	17	0
	Canada	Toronto	2871	0	0	30	784	16	24	0	0	9	0	58	60	0	0	7	0	0	11	0	381	0	0	16	0	0	464	0	0	0	304	0	386	0	0	0	0	122	0	30	0	0	127	0	10	0	32	0
	Costa Rica	San Jose	27955	31	0	525	9733	91	116	74	0	6	857	0	200	288	0	0	0	0	278	0	2015	28	0	79	11	13	10958	0	1390	0	307	0	0	0	12	31	0	137	0	530	0	0	0	0	246	0	0	0
	Mexico	P. Vallarta	2313	0	0	0	0	29	0	0	26	0	0	0	36	23	0	0	0	0	12	0	522	0	0	37	0	0	650	0	0	0	321	0	470	0	0	0	0	24	0	142	0	0	0	0	10	0	10	0
	Panama	Panama City	4295	0	0	41	1563	5	0	0	0	0	0	0	27	0	0	0	0	0	23	0	259	0	0	9	0	0	1462	0	0	0	107	0	737	0	0	0	0	20	0	16	0	0	0	0	24	0	0	2
	USA	Denver	6530	17	0	58	429	67	404	0	0	37	0	31	33	489	0	30	0	18	999	0	500	12	0	151	0	0	1112	0	351	0	115	0	70	0	39	0	15	126	0	140	396	0	600	55	76	0	144	17
	USA	Iowa City	5269	3	0	86	0	118	728	37	117	55	0	0	0	850	0	17	103	0	780	59	354	6	0	124	0	0	627	28	90	0	0	2	0	0	42	174	0	59	0	201	0	0	223	40	21	0	325	0
	USA	Las Vegas	5253	8	0	92	0	120	696	22	62	46	0	30	0	455	4	31	0	0	0	210	292	41	0	296	0	0	1414	44	194	0	0	0	0	0	19	189	16	451	0	183	114	0	0	54	44	0	114	13
	USA	Dallas	10165	9	0	77	0	181	1113	0	0	47	0	159	35	508	0	30	0	0	2611	0	956	26	0	538	0	0	1510	28	640	0	48	0	0	0	33	0	20	577	0	115	83	0	459	68	164	0	127	3
	USA	Missouri - St Louis	241	0	0	0	87	0	0	0	0	0	0	29	0	0	0	0	0	0	11	0	104	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0
	USA	Miami	464	0	0	0	214	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	93	0	0	5	0	0	50	0	0	0	0	0	95	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
	USA	New York City	2575	0	0	13	995	5	0	0	0	3	0	0	16	0	0	2	0	0	55	0	230	0	0	20	0	0	905	0	0	0	82	0	104	0	0	0	0	79	0	57	0	0	0	0	8	0	0	0
Oceani	Australia	Sydney	649	0	0	0	135	47	0	0	0	2	0	0	19	10	0	0	0	0	38	0	26	0	0	4	0	0	0	0	24	0	0	0	46	0	0	0	0	0	0	0	279	0	0	0	0	0	19	0
	Australia	Melbourne	329	0	0	0	0	15	0	0	0	0	0	0	0	81	0	0	0	0	49	0	0	0	0	0	0	0	42	0	0	0	126	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0
	Indonesia	Bandung	8697	0	0	0	5564	6	33	0	0	0	0	0	390	0	0	0	0	0	0	0	208	0	0	24	0	0	1295	0	0	0	235	0	926	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0
South America	Antigua	St John's	14811	0	0	92	4811	17	25	0	0	0	0	0	287	0	0	0	0	0	35	0	658	0	0	18	0	0	2465	0	429	89	249	0	4945	344	41	0	0	233	0	36	0	0	32	0	7	0	0	0
	Argentina	Buenos Aires	17313	5	0	156	5131	74	33	0	0	4	0	0	421	50	0	4	87	35	121	0	0	0	0	59	0	0	5872	0	252	0	409	0	3916	260	0	52	0	53	0	247	0	0	0	0	60	0	10	0
	Bolivia	La Paz	108828	0	0	206	5932	219	137	0	0	0	586	160	217	0	0	0	0	0	106	0	2287	0	0	364	795	0	25371	1686	943	0	384	0	64371	0	0	630	0	28	0	3150	0	0	75	0	1180	0	0	0
	Brazil	Natal	2226	0	0	15	0	33	0	0	21	0	0	0	0	0	0	0	0	0	1973	27	23	0	0	0	0	0	0	0	50	52	0	0	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Brazil	Americana	3015	0	0	60	1376	27	0	0	0	0	0	0	40	0	31	0	0	0	13	0	0	0	0	31	0	0	1160	0	0	0	173	0	88	0	10	0	0	0	0	0	0	0	0	0	0	0	6	0
	Chile	Santiago	10515	11	0	299	1433	664	217	0	0	11	117	50	416	0	0	0	0	21	389	0	267	6	0	171	0	0	4309	368	244	0	0	0	39	784	19	11	0	62	0	106	0	0	301	17	105	0	78	0
	Colombia	Cali	3194	0	0	0	831	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	870	0	270	0	41	0	1126	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0
	Ecuador	Guayaquil	7064	0	0	46	1329	56	40	0	0	0	0	0	45	19	0	0	0	0	30	0	207	0	0	62	0	0	2147	61	243	0	71	0	2088	219	0	12	0	33	0	274	0	0	0	0	82	0	0	0
	Peru	Huancayo	7168	0	0	59	3260	6	0	0	0	0	0	0	25	0	0	0	0	0	23	0	198	0	0	13	0	0	1537	0	0	0	129	0	1853	0	0	12	0	21	0	15	0	0	0	0	15	0	0	2
	Uruguay	Montevideo	22831	0	0	220	7265	95	0	0	0	10	578	0	258	111	0	18	0	0	177	0	559	0	0	22	0	13	7648	0	0	0	699	0	4897	0	0	97	0	0	0	94	0	0	0	0	69	0	0	0

Table I3. Detailed description of the mixtures used in the experiment: ratio [%] of occurrence of each compound for each of the 7 treatments and PhAC concentration [ng L^{-1}] of each mixture (3 different exposure for treatment A, B, D, E and G, and 2 exposures for treatments C and F). The compounds occurring with the highest ratio for each of the 7 treatments are highlighted with shades of the corresponding color.

Mixture	Total concentration [ng/L]	Amitriptyline	Artemisinin	Atenolol	Caffeine	Carbamazepine	Ceterizine	Cimetidine	Ciprofloxacin	Citalopram	Clarithromycin	Codeine	Cotinine	Desvenlafaxine	Diazepam	Diltiazem	Enrofloxacin	Erythromycin	Fexofenadine	Fluconazole	Gabapentin	Hydrocodone	Lidocaine	Loratadine	Metformin	Metronidazole	Naproxen	Nevirapine	Nicotine	Paracetamol	Propranolol	Ranitidine	Salbutamol	Sitagliptin	Sulfamethoxazole	Thiabendazole	Tramadol	Triamterene	Trimethoprim
A-mid concentration ratio [%]	100%	0.0%	0.0%	2.1%	40.2%	1.5%	0.6%	0.2%	0.0%	0.2%	0.0%	2.8%	0.8%	1.0%	0.0%	0.0%	0.0%	0.0%	1.1%	0.0%	2.0%	0.2%	0.1%	0.0%	25.4%	0.0%	0.0%	0.3%	0.7%	12.3%	0.0%	0.0%	0.0%	0.0%	6.8%	0.0%	0.0%	0.0%	1.5%
A-mid	12272	0	0	256	4932	180	79	30		29	0	341	100	127	0	0	0	0	136	0	247	24	16	0	3120	0	0	40	91	1509	0	0	0	0	830	0	0	0	185
A-low	464	0	0	10	186	7	3	1	0	1	0	13	4	5	0	0	0	0	5	0	9	1	1	0	118	0	0	2	3	57	0	0	0	0	31	0	0	0	7
A-high	52365	0	0	1091	21046	769	338	128	0	124	0	1453	426	544	0	0	0	0	579	0	1054	101	68	0	13312	0	0	172	388	6437	0	0	0	0	3543	0	0	0	791
B-mid concentration ratio [%]	100%	0.2%	0.0%	1.7%	7.1%	2.1%	4.3%	11.2%	4.1%	0.7%	4.0%	0.4%	0.2%	3.8%	0.0%	0.4%	0.0%	0.7%	3.4%	0.0%	13.7%	1.2%	1.1%	0.5%	18.5%	0.1%	2.0%	0.0%	0.5%	0.3%	0.4%	1.1%	0.1%	8.8%	1.0%	2.2%	3.1%	0.1%	0.5%
B-mid	7965	14	0	132	567	169	340	890	330	57	318	35	16	305	0	31	0	57	267	0	1088	92	90	41	1477	8	162	0	36	26	30	91	9	699	77	177	248	9	37
B-low	329	1	0	5	23	7	14	37	14	2	13	1	1	13	0	1	0	2	11	0	45	4	4	2	61	0	7	0	1	1	1	4	0	29	3	7	10	0	2
B-high	23846	42	0	396	1696	507	1017	2663	987	172	951	104	49	913	0	92	0	172	800	0	3257	276	269	122	4423	25	486	0	108	77	89	272	28	2094	229	530	743	26	112
C-mid concentration ratio [%]	100%	0.1%	0.0%	0.0%	0.0%	0.2%	0.7%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%	0.0%	1.5%	0.0%	9.5%	73.4%	3.2%	0.0%	0.2%	0.0%	0.2%	10.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
C-mid	10052	7	0	0	0	15	68	0	30	0	0	0	0	0	26	0	0	0	0	37	0	0	146	0	950	7375	325	0	23	0	22	1029	0	0	0	0	0	0	0
C-high	21564	14	0	0	0	33	146	0	65	0	0	0	0	0	55	0	0	0	0	79	0	0	313	0	2038	15821	698	0	50	0	46	2207	0	0	0	0	0	0	0
D-mid concentration ratio [%]	100%	0.0%	0.0%	0.7%	0.0%	0.1%	0.6%	0.3%	0.4%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.8%	0.0%	68.8%	0.2%	1.1%	0.0%	0.1%	0.0%	22.6%	0.0%	0.8%	0.0%	1.7%	1.1%	0.0%	0.1%	0.0%	0.3%	0.1%	0.0%	0.0%	0.0%	0.0%
D-mid	14176	2	0	100	0	15	84	44	53	0	35	0	0	0	0	0	116	0	9746	26	152	0	9	0	3207	0	113	0	247	154	0	10	0	43	11	0	0	0	5
D-low	2226	0	0	16	0	2	13	7	8	0	6	0	0	0	0	0	18	0	1530	4	24	0	1	0	504	0	18	0	39	24	0	2	0	7	2	0	0	0	1
D-high	40317	7	0	285	0	43	238	124	150	0	100	0	0	0	0	0	331	0	27720	74	433	0	26	0	9122	0	322	0	703	439	0	29	0	124	30	0	0	0	15
E-mid concentration ratio [%]	100%	0.0%	0.0%	1.6%	8.9%	1.1%	1.6%	0.7%	0.0%	0.0%	0.0%	0.0%	1.9%	0.0%	0.1%	0.0%	0.0%	0.0%	0.8%	0.0%	4.0%	0.0%	0.0%	0.0%	14.7%	0.0%	0.0%	0.0%	7.8%	54.3%	0.0%	0.0%	0.0%	1.8%	0.0%	0.0%	0.6%	0.0%	0.0%
E-mid	2992	0	0	49	265	32	49	22	0	0	0	0	56	0	4	0	0	0	25	0	121	0	0	0	441	0	0	0	233	1623	0	0	0	54	0	0	17	0	0

Mixture	Total concentration [ng/L]	Amitriptyline	Artemisinin	Atenolol	Caffeine	Carbamazepine	Ceterizine	Cimetidine	Ciprofloxacin	Citalopram	Clarithromycin	Codeine	Cotinine	Desvenlafaxine	Diazepam	Diltiazem	Enrofloxacin	Erythromycin	Fexofenadine	Fluconazole	Gabapentin	Hydrocodone	Lidocaine	Loratadine	Metformin	Metronidazole	Naproxen	Nevirapine	Nicotine	Paracetamol	Propranolol	Ranitidine	Salbutamol	Sitagliptin	Sulfamethoxazole	Thiabendazole	Tramadol	Triamterene	Trimethoprim
E-low	237	0	0	4	21	3	4	2	0	0	0	0	4	0	0	0	0	0	2	0	10	0	0	0	35	0	0	0	18	129	0	0	0	4	0	0	1	0	0
E-high	108828	0	0	1787	9650	1176	1789	800	0	0	0	0	2023	0	163	0	0	0	913	0	4385	0	0	0	16027	0	0	0	8486	59042	0	0	0	1957	0	0	629	0	0
F-mid concentration ratio [%]	100%	0.0%	0.0%	0.0%	36.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	11.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	4.6%	0.0%	43.2%	0.0%	1.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.2%	0.0%	0.0%	0.0%	0.0%
F-mid	241	0	0	0	87	0	0	0	0	0	0	29	0	0	0	0	0	0	11	0	104	0	3	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0
F-high	694	0	0	0	250	0	0	0	0	0	0	82	0	0	0	0	0	0	32	0	300	0	8	0	0	0	0	0	0	0	0	0	0	0	23	0	0	0	0
G-mid concentration ratio [%]	100%	0.0%	0.2%	0.3%	15.1%	0.5%	1.6%	0.0%	3.5%	0.0%	0.0%	0.0%	0.8%	1.6%	0.0%	0.0%	2.0%	0.0%	0.5%	0.0%	1.9%	0.0%	0.0%	0.0%	58.6%	0.0%	2.9%	0.0%	0.5%	8.3%	0.0%	0.0%	0.0%	1.3%	0.0%	0.0%	0.0%	0.0%	0.2%
G-mid	2823	0	7	9	427	15	45	0	99	0	0	0	23	45	0	0	56	0	15	0	53	0	0	0	1655	0	82	0	15	233	0	0	0	38	0	0	0	0	5
G-low	252	0	1	1	38	1	4	0	9	0	0	0	2	4	0	0	5	0	1	0	5	0	0	0	148	0	7	0	1	21	0	0	0	3	0	0	0	0	0
G-high	12822	0	30	40	1939	70	207	0	449	0	0	0	104	207	0	0	253	0	69	0	241	0	0	0	7516	0	374	0	70	1060	0	0	0	172	0	0	0	0	22

Table I4. Plant growth results: days each plant needed to sprout (d).

Replicate #	Control	A			B			C		D			E			F		I		
		low	mid	high	low	mid	high	mid	high	low	mid	high	low	mid	high	mid	high	low	mid	high
1	2	2	2	2	2	2	4	2	2	2	2	2	2	3	2	2	3	2	2	2
2	2	2	2	2	2	2	2	2	3	2	2	2	2	2	2	2	2	2	2	2
3	2	2	2	3	2	2	3	2	2	2	2	2	2	2	2	2	2	2	2	2
4	2	2	2	2	2	2	2	2	2	3	2	2	3	2	2	3	2	2	2	5
5	2	2	2	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
average	2.0	2.0	2.0	2.4	2.0	2.0	2.6	2.0	2.2	2.2	2.0	2.0	2.2	2.2	2.0	2.2	2.2	2.0	2.0	2.6
st.dev	0.0	0.0	0.0	0.5	0.0	0.0	0.9	0.0	0.4	0.4	0.0	0.0	0.4	0.4	0.0	0.4	0.4	0.0	0.0	1.3
comparison between control and each mixture	-	0%	0%	+20%	0%	0%	+30%	0%	+10%	+10%	0%	0%	+10%	+10%	0%	+10%	+10%	0%	0%	+30%
comparison between control and each treatment	-	+6.7%			+10.0%			+5.0%		+3.3%			+6.7%			+10.0%		+10.0%		
one-way ANOVA, multiple comparison																				
overall p value	0.544																			
significance level (p) between control and each treatment		>0.999	>0.999	0.997	>0.999	>0.999	0.849	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.849
Significant ($p<0.05$)?		NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
two-way ANOVA, multiple comparison																				
significance level (p) between control and each treatment (grouped)		0.961			0.815			NA		0.998			0.961			NA		0.815		
Significant ($p<0.05$)?		NO			NO			NA		NO			NO			NA		NO		

Table I5. Plant growth results: days to see 4 leaves coming out (d).

Replicate #	Control	A			B			C		D			E			F		G		
		low	mid	high	low	mid	high	mid	high	low	mid	high	low	mid	high	mid	high	low	mid	high
1	11	11	12	12	12	12	12	13	12	12	11	13	12	11	11	12	12	10	13	12
2	12	11	13	13	12	12	13	11	12	12	10	15	12	11	11	10	12	11	12	12
3	10	13	13	13	10	12	14	12	11	12	13	12	12	12	12	13	14	12	13	12
4	10	12	12	12	13	13	11	12	12	12	12	14	12	12	14	13	12	12	10	13
5	11	11	12	12	13	13	13	11	13	11	11	13	13	13	11	13	12	12	12	12
average	10.8	11.6	12.4	12.4	12.0	12.4	12.6	11.8	12.0	11.8	11.4	13.4	12.2	11.8	11.8	12.2	12.4	11.4	12.0	12.2
st.dev	0.8	0.9	0.5	0.5	1.2	0.5	1.1	0.8	0.7	0.4	1.1	1.1	0.4	0.8	1.3	1.3	0.9	0.9	1.2	0.4
comparison between control and each mixture	-	+7.4%	+14.8%	+14.8%	+11.1%	+14.8%	+16.7%	+9.3%	+11.1%	+9.3%	+5.6%	+24.1%	+13.0%	+9.3%	+9.3%	+13.0%	+14.8%	+5.6%	+11.1%	+13.0%
comparison between control and each treatment	-	+12.3%			+14.2%			+10.2%		+13.0%			+10.5%			+13.9%		+9.9%		
one-way ANOVA, multiple comparison																				
overall p value		0.047																		
significance level (p) between control and each treatment		0.998	0.402	0.402	0.862	0.402	0.206	0.970	0.862	>0.999	>0.999	0.004	0.649	0.970	0.970	0.649	0.402	>0.999	0.862	0.649
Significant (p<0.05)?		NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	NO	NO	NO	NO	NO	NO	NO	NO
two-way ANOVA, multiple comparison																				
significance level (p) between control and each treatment (grouped)		0.002			<0.001			NA		<0.001			0.011			NA		0.021		
Significant (p<0.05)?		YES			YES			NA		YES			YES			NA		YES		

Table I6. Plant growth results: plant biomass weight (μg).

Replicate #	Control	A			B			C		D			E			F		G		
		low	mid	high	low	mid	high	mid	high	low	mid	high	low	mid	high	mid	high	low	mid	high
1	22.9	15.6	14.0	13.0	14.8	12.9	11.3	17.9	14.4	11.1	18.1	10.8	19.7	16.0	10.4	10.2	11.8	12.9	12.0	11.0
2	23.2	16.2	16.0	17.1	15.6	13.1	14.5	18.1	17.4	12.9	21.2	13.0	21.0	16.5	12.3	10.3	16.1	16.0	12.4	12.9
3	24.4	17.3	16.3	17.5	17.3	13.8	14.9	18.9	17.5	13.5	21.4	13.0	23.9	16.9	13.5	10.8	20.0	16.8	12.6	13.9
4	26.9	19.0	19.2	17.7	18.6	14.2	15.1	20.2	17.5	14.0	21.8	19.6	24.3	19.1	15.2	12.0	21.5	19.3	12.9	14.6
5	27.3	19.2	21.3	18.9	21.9	15.0	16.6	20.2	18.3	14.4	22.3	19.6	27.5	21.1	16.7	12.8	22.3	24.9	14.0	15.8
average	24.9	17.5	17.4	16.8	17.6	13.8	14.5	19.0	17.0	13.2	21.0	15.2	23.3	17.9	13.6	11.2	18.3	18.0	12.8	13.6
st.dev	2.1	1.6	2.9	2.2	2.8	0.8	1.9	1.1	1.5	1.3	1.7	4.1	3.1	2.1	2.4	1.1	4.4	4.5	0.7	1.8
comparison between control and each mixture	-	-29.9%	-30.4%	-32.5%	-29.3%	-44.7%	-41.9%	-23.7%	-31.8%	-47.2%	-16.0%	-39.2%	-6.7%	-28.2%	-45.4%	-55.1%	-26.5%	-27.9%	-48.8%	-45.4%
comparison between control and each treatment	-	-31.0%			-38.6%			-27.7%		-34.1%			-26.7%			-40.8%		-40.7%		
one-way ANOVA, multiple comparison																				
overall p value		<0.001																		
significance level (p) between control and each treatment		0.001	0.001	<0.001	0.002	<0.001	<0.001	0.035	<0.001	<0.001	0.543	<0.001	>0.999	0.003	<0.001	<0.001	0.009	0.004	<0.001	<0.001
Significant ($p<0.05$)?		YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	NO	YES	YES	YES	YES	YES	YES	YES
two-way ANOVA, multiple comparison																				
significance level (p) between control and each treatment (grouped)		<0.001			<0.001			NA		<0.001			<0.001			NA		<0.001		
Significant ($p<0.05$)?		YES			YES			NA		YES			YES			NA		YES		

Table I7. Plant growth results: plant root length (mm).

Replicate #	Control	A			B			C		D			E			F		G		
		low	mid	high	low	mid	high	mid	high	low	mid	high	low	mid	high	mid	high	low	mid	high
1	106	62	73	74	54	81	84	74	70	63	80	53	81	54	41	65	44	79	52	60
2	84	80	79	75	85	77	82	72	81	41	51	35	45	56	47	47	89	69	50	38
3	67	80	86	85	83	60	72	69	76	59	79	54	79	86	59	70	92	40	56	50
4	91	89	50	41	52	85	65	61	54	61	73	80	72	76	36	51	91	115	50	65
5	78	82	51	62	80	70	43	77	79	60	72	44	50	64	70	57	66	48	46	74
average	85.2	78.6	67.8	67.4	70.8	74.6	69.2	70.6	72.0	56.8	71.0	53.2	65.4	67.2	50.6	58.0	76.4	70.2	50.8	57.4
st.dev	14.6	10.0	16.5	16.9	16.4	9.9	16.5	6.1	10.9	9.0	11.7	16.8	16.8	13.6	13.8	9.5	21.1	29.5	3.6	13.9
comparison between control and each mixture	-	-7.7%	-20.4%	-20.9%	-16.9%	-12.4%	-18.8%	-17.1%	-15.5%	-33.3%	-16.7%	-37.6%	-23.2%	-21.1%	-40.6%	-31.9%	-10.3%	-17.6%	-40.4%	-32.6%
comparison between control and each treatment	-		-16.4%			-16.0%		-16.3%			-29.2%			-28.3%		-21.1%			-30.2%	
one-way ANOVA, multiple comparison																				
overall p value	0.017																			
significance level (p) between control and each treatment		>0.999	0.944	0.932	0.992	>0.999	0.975	0.990	0.997	0.249	0.993	0.103	0.925	0.846	0.049	0.320	>0.9999	0.987	0.052	0.284
Significant ($p<0.05$)?		NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	NO	NO	NO	NO	NO
two-way ANOVA, multiple comparison																				
significance level (p) between control and each treatment (grouped)			0.136			0.150		NA			<0.001			<0.001		NA			<0.001	
Significant ($p<0.05$)?			NO			NO		NA			YES			YES		NA			YES	

Table I8. Plant growth results: plant shoot height (mm).

Replicate #	Control	A			B			C		D			E			F		G		
		low	mid	high	low	mid	high	mid	high	low	mid	high	low	mid	high	mid	high	low	mid	high
1	44	53	41	46	49	26	37	54	48	47	48	33	32	58	47	42	37	44	38	46
2	45	46	50	46	49	40	40	53	50	44	51	37	46	47	50	46	47	44	41	42
3	53	42	40	43	42	50	36	50	51	43	41	51	44	50	51	45	40	44	44	51
4	48	42	47	52	52	42	43	53	43	42	47	45	44	43	39	40	46	50	45	43
5	50	48	49	52	45	49	38	50	39	44	54	39	41	36	49	40	36	50	45	42
average	48.0	46.2	45.4	47.8	47.4	41.4	38.8	52.0	46.2	44.0	48.2	41.0	41.4	46.8	47.2	42.6	41.2	46.4	42.6	44.8
st.dev	3.7	4.6	4.6	4.0	3.9	9.6	2.8	1.9	5.1	1.9	4.9	7.1	5.5	8.2	4.8	2.8	5.1	3.3	3.0	3.8
comparison between control and each mixture	-	-3.7%	-5.4%	-0.4%	-1.3%	-13.8%	-19.2%	8.3%	-3.7%	-8.3%	0.4%	-14.6%	-13.8%	-2.5%	-1.7%	-11.3%	-14.2%	-3.3%	-11.3%	-6.7%
comparison between control and each treatment	-		-3.2%			-11.4%		2.3%			-7.5%			-6.0%		-12.7%			-7.1%	
one-way ANOVA, multiple comparison																				
overall p value		0.008																		
significance level (p) between control and each treatment		>0.999	>0.999	>0.999	>0.999	0.835	0.280	0.999	>0.999	0.999	>0.999	0.760	0.835	>0.999	>0.999	0.968	0.799	>0.999	0.968	>0.999
Significant ($p<0.05$)?		NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
two-way ANOVA, multiple comparison																				
significance level (p) between control and each treatment (grouped)			0.959			0.041		NA			0.366			0.618		NA			0.431	
Significant ($p<0.05$)?			NO			YES		NA			NO			NO		NA			NO	

Table I9. Plant growth results: plant shoot height (mm).

Replicate #	Control	A			B			C		D			E			F		G		
		low	mid	high	low	mid	high	mid	high	low	mid	high	low	mid	high	mid	high	low	mid	high
1	44	53	41	46	49	26	37	54	48	47	48	33	32	58	47	42	37	44	38	46
2	45	46	50	46	49	40	40	53	50	44	51	37	46	47	50	46	47	44	41	42
3	53	42	40	43	42	50	36	50	51	43	41	51	44	50	51	45	40	44	44	51
4	48	42	47	52	52	42	43	53	43	42	47	45	44	43	39	40	46	50	45	43
5	50	48	49	52	45	49	38	50	39	44	54	39	41	36	49	40	36	50	45	42
average	48.0	46.2	45.4	47.8	47.4	41.4	38.8	52.0	46.2	44.0	48.2	41.0	41.4	46.8	47.2	42.6	41.2	46.4	42.6	44.8
st.dev	3.7	4.6	4.6	4.0	3.9	9.6	2.8	1.9	5.1	1.9	4.9	7.1	5.5	8.2	4.8	2.8	5.1	3.3	3.0	3.8
comparison between control and each mixture	-	-3.7%	-5.4%	-0.4%	-1.3%	-13.8%	-19.2%	8.3%	-3.7%	-8.3%	0.4%	-14.6%	-13.8%	-2.5%	-1.7%	-11.3%	-14.2%	-3.3%	-11.3%	-6.7%
comparison between control and each treatment	-	-3.2%			-11.4%			2.3%		-7.5%			-6.0%			-12.7%		-7.1%		
one-way ANOVA, multiple comparison																				
overall p value		0.002																		
significance level (p) between control and each treatment		>0.999	0.848	0.933	0.989	0.955	0.484	>0.999	0.989	0.1033	0.995	0.014	0.397	0.887	0.044	0.098	0.983	0.968	0.011	0.148
Significant ($p<0.05$)?		NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	NO	NO	YES	NO	NO	NO	YES	NO
two-way ANOVA, multiple comparison																				
significance level (p) between control and each treatment (grouped)		0.056			0.008			NA		<0.001			<0.001			NA		<0.001		
Significant ($p<0.05$)?		NO			YES			NA		YES			YES			NA		YES		

List of pubblications

With the exception of the results discussed in Chapter 7, all the findings reported in this PhD thesis have been published in scientific journals as follows:

1. Ghirardini, A., Verlicchi, P. 2019. A review of selected microcontaminants and microorganisms in land runoff and tile drainage in treated sludge-amended soils. *Science of the Total Environment*. 655, 939-957.

2. Verlicchi, P., Ghirardini, A. 2019. Occurrence of micropollutants in wastewater and evaluation of their removal efficiency in treatment trains: The influence of the adopted sampling mode. *Water (Switzerland)*. 11, 1152.

3. Ghirardini, A., Grillini, V., Verlicchi, P. 2020. A review of the occurrence of selected micropollutants and microorganisms in different raw and treated manure – Environmental risk due to antibiotics after application to soil. *Science of the Total Environment*. 707, 136118.

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Review

A review of selected microcontaminants and microorganisms in land runoff and tile drainage in treated sludge-amended soils

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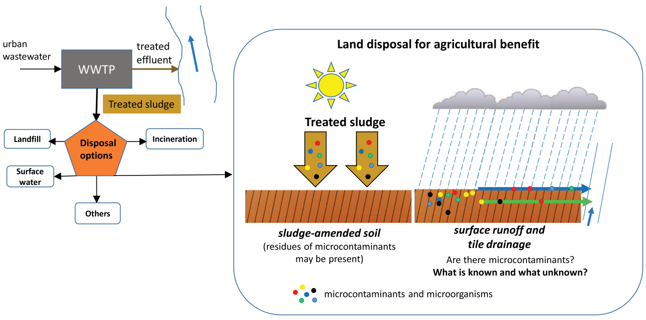
HIGHLIGHTS

- Runoff in sludge-amended soil is a source of pollutants in surface water.
- Occurrence of microcontaminants in sludge-amended soil runoff was reviewed.
- Ibuprofen, tonalide and gemfibrozil exhibited the highest concentrations.
- Occurrence in runoff was found lower than in secondary effluent and in surface water.
- Sludge application strategy greatly influences land runoff quality.

GRAPHICAL ABSTRACT



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ABSTRACT

The objective of this study is to provide a snapshot of the quality of surface runoff and tile drainage in sludge-amended soil in terms of 57 microcontaminants, including pharmaceuticals, hormones and fragrances, and 5 different species of bacteria. It also discusses the main factors affecting their occurrence (soil characteristics, applied sludge load and rate, sludge application method, rain intensity and frequency). It is based on 38 investigations carried out by different research groups in Canada, Australia, the USA and Ireland. The most frequently investigated compounds were hormones, the antiseptics triclosan and triclocarban, the analgesics and anti-inflammatories acetaminophen, ibuprofen and naproxen, the antibiotic sulfamethoxazole, the lipid regulator gemfibrozil and the psychiatric drug carbamazepine. Of all the bacteria, *E. coli* was the most monitored species. It was found that concentrations of the studied pollutants in surface runoff and tile drainage may vary, depending on many factors. They are generally lower than those observed in the secondary municipal effluent and in surface water, but their contribution to the deterioration of surface water quality might be relevant, mainly in wide rural areas. In this context, the reported data or their ranges represent an attempt to provide reference thresholds and bands of observed concentrations for a rough estimation of the contribution made by the release of the selected pollutants into surface water bodies via surface runoff and tile drainage.

Abbreviations: AOX, adsorbable organic halides; AWI, anthropogenic waste indicators; CEC, cation exchange capacity; dm, dry matter; DMB, dewatered municipal biosolids; D_{ow}, octanol-water distribution coefficient; FC, fecal coliforms; HM, heavy metal; K_{ow}, octanol-water partition coefficient; LAS, Linear Alkylbenzene; LMB, liquid municipal biosolids; LOD, limit of detection; LOQ, limit of quantification; MEC, measured environmental concentration; MW, molecular weight; PAH, polycyclic aromatic hydrocarbons; PCB, polychlorinated biphenyl; PCDD/F, dioxins and furans; pK_a, dissociation constant; PPCPs, pharmaceutical and personal care products; RL, reporting limit; SD, standard deviation; TC, total coliforms; u.o.m., unit of measure.

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Contents

1. Introduction

Intense rainfall events detach and transport fine and low-density particles in land runoff, which may contain different kinds of pollutants. In addition, rain infiltrating dry soils can also leachate natural and anthropogenic substances (contaminants) retained and accumulated in the soil matrix and convey them to other environmental compartments, including groundwater and surface water bodies.

Contaminant accumulation in/on soil can be due to different routes. The most important contributions come from the disposal of treated sludge (Torri and Cabrera, 2017), the disposal of manure (Segat et al., 2015), land irrigation with reclaimed wastewater (Dodgen and Zheng, 2016; Pedersen et al., 2003, 2005; Xu et al., 2009), and land irrigation with surface water containing these contaminants (Calderón-Preciado et al., 2011).

Land disposal of treated municipal sludges is a common practice in many countries worldwide (Inglezakis et al., 2014; Kelessidis and Stasinakis, 2012). In the following the term *biosolids* will be used as an alternative of treated sludges: the term biosolids was introduced in 1991 in the USA by the Water Environment Federation (WEF, 2005) to distinguish raw, untreated sewage sludge from treated and tested sewage sludge, which could legally be used for agricultural benefits.

This method of disposal can positively contribute to the improvement of soil properties and fertility (Clarke and Smith, 2011) due to the presence of nutrients and other substances in the sludges able to improve soil porosity or permeability (amendant effects), to favor aggregation of the main soil constituents (conditioning effects), to change chemical and physical soil properties (corrective effects) and/or to provide elements in assimilable or available form for plants (fertilizing effects).

Sewage sludge not only provides soil with organic matter, but it also increases infiltration, reduces the possibility of soil erosion (Lucid et al., 2014) and increases agronomic productivity (Samaras et al., 2008; Tsadilas et al., 2005; Zartman et al., 2012). Its use for agricultural benefits also addresses European Union policy on sustainability and the recycling of resources (COM, 2014).

In the case of intense rain events, surface runoff could seriously impact on near surface water bodies and affect their quality in terms of suspended solids, nutrients, and bacteria, as well as other emerging contaminants.

In addition, in those regions where natural field drainage can adversely affect crop production activities, it may interfere with the groundwater if the groundwater system is near the surface on a year round basis, as could be the case in any of the poorly drained soils in eastern Ontario (Lapen et al., 2008a,b, 2018). There, tile drains are placed on agricultural areas within 1 m of the soil surface in order to collect draining water and short-circuit it to the adjacent surface water streams or rivers. This stream is then conveyed to a surface water body.

In the last few years, issues related to land runoff as well as tile drainage quali-quantitative characteristics have caused increasing concern. For the most part, attention has been paid to macro-pollutants (suspended solids, organic substances, nitrogen and phosphorus compounds) (Paule et al., 2014), heavy metals (Hosseini Koupaie and

Eskicioglu, 2015), and pesticides (Torri and Cabrera, 2017). Some investigations have strongly focused on the environmental risks posed by the presence of nutrients and heavy metals due to the land application of treated sludge, as well as manure (Eldridge et al., 2009; Peyton et al., 2016; Jia et al., 2015; Bai et al., 2016). Some studies have also addressed mobilization in the runoff of bacteria applied to soil with the treated sludge. It has been highlighted that the soil environment is hostile for their development and that their survival time, following land application, is around 2–4 months (Brennan et al., 2012). As a consequence, bacteria are more likely to be transported into receiving water courses after rainfall events.

Recent studies have highlighted the occurrence of microcontaminants, mainly pharmaceuticals and personal care products (PPCPs) in treated sludges and underlined the importance of more thorough investigations into the fate of these pollutants once spread on soil (Verlicchi and Zambello, 2015). In this way, contaminants could reach surface water bodies and in some cases they could pose a potentially acute and chronic risk for aquatic life (Clarke and Cummins, 2015), or deteriorate the quality of freshwater reserves used for potable needs (Clarke et al., 2016).

From a legislative view point, different scenarios exist. The reuse of biosolids is not allowed in Belgium, Romania and Switzerland (Healy et al., 2017). In other countries it is possible (many European countries, New South Wales-Australia, Ontario-Canada, and the USA) and the current legal requirements regarding sludge disposal on soil commonly concern maximum sludge concentrations and/or maximum sludge loads for organic matter and nutrients, heavy metals, selected pesticides, and organic microcontaminants such as AOX, PCB, PCDD/F, as well as chemical-physical characteristics of the receiving soil, and the maximum quantity of sludge to be disposed on soil on a yearly basis (Mininni et al., 2015; Kelessidis and Stasinakis, 2012; Le Blanc et al., 2008).

To date, no legal requirement has been set concerning the maximum permitted concentrations or loads for PPCPs (Kelessidis and Stasinakis, 2012; Lu et al., 2012; Le Blanc et al., 2008). With regard to microorganisms, only a few States have standards regarding the maximum concentrations in sewage sludge to be disposed of on soil, and these mainly concern *Salmonella* and *E. coli* (Lu et al., 2012; Mininni et al., 2015).

As reported above, reclaimed water reuse for irrigation purposes may also contribute to the introduction of residues of microcontaminants of emerging concern into the soil (Kinney et al., 2006; Martínez-Piernas et al., 2018). The benefits due to the reduced demand for fresh water and the supply of nutrients (occurring in reclaimed water) are counterbalanced by the potential contamination risk to water and plants by still unregulated pollutants (Christou et al., 2017; Wu et al., 2015). This reuse practice is of great interest, principally for regions characterized by water scarcity (among them Spain, Cyprus, Libya, and Jordan) and/or frequent periods of drought (Morocco, Algeria, and Tunisia). The contribution may be relevant but also limited to specific case studies.

This review aims to provide a snapshot of the chemical characteristics of surface runoff and also the leachate in sludge-amended soil with regard to investigated PPCPs and bacteria species. A further objective is to investigate the main factors affecting them (compound properties, soil characteristics, applied sludge load and flow rate, sludge application method, rain intensity and frequency). The idea is to provide reliable data on the quality of surface runoff and tile drainage leading to an assessment of the potential contribution of these streams to the quality of the surface water body during intense rain events. This review also underlines the strengths and weaknesses of available studies, the gaps in current knowledge and the research fields requiring further investigation. The reported data or their ranges could represent reference thresholds or bands of observed concentrations for a rough estimation of the contribution made by the release of the selected pollutants into surface water bodies via surface runoff.

1.1. Framework of the study

This study provides an overview of chemical characteristics in terms of concentrations of a selection of microcontaminants and microorganisms (Table 1) in the water streams (surface runoff and tile drainage) which, due to rain events, leave agricultural soils where treated sludge (=biosolids) has previously been applied. The review is based on a collection of 16 papers, published between 1980 and 2017, referring to 38 investigations into the occurrence of 57 PPCPs and 5 species of microorganisms (*E. coli*, Fecal coliform, Total coliform, Fecal streptococcus, and *Clostridium perfringens*), in land runoff or in tile drainage after the disposal of treated municipal sludge onto soil.

Selected investigations differ in at least one of the following issues: (i) soil type, (ii) municipal sludge type (depending on the treatment it was subjected to), (iii) sludge application method, (iv) sludge application rate, (v) investigated water stream (runoff or tile drainage), and (vi) rainfall frequency pattern. One study may include more than one investigation.

The 38 investigations were carried out in Ireland (8), the USA (15), Canada (11) and Australia (4) and most of the research groups belong to agricultural research centers. Investigations into land runoff in the case of manure applied on soil were not included, since manure disposal on rural land is subject to specific regulations from country to country and many types of manures are available depending on the animals (cattle, pigs, chickens, sheep, etc.). Table 2 reports the main characteristics of the studies included in the review with the number of investigations specified for each study. Their aims and scope and the principal issues addressed are also underlined.

Fig. S1 shows how these studies are temporally and spatially correlated and whether they include common research groups; Table S1 details the main characteristics of the reviewed 38 investigations with regard to field/plots, soil, sludge, rainfall, sampling strategy and the main findings. Investigations referring to the spiking of soil with specific microcontaminants (among them Davis et al., 2006) were not included, as according to Al-Rajab et al., 2009, the effect of the presence of treated sludge (the matrix containing microcontaminants) strongly influences the fate and behaviour of such contaminants in the soil.

Bearing in mind the definition sets in chemical engineering manuals, leachate is the liquid stream obtained from a leaching process, that is a unit operation consisting of a mass (and energy) transport from a solid phase (the soil) to a liquid one (the water leaving the soil) when they come into contact. With regard to Fig. 1, once the rain starts, dry soil begins to retain water within its macro- and micro-pores. Water tends to percolate (generating the *leachate* or *percolate*) and in the case of prolonged rain events, when the soil becomes saturated, the water starts moving (flowing) on the soil surface, according to its slope, generating the so-called *surface runoff*. Sludge retained on the land surface is also subject to light exposure and photodegradation processes may occur, changing the characteristics of the sludge.

With regard to percolation of the water through the soil, if this stream is intercepted by pipes (drains), the water flow which spills out is called *tile drainage*. Fig. 1 shows the different water flow paths on soil in the case of precipitation and it also shows the potential degradation/removal mechanisms pollutants and microorganisms occurring in the soil may undergo. Fig. 1 is the conceptual scheme this study will refer to. In particular, it will investigate surface runoff and tile drainage characteristics in treated municipal sludge-amended soil: the two streams which can rapidly reach the receiving water stream, affecting its quality.

Section 2.2 deals with sludge application on soil in terms of sludge types based on the treatment before application, sludge application methods (compared in Table 3), the maximum loads allowed by the different regulations, and a brief overview of the legal requirements (standards for specific pollutants and in particular for microorganisms, Section 2.2.3, with further details provided in Tables S3 and S4).

Table 1
Pharmaceutical compounds, hormones, fragrances and microorganisms included in this study.

Therapeutic class	Pharmaceutical compound/species	MW	Chemical formula	pK_a	LogK_{ow}	# papers	Reference
Analgesics/anti-inflammatories (A) (3)	Acetaminophen	151.2	$C_8H_9NO_2$	9.38	0.46–0.49	5	Edwards et al., 2009; Gottschall et al., 2012; Lapen et al., 2008b; Sabourin et al., 2009; Topp et al., 2008b
	Ibuprofen	206.3	$C_{13}H_{18}O_2$	4.94	3.97	5	Edwards et al., 2009; Gottschall et al., 2012; Lapen et al., 2008b; Sabourin et al., 2009; Topp et al., 2008b
	Naproxen	230.3	$C_{14}H_{14}O_3$	4.15	3.18	5	Edwards et al., 2009; Gottschall et al., 2012; Lapen et al., 2008b; Sabourin et al., 2009; Topp et al., 2008b
Antibiotics (B) (7)	4-Epitetracycline	444.4	$C_{22}H_{24}N_2O_8$	3.3	−1.37	1	Gottschall et al., 2012
	Ciprofloxacin	331.3	$C_{17}H_{18}FN_3O_3$	6.09	0.28	1	Gottschall et al., 2012
	Ofloxacin	3614.	$C_{18}H_{20}FN_3O_4$	pK_{a1}: 5.97 pK_{a2}: 9.28	−0.39	1	Gottschall et al., 2012
	Oxytetracycline	460.4	$C_{22}H_{24}N_2O_9$	3.27	−0.9	1	Gottschall et al., 2012
	Sulfamethoxazole	253.3	$C_{10}H_{11}N_3O_3S$	pK_{a1}: 1.60 pK_{a2}: 5.70	0.89	5	Edwards et al., 2009; Lapen et al., 2008b; Pedersen et al., 2005; Sabourin et al., 2009; Topp et al., 2008b
	Sulfapyridine	249.3	$C_{11}H_{11}N_3O_2S$	8.43	0.35–0.9	1	Lapen et al., 2008b;
	Tetracycline	444.4	$C_{22}H_{24}N_2O_8$	3.3	−1.37	1	Gottschall et al., 2012
Antifungals (C) (1)	Miconazole	416.1	$C_{18}H_{14}Cl_4N_2O$		6.1	1	Gottschall et al., 2012
Antihistamines (D) (1)	Diphenhydramine	255.4	$C_{17}H_{21}NO$	8.98	3.27	1	Gottschall et al., 2012
Antiseptics (E) (2)	Triclocarban	345.6	$C_{13}H_9Cl_3N_2O$	12.7	4.9	5	Edwards et al., 2009; Giudice and Young, 2011; Gottschall et al., 2012; Healy et al., 2017; Sabourin et al., 2009
	Triclosan	289.5	$C_{12}H_7Cl_3O_2$	7.9	4.76	8	Edwards et al., 2009; Giudice and Young, 2011; Gottschall et al., 2012; Gray et al., 2017; Healy et al., 2017; Lapen et al., 2008b; Sabourin et al., 2009; Topp et al., 2008b
Beta-blockers (F) (1)	Atenolol	266.3	$C_{14}H_{22}N_2O_3$	9.6	0.16	4	Edwards et al., 2009; Lapen et al., 2008b; Sabourin et al., 2009; Topp et al., 2008b
Fragrances and PCPs ingredients (G) (5)	Galaxolide (HHCB)	258.4	$C_{18}H_{26}O$		5.9	1	Gray et al., 2017
	Indole	117.2	C_8H_7N	−2.4	2.14	1	Gray et al., 2017
	Menthol	156.3	$C_{10}H_{20}O$		3.2	1	Gray et al., 2017
	Skatole (3-menthyl-1*H*-indole)	131.2	C_9H_9N		2.6	1	Gray et al., 2017
	Tonalide (AHTN)	258.4	$C_{18}H_{26}O$		5.7	1	Gray et al., 2017
Hormones (H) (22)	11-Ketotestosterone	302.4	$C_{19}H_{26}O_3$		1.92	1	Yang et al., 2012
	17-α-Estradiol (Alfatradiol)	272.4	$C_{18}H_{24}O_2$		3.94–4.01	2	Gottschall et al., 2013; Yang et al., 2012
	17-Beta-estradiol (or estradiol)	272.4	$C_{18}H_{24}O_2$		3.94–4.01	2	Gottschall et al., 2013; Yang et al., 2012
	α-Dihydroequilin	270.4	$C_{18}H_{22}O_2$			1	Gottschall et al., 2013
	Androstenedione	286.4	$C_{19}H_{26}O_2$		2.75–2.76	2	Gottschall et al., 2013; Yang et al., 2012;
	Androsterone	290.4	$C_{19}H_{30}O_2$		3.69	1	Gottschall et al., 2013
	cis-Androsterone	290.4	$C_{19}H_{30}O_2$		3.07–3.69	1	Yang et al., 2012
	Desogestrel	310.5	$C_{22}H_{30}O$		5.65	1	Gottschall et al., 2013
	Diethylstilbestrol	268.4	$C_{18}H_{20}O_2$		5.07–5.64	1	Yang et al., 2012
	Dihydrotestosterone (Stanolone)	290.4	$C_{19}H_{30}O_2$		3.07–3.55	1	Yang et al., 2012
	Epitestosterone	288.4	$C_{19}H_{28}O_2$		3.27–3.32	1	Yang et al., 2012
	Equilenin	266.3	$C_{18}H_{18}O_2$		3.93	2	Gottschall et al., 2013; Yang et al., 2012
	Equilin	268.4	$C_{18}H_{20}O_2$		3.35	2	Gottschall et al., 2013; Yang et al., 2012
	Estradiol benzoate	376.5	$C_{25}H_{28}O_3$			1	Gottschall et al., 2013
	Estriol	288.4	$C_{18}H_{24}O_3$	10.54	2.45–3.67	2	Gottschall et al., 2013; Yang et al., 2012
	Estrone	270.4	$C_{18}H_{22}O_2$		3.13–3.43	2	Gottschall et al., 2013; Yang et al., 2012
	Ethinyl estradiol	296.4	$C_{20}H_{24}O_2$	10.4	3.67–4.15	3	Giudice and Young, 2011; Gottschall et al., 2013; Yang et al., 2012
	Mestranol	310.4	$C_{21}H_{26}O_2$		4.61–4.68	2	Gottschall et al., 2013; Yang et al., 2012
	Norethindrone	298.4	$C_{20}H_{26}O_2$		2.97–2.99	2	Gottschall et al., 2013; Yang et al., 2012
	Norgestrel (Levonorgestrel)	312.5	$C_{21}H_{28}O_2$		3.48	1	Gottschall et al., 2013
	Progesterone	314.5	$C_{21}H_{30}O_2$		3.67–3.87	2	Gottschall et al., 2013; Yang et al., 2012
	Testosterone	288.4	$C_{19}H_{28}O_2$		3.27–3.32	2	Gottschall et al., 2013; Yang et al., 2012
Lipid regulators (I) (1)	Gemfibrozil	250.3	$C_{15}H_{22}O_3$	4.5	4.77	5	Edwards et al., 2009; Gottschall et al., 2012; Lapen et al., 2008b; Sabourin et al., 2009; Topp et al., 2008b
Stimulant (J) (2)	Caffeine	194	$C_8H_{10}N_4O_2$	10.4	−0.07	1	Sabourin et al., 2009
	Cotinine	176.2	$C_{10}H_{12}N_2O$		0.07	5	Edwards et al., 2009; Gottschall et al., 2012; Lapen et al., 2008b; Sabourin et al., 2009; Topp et al., 2008b
Psychiatric drugs (K) (12)	Bupropion	239.7	$C_{13}H_{18}ClNO$	8.22	3.85	1	Gottschall et al., 2012
	Carbamazepine	236.3	$C_{15}H_{12}N_2O$	13.9	2.45	5	Edwards et al., 2009; Gottschall et al., 2012; Lapen et al., 2008b; Sabourin et al., 2009; Topp et al., 2008b
	Citalopram	324.4	$C_{20}H_{21}FN_2O$		3.5	1	Gottschall et al., 2012
	Desmethyl citalopram	310.4	$C_{19}H_{19}FN_2O$		2.8	1	Gottschall et al., 2012;
	Desmethyl sertraline	320.2	$C_{17}H_{15}Cl_2NO$		4.5	1	Gottschall et al., 2012
	Desvenlafaxine	263.4	$C_{16}H_{25}NO_2$	pK_{a1}: 9.45 pK_{a2}: 10.66	2.72	1	Gottschall et al., 2012
	Fluoxetine	309.3	$C_{17}H_{18}F_3NO$	9.5	4.05	3	Edwards et al., 2009; Gottschall et al., 2012; Lapen et al., 2008b
	Norfluoxetine	295	$C_{16}H_{16}F_3NO$		3.5	1	Gottschall et al., 2012
	Norvenlafaxine	263.4	$C_{16}H_{25}NO_2$		3	1	Gottschall et al., 2012

Table 1 (*continued*)

Therapeutic class	Pharmaceutical compound/species	MW	Chemical formula	pK_a	LogK_{ow}	# papers	Reference
	Paroxetine	329.4	$C_{19}H_{20}FNO_3$	9.6	1.23	1	Gottschall et al., 2012
	Sertraline	30.,2	$C_{17}H_{17}Cl_2N$		5.1	1	Gottschall et al., 2012
	Venlafaxine	277.4	$C_{17}H_{27}NO_2$	10.09	3.2	1	Gottschall et al., 2012

Group	Species	# papers	Ref
Bacteria (5)	*Clostridium perfringens*	2	Gottschall et al., 2013; Lapen et al., 2008a
	Escherichia coli	5	Atalay et al., 2007; Eldridge et al., 2009; Gottschall et al., 2013; Lapen et al., 2008a; Peyton et al., 2016
	Fecal coliforms	2	Dunigan and Dick, 1980; Wallace et al., 2014
	Fecal streptococcus	1	Gottschall et al., 2013
	Total coliforms	3	Atalay et al., 2007; Gottschall et al., 2013; Peyton et al., 2016

This study goes on to present the characteristics of the soil which can affect the runoff/tile drainage quality, the size of the plots and the characteristics of the rainfall events of the investigations. Special attention was also paid to data reliability and accuracy (Section 2.5), and an analysis of the reviewed studies can be found in Table S1. The results are reported in graphs which show measured concentrations of selected PPCPs and bacteria in surface runoff and tile drainage as well as background concentrations in the absence of sludge application (when available). In the Supplementary materials section, details are available in terms of a descriptive statistical analysis of the concentrations observed in surface runoff (Table S5) and tile drainage (Table S6).

Discussion of the results focuses mainly on the influence of the factors affecting runoff/tile drainage concentrations:

- compound characteristics;
- soil characteristics (matrix, pH, organic matter, organic carbon, cationic exchange capacity);
- sludge properties (CEC, moisture, pH, chemical composition) and its application rate;
- applied pollutant load;
- application method;
- application depth;
- applied water volume.

Moreover, discussion of the collected results also refers to the ranges of measured concentrations of selected contaminants in secondary effluents, anaerobically digested sludges, runoff of rural soil irrigated with reclaimed water and surface water. The literature ranges to which the comparison refers are reported in Tables S2 and S5.

The study concludes with a list of the lessons learned from past investigations, the main gaps in the investigations and the issues requiring further study.

2. Materials and methods

2.1. Compounds included in the review and main investigations

Investigated chemical compounds belong to 11 different therapeutic classes or groups: analgesics and anti-inflammatories (class A including 3 compounds); antibiotics (class B with 7 compounds); antifungals (class C, 1 compound); antihistamines (class D, 1 compound); antiseptics (class E, 2 compounds); beta-blockers (class F, 1 compound); fragrances (class G, 5 compounds), hormones (class H, 22 compounds), lipid regulators (class I, 1 compound); stimulants (class J, 2 compounds) and psychiatric drugs (class K, 12 compounds).

Table 1 compiles all of these with the main chemical characteristics (molecular weight MW, chemical formula, pK_a, LogK_{ow}), which are useful for analyzing or predicting their fate/behaviour once on the soil following sludge disposal, the number of related studies and corresponding references are also provided.

The last group refers to bacteria commonly monitored in municipal wastewater treatments: *Clostridium perfringens*, *Escherichia coli*, Fecal coliforms, Fecal streptococci and Total coliforms.

2.2. Sludge application on soil – sludge types, application methods, authorized loads, and legal requirements

2.2.1. Sludge types

Based on the studies under review, it was found that sludge intended for land disposal was generally anaerobically digested and, in many cases, dewatered. In a few cases it was subjected to further treatments: high temperature drying (Eldridge et al., 2009; Giudice and Young, 2011; Healy et al., 2017; Peyton et al., 2016), gamma irradiation (Eldridge et al., 2009), centrifugal dewatering (Gottschall et al., 2012) or lime stabilization (Healy et al., 2017; Peyton et al., 2016). With regard to the selected micro-contaminants and bacteria, the concentrations in the applied sludge were included in the ranges reported in Table S2, even in the case of sludges spiked with micro-contaminants, with just two exceptions referring to acetaminophen and ibuprofen (in both cases final concentration was higher than the maximum literature value).

According to Sabourin et al. (2009) it was assumed that the sludge is considered liquid (often called liquid municipal biosolids LMB) if its solid content is <18%, and dewatered (dewatered municipal biosolid DMB) if its solid content is higher than 18%. In the different investigations, the sludge had a largely different solid content; on the basis of 11 of the 16 studies which reported sludge composition in detail, the solid content varied between <18% and 91.6% (granulated high temperature dried sludge in Eldridge et al., 2009).

2.2.2. Application methods

Four different disposal methods were followed according to the collected studies. Table 3 shows the main sludge disposal strategies followed in the investigations under review, with a diagram and description for each of them, along with information regarding the papers dealing with them.

Table S1 includes many details referring to sludge application in the different investigations. In particular, as well as compiling sludge properties (pH, CEC, moisture/solid content) and treatment, it also details the application rate (dry matter kg/ha), application method and application depth.

2.2.3. Authorized concentrations and loads and other legal requirements

Regulations in force place great attention on the concentrations of heavy metals, microorganisms, some organic microcontaminants (including AOX, PAH, PCB, PCDD/F) and define limits of their concentrations in the treated sludge intended for land disposal.

In addition, they set maximum rates of sludge to be applied on soil (kg/ha year), maximum rate of nutrients (kg nutrient/ha year) and heavy metals (kg HM/ha year) in sludge. Some of them also set the maximum concentrations of heavy metals in soil (mg HM/kg dm).

Table 2
A brief presentation of the studies included in this review, in terms of their main characteristics, aims, scope of the investigations and number of investigations (see Table S1 for details).

Reference		Main characteristics of the investigations
Atalay et al., 2007	USA	Lab investigations were carried out into the occurrence of 2 microorganisms (*E. coli* and Total coliforms), nutrients and heavy metals in **surface runoff** after treated sludge applications. Air-dried sludge was applied at a rate of 2240 kg/ha and mixed with the top 5 cm of the soil bed, on two different soils (a clay loam soil and a sandy loam soil called, respectively, Cullen and Bojac, see Table S1) to compare the influence of soil on runoff quality. Investigation fields consisted of 12 microplots (2 soils × 2 treatments × 3 replicates) - tilted aluminium beds (0.8 m × 1.9 m = 1.62 m^2 each) set up in an environmentally controlled greenhouse and used both for treatment and to control the investigation. Rainfall simulation (deionized water at a rate of 65 mm/h for 45 min) took place immediately before the sludge application (control investigation) and immediately after (treatment investigation), resulting in 2 samples (each with 3 replicates) per plot available for chemical and microbiological analyses. Number of investigations: 2
Dunigan and Dick, 1980	USA	On-field investigations were carried out on the occurrence of nutrients and Fecal coliforms in **surface runoff** in sludge-amended soil. Treated municipal sludge was applied at different rates (14.8, 16.2, and 28.9 tons/ha) and the concentrations of bacteria were monitored during the following weeks in order to evaluate their temporal variations. Investigation fields consisted of triplicate plots and a control one. Rainfall was simulated by applying deionized water at a rate of 1.11 cm/h for 2 h. Number of investigations: 3
Edwards et al., 2009	Canada	On-field investigations were carried out on the occurrence (concentrations) and mass loads of 11 PPCPs in agricultural **tile drainage** systems following sludge application. Monitoring lasted approximately 162 days. Dewatered (centrifugated) anaerobically digested sludge was applied to loam soil at a rate of 8000 kg/ha and mixed with the soil bed using two different methods (to compare the results): tilling with the top 10 cm of the soil and direct injections at a depth of 11 cm. The aim was to test the capacity to break DMB solid/aggregates apart and the effect of the atmosphere exposure and the soil environment on the PPCPs. Investigation fields consisted of 8 plots (100 m × 15 m = 1500 m^2 each) in a field with tiles placed 0.8 m below the soil surface and spaced 15 m apart; 2 of them were hydraulically isolated and used for the control investigation (they never received DMB). Sampling occurred after a real rain event (in the case of a rainfall depth of 5 mm/h in summer and 7 mm/24 h in fall gathered in a rainwater collection vessel) which took place in the study period, with a total depth of 413 mm. Samples were collected with an automatic water sampler when a rainfall with a depth of 5 mm/h (summer) and 7 mm/day (fall) was gathered in a rain collection vessel. Samples were taken more frequently near the trigger followed by a gradual reduction (sample intervals were initially every 15 min, then every 30, 60, 90 and 120 min) Number of investigations: 2
Eldridge et al., 2009	Australia	On-field investigations were carried out into the occurrence of *E. coli* and nutrients in **surface runoff** after surface spreading of 2 types of dewatered sludges (irradiated, non-irradiated and granulated biosolids) and a manure (poultry) on a silty clay loam soil covered by turf. Sludges and manure were spread on the surface. The applied sludges were a high temperature dried sludge (at a rate of 4500 kg/ha, DMB1), a high temperature dried sludge that received gamma irradiation (pathogen free, at a rate of 4500 kg/ha, DMB2), and a poultry litter (at a rate of 5150 kg/ha). Investigation fields consisted of three replicate plots for four scenarios, resulting in 12 microplots (1 m × 2 m = 2 m^2 each) in a field with a slope of 10%. The four scenarios were an untreated control plot; poultry application, DMB1 application and DMB2 application. Rainfall simulation (potable water at a rate of 90 mm/h for 30 min) took place 7 days after application; 2 samples were collected for each plot - one for the first 3 L of runoff (first flush) and another for the total runoff volume. Number of investigations: 3
Giudice and Young, 2011	USA	Investigations were carried out into the occurrence of endocrine-disrupting compounds and heavy metals in **surface runoff** after sludge application for approximately 31 days. Dewatered (and thermally dried) anaerobically digested sludge was applied to sandy loam soil at a rate of 22,500 kg/ha and mixed with the top 7–15 cm of the soil bed. The investigation fields consisted of 3 replicated plots (2 m × 1 m × 0.38 m depth each) which were built in a field with a slope of 3,5–4% and used for control (before sludge application) and for runoff analysis (after sludge application, for three different simulated rain events) as well as leachate analysis. Rainfall simulations (carbon filtrated well water at a rate of 60 mm/h until runoff occurred) took place 5 days before application (control) and 3, 9, and 24 days after application in the three plots. Six runoff samples (4 L) were collected after each rainfall simulation and their cumulative volume was investigated for the analytes of interest. A single 2.5-L leachate sample was withdrawn at the end of each simulation from the composite reservoir collecting the generated leachate (**tile drain** depth = 0.38 m; space between tiles = 0.025 m). Number of investigations: 2
Gottschall et al., 2012	Canada	An on-field investigation into the occurrence of 26 PPCPs in **tile drainage** (PPCPs in soil matrix, groundwater and wheat grain grown on the field) after sludge application was carried out for approximately 365 days. Dewatered (centrifugated) anaerobically digested sludge was applied to loam soil at a rate of 22,000 kg/ha and mixed with the top 20 cm of the soil bed. Investigation fields consisted of 2 macroplots (3 ha each) in an agricultural field located in Ontario, Canada, which was fallow the year before the investigation. Tiles were placed 1.1–1.2 m below the soil surface and spaced 15 m apart. The first macroplot represents the control system and the second the treatment system (where sludge was applied). Real rainfall occurred in the study period with a total depth of 1070 mm. Samples were time-proportionally collected at the bottom of the tiles when a trigger (adjusted depending on weather and soil water content) occurred. A total of 10 hydrograph event samples were selected for analysis. Number of investigations: 1
Gottschall et al., 2013	Canada	A commercial field-scale investigation was carried out into the occurrence of 17 PPCPs (hormones), 3 pathogens and 10 sterols in **tile drainage**, in the surface soil core, DMB aggregates mixed with soil, groundwater and wheat grain after sludge application in a real agricultural field (the same as that of Gottschall et al., 2012) for approximately 365 days. The aim was to study the long-term persistence of the selected compounds in the environmental matrices and to correlate the occurrence of fecal bacteria with sterols. Dewatered (centrifugated) anaerobically digested sludge was applied to loam soil at a rate of 22,000 kg/ha and mixed with the top 20 cm of the soil bed. Investigation fields consisted of the same 2 macroplots (3 ha each) as in the agricultural field described in Gottschall et al. (2012), with tiles positioned 1.1–1.2 m below the soil surface and spaced 15 m apart. 1 of them was isolated and used for the control investigation. Real rainfall occurred in the study period with a total depth of 1070 mm. Samples were time-proportionally collected at the bottom of the tiles when a trigger (adjusted depending on weather and soil water content) occurred. A total of 8 hydrograph event samples were selected for analysis. Number of investigations: 1
Gray et al., 2017	USA	On-field investigations were carried out into the occurrence of a wide spectrum of anthropogenic waste indicators (including 6 PPCPs) in **surface runoff** after sludge application on an agricultural field in Colorado for approximately 40 days. The site had not previously been treated with biosolids. Dewatered anaerobically digested sludge was applied on loamy sand soil at a rate of 3500 kg/ha and mixed with the top 15 cm of the soil bed. The investigation fields consisted of 5 microplots (6 m^2 each) in a field with a slope of 2.1–3%; each one was used both for treatment and control investigations. Rainfall simulations (application of hormone-free well water at a rate of 65 mm/h, corresponding to a 100-year simulated rain event) took place 5 days before (control plot) and 1, 8, and 35 days after application in the three plots. In the remaining two plots, rainfalls were conducted only on day 35 in order to evaluate the fate of compounds in the absence of repeated rainfall events. The same plots and similar operational conditions were used in a previous investigation by Yang et al., 2012 Number of investigations: 2

Table 2 (*continued*)

Reference		Main characteristics of the investigations
Healy et al., 2017	Ireland	Investigations were carried out into the occurrence of 2 PPCPs (TCS and TCC) in **surface runoff** after sludge application in a field experiment lasting approximately 15 days. In order to compare different types of behaviour, three differently treated sludges were spread on the surface of loam soil: an anaerobically digested sludge (at a rate of 6727 kg/ha), a thermally dried sludge (at a rate of 2683 kg/ha), and a lime stabilized sludge (at a rate of 29,536 kg/ha). The investigation fields consisted of replicated (n = 3) hydraulically isolated microplots (0.4 m × 0.9 m = 0.36 m^2 each) in a field with a slope of 2.8–3.7% (without controls). Each microplot was equipped with a channel collecting all the runoff during a rain event. Rainfall simulations (at a rate of 11 mm/h) took place 1, 2, and 15 days after sludge application in the same plots. Each rainfall lasted 30 min from the time of the first occurrence of surface runoff. Number of investigations: 3
Lapen et al., 2008b	Canada	On-field investigations carried out on the occurrence of 11 PPCPs in **tile drainage** after sludge application were performed in Ontario, Canada, for approximately 46 days. Liquid anaerobically digested sludge was applied on silty clay loam soil at a rate of 93,500 L/ha and mixed with the soil bed with two different approaches (in order to compare the influence on tile drain quality): tilling with the top 10 cm of the soil (subsurface spreading) and one-pass aeration tilling with the top 11 cm (surface spreading). Most of the selected PPCPs were spiked in the sludge before soil application as their concentration was found to be below the detection limits. The investigation fields consisted of three plot replications for each application type with one control bed, giving a total of 8 plots (740 m^2 each) in a field with tiles positioned 0.8 m below the soil surface and placed 15 m apart. Real rainfall occurred in the study period with a total depth of 124 mm. Samples were time-proportionally collected at the bottom of the tiles when a trigger occurred. Number of investigations: 2
Lapen et al., 2008a	Canada	On-field investigations into the occurrence of *E. coli* and *C. perfringens* as well as nutrients in **tile drainage** after sludge application were carried out in Ontario, Canada, for approximately 46 days. Liquid anaerobically digested sludge was applied on silty clay loam soil at a rate of 93,500 L/ha and mixed with the soil bed using two different methods (in order to compare the influence on the loss of microorganisms): tilling with the top 10 cm of the soil and one-pass aeration tilling with the top 11 cm. The investigation fields consisted of three plot replications for each application type with one control bed, for a total of 8 plots (740 m^2 each) in a field with tiles positioned 0.8 m below the soil surface and placed 15 m apart. Real rainfall occurred in the study period with a total depth of 124 mm. Samples were time-proportionally collected at the bottom of the tiles when a trigger occurred. Number of investigations: 2
Peyton et al., 2016	Ireland	Investigations into the occurrence of 2 microorganisms, nutrients and metals in **surface runoff** after sludge application were carried out in Ireland, for approximately 15 days. Five different sludges were applied on the surface of loam soil and the resulting runoff was compared: an anaerobically digested sludge from the UK (at a rate of 6775 kg/ha), an anaerobically digested sludge from EIRE (at a rate of 6727 kg/ha), a thermally dried sludge (at a rate of 2683 kg/ha), a lime stabilized sludge (at a rate of 29,536 kg/ha) and a dairy cattle slurry (at a rate of 80,000 kg/ha). The investigation fields consisted of 30 microplots (0.9mx0.4 m = 0.36 m^2 each) in a field with a slope of 2.8–3.7% in order to compare six different scenarios (treatment with one sludge type + control). For this reason, 6 of them were isolated and used for the control investigation. Rainfall simulations (at a rate of 11 mm/h) took place 1, 2, and 15 days after application in the same plots. The first and the last 50 mL of runoff occurring on each plot were collected (2 samples per plot). Number of investigations: 5
Sabourin et al., 2009	Canada	An on-field investigation into the occurrence of 13 PPCPs in **surface runoff** after sludge application was carried out in Ontario, Canada, for approximately 36 days. Dewatered (centrifugated) anaerobically digested sludge was applied on silt loam soil at a rate of 8000 kg/ha and mixed with the top 15 cm of the soil bed. The investigation fields consisted of 30 microplots (2 m × 3 m = 6 m^2 each) in a field with a slope of 7%. 5 of them were isolated and used for a control investigation (no sludge applied on them). A group of 5 (+1 control) microplots was considered for each rain event which took place 1, 3, 7, 21 and 36 days after sludge application. Rainfall simulations consisted of ozonated groundwater at a rate of 4.1 mm/min. One runoff sample was collected for each plot. Number of investigations: 1
Topp et al., 2008b	Canada	Investigations into the occurrence of 9 PPCPs in **surface runoff** after sludge application were carried out in Ontario in a real field for approximately 266 days. Liquid anaerobically digested sludge and ozonated groundwater spiked with pharmaceuticals were applied on silt loam soil (slope 5%) at a rate of 93,500 L/ha and mixed with the soil bed. Sludge was amended to soil using two different approaches: by tilling with the top 15 cm of the soil and by subsurface injections at a depth of 10 cm. Spiked ozonated groundwater was added to the plots only by subsurface injection at a depth of 10 cm. The investigation fields consisted of 75 microplots (2 m × 1 m = 2 m^2 each) in a field with a slope of 5%. The “control” plots were the 25 receiving spiked water but none of the plots were tested without PPCPs sources. Rainfall simulations (ozonated groundwater) took place 1, 3, 7, 21, 36 or 266 days after application in plots that had never received rainfall before to investigate the degradation/adsorption effects. Rainfall simulated events lasted until a minimum of 10 L of runoff had been collected in each microplot. Number of investigations: 3
Wallace et al., 2014	USA	Investigations into the occurrence of microorganisms and nutrients in **surface runoff** after dewatered anaerobically digested sludge application were carried out in Missouri for approximately 54 days. Sludge and mineral fertilizer were applied (to compare results) on silt loam soil and on the same soil but with a vegetation strip buffer (to compare the buffer ability in reducing key compound losses in runoff) with surface spreading applications. Four investigations occurred: untreated control plot; low rate (1664 kg/ha) of biosolids with 1 m of vegetative filter; low rate (1664 kg/ha) of biosolids without filter, and high rate of biosolids with 1 m of vegetative filter (3328 kg/ka). The investigation fields consisted of operating and control plots (1.5 m × 2 = 3 m^2 each) in a field of a slope of 3–6%. For each experiment, four replicates were conducted. Rainfall simulations (deionized water at a rate of 70 mm/h) took place immediately after application. One sample per plot was collected. Number of investigations: 5
Yang et al., 2012	USA	An on-field investigation was carried out in Colorado into the occurrence of 17 PPCPs (hormones) and 2 sterols in **surface runoff** after sludge application for approximately 40 days. Dewatered anaerobically digested sludge was applied on loamy sand soil at a rate of 3500 kg/ha and mixed with the top 15 cm of the soil bed. An analysis of the hormone partitioning between the dissolved phase and suspended-particle bond phase was reported. The investigation fields consisted of the microplots (6 m^2 each) in a field with a slope of 2.1–3%; used both for treatment and control investigations. Rainfall simulations (hormone-free well water at a rate of 65 mm/h) took place 5 days before (control) and 1, 8, and 35 days after application in the same plots. 3 composite samples (early, middle and late rain events) were collected per plot for the control (5 days before sludge application) and on the first, 8th and 35th day. Since on day 35 only two plots were monitored, a total of 33 composite samples were available. Number of investigations: 1

Table S3 summarizes the main characteristics of the regulations in the European Community and in countries including those where most of the investigations under review took place (Ireland, Italy, New South Wales-Australia, Ontario-Canada, and the USA).

Table S4 compares the limits for the different microorganisms in treated sludge to be fulfilled in the case of land disposal in many European countries, as well as in New South Wales (Australia), Ontario (Canada) and the USA.

It is important to highlight that most of the current regulations clearly define spreading and tilling procedures, the maximum slope values, and pH and CEC in soil. In addition, some legislations require that a minimum distance from waterways and a minimum depth from the aquifer is respected with regard to the plot size where sludge is applied (Table S3).

Limits referring to new contaminants of emerging interest, including pharmaceuticals, hormones, and fragrances of interest in this review have not yet been set.

2.3. Characteristics of the soils in the investigations included in the review

The texture of the soils where investigations took place is reported in Fig. 2 in the well-known texture triangle. A rapid glance shows the percentage of the main soil components (sand:silt:clay) in each case study. The legend on the right reports the studies shown in the diagram.

2.4. Plot size, precipitation and sampling strategy

2.4.1. Plot size

Concerning land runoff, investigations took place in plots whose size was in the range 0.36–6 m^2 (0.36 m^2, 1.62 m^2, 2 m^2, 3 m^2, 4.5 m^2, and 6 m^2). Tile drainage experiments were carried out in wider surface plots: 740 m^2, 1500 m^2 and 3 ha, with the only exception being the investigation carried out by Giudice and Young (2011), which was carried out in 3 plots of 2 m^2. 32 of the 38 investigations included a control plot where sludge was not amended in order to compare the quality of surface runoff/tile drainage without sludge application - only the investigations carried out by Healy et al. (2017) and Topp et al. (2008b) did not include such a plot.

2.4.2. Rainfall type, intensity, duration and frequency

In 30 of the 38 investigations, rainfall was artificial (ozonated groundwater, carbon filtrated groundwater, deionized water, or drinking water) and after the simulated rain event, runoff samples were collected. The rain intensity was in the range of 11–90 mm/h and the chosen intensity corresponded to rain with a specified return period, typical of the country where the investigation was being carried out - 2 years in Missouri (Wallace et al., 2014) and 100 years in Canada and in the USA (Sabourin et al., 2009; Gray et al., 2017).

The duration of the rain events was clearly defined in some studies (30 min by Eldridge et al., 2009; 45 min in Atalay et al., 2007), whereas in others it was a defined period from the first occurrence of runoff (for instance 30 min in Healy et al., 2017, Peyton et al., 2016) or related to the desired runoff volume to be collected during the investigation (Giudice and Young, 2011; Topp et al., 2008b; Sabourin et al., 2009; Wallace et al., 2014).

With regard to frequency, artificial rainfall was applied on different days: often some days before sludge application (control step) and then after sludge application with a different frequency pattern, covering a period ranging from a few days to one year. In the case of only one investigation was the interval as long as 266 days (Topp et al., 2008b), while in all the others it was <54 days (Table S1).

Rain was applied in the same plot to assess pollutant mobilization following rain events (Atalay et al., 2007; Eldridge et al., 2009; Giudice and Young, 2011; Gray et al., 2017; Healy et al., 2017; Peyton et al., 2016; Wallace et al., 2014, and Yang et al., 2012) or in different plots with different frequencies to evaluate the contribution of degradation and sequestration of the investigated compounds after different prolonged dry periods (Sabourin et al., 2009; Topp et al., 2008b).

In only 5 studies, all referring to tile drainage tests (Edwards et al., 2009; Gottschall et al., 2012, 2013; Lapen et al., 2008a,b), was the rainfall real, with its total depth varying between 124 mm (Lapen et al., 2008a,b) and 1070 mm (Gottschall et al., 2012, 2013). Investigations lasted from 46 days (Lapen et al., 2008a,b) to 365 days (Gottschall et al., 2012, 2013).

Many other details for the different investigations are reported in Table S1.

2.4.3. Sampling mode and frequency

Different sampling strategies were applied in surface runoff and details are reported in Table S1. Not all the studies clearly reported the description of the sampling mode and frequency, and analysis may refer to grab or composite samples.

In other studies, composite samples were collected and derived from the mixture of samples withdrawn from different plots (Sabourin et al., 2009; Giudice and Young, 2011). In Yang et al. (2012) analyses were performed on composite samples referring to different phases of the runoff (early, medium and late runoff).

Regarding tile drainage, analyses were on grab samples - water samples were collected during real rainfall events by means of in-line water flow control structures on the tile drain headers for each field section under study (Edwards et al., 2009; Gottschall et al., 2012, 2013, Lapen et al., 2008a,b).

2.5. Accuracy and uncertainty of the collected data

The collected data reported in graphs and tables in the manuscript and in the Supplementary material section come not only from tables, but also graphs and in this case, the uncertainties associated with the values add to the uncertainties due to sampling and analysis (as discussed in Verlicchi and Zambello, 2016), even in cases where the reading of the data was quite accurate. If a value was reported below its limit of detection (LOD) or its reporting limit (RL) it was assumed equal to the LOD or the RL and if it was reported below its limit of quantification (LOQ), it was assumed to be half its LOQ value. Table S1 reports the details provided referring to the experimental campaigns and a rough estimation (good, modest or poor) of the accuracy/reliability of the reported data, the description of sampling strategies and the adopted equipment.

3. Results

3.1. Ranges of the concentration of selected compounds in land runoff and tile drainage samples

A rapid glance at Table S1 highlights that 29 of the 38 investigations refer to surface runoff monitoring, only 9 investigations monitored tile drainage, 21 investigations dealt with bacteria and 17 with pharmaceuticals, hormones and fragrances.

Figs. 3 and 4 report the measured concentrations found for the selected pollutants in land runoff and in tile drainage samples in the case of sludge-amended soil. Where available, the background concentrations (control concentrations related to runoff or tile drainage from soil where sludge was not applied) of the compounds are also reported for comparison and analysis. Generally, these concentrations refer to water samples resulting from artificial rainfall applied some days before sludge application.

With regard to land runoff, it emerges that 33 compounds were monitored in the different investigations. The most studied compounds were hormones (class H), with ethinyl estradiol at the top with 37 measures, followed by all the other hormones with 33 values. The antiseptics triclosan and triclocarban and the analgesic ibuprofen were frequently monitored (values of 26 and 20, respectively), while fewer

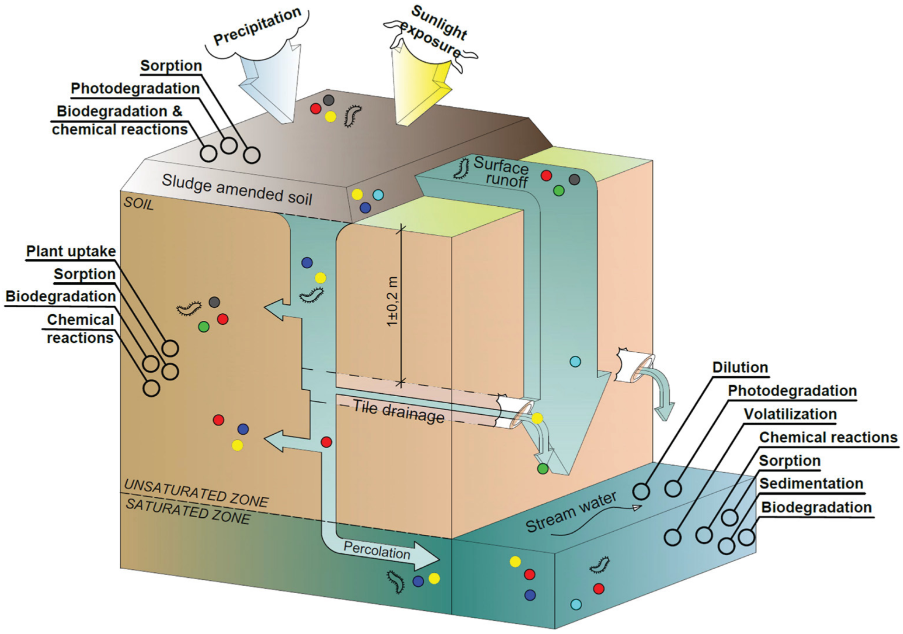
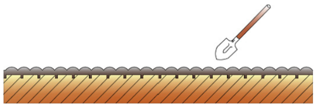
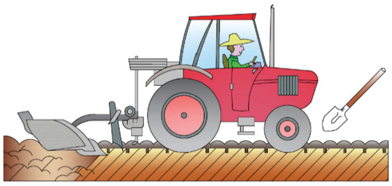
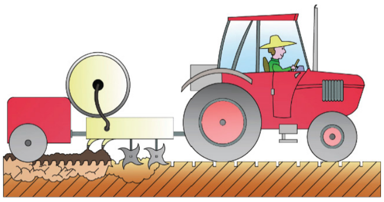
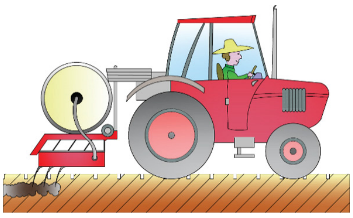


Fig. 1. The two water streams leaving soil in the case of rain events (surface runoff and tile drainage) and the main removal mechanisms for microcontaminants (PPCPs and microorganisms) occurring within the soil.

than 10 values were recorded for the remaining 14 compounds. MECs referring to hormones were collected in investigations tackling the application of dewatered anaerobically digested sludge on loamy sand and sandy loam soils. The data comes from the studies by Giudice and Young (2011) and Yang et al. (2012), which correspond to investigation numbers 4, 5, 6 and 7 in Fig. 2. Table S5 reports the number of data for

Table 3
The application strategies for sludge disposal in agricultural soils considered in this review, the typical application depth, and relative references.

Application method	Graphical description	Application depth	Description	References
Land or surface spreading		0 cm	With *land* or *surface spreading*, biosolids are deposited on the land surface without other operations.	Dunigan and Dick, 1980; Eldridge et al., 2009; Healy et al., 2017; Peyton et al., 2016; Wallace et al., 2014
Tilling		5–20 cm	In the case of *tilling*, sludge is spread on soil and then it is amended with the first 20–30 cm of soil within 20–24 h.	Atalay et al., 2007; Edwards et al., 2009; Giudice and Young, 2011; Gottschall et al., 2012, 2013; Gray et al., 2017; Lapen et al., 2008a,b; Sabourin et al., 2009; Topp et al., 2008b; Yang et al., 2012
One-pass aeration tilling		13–15 cm	In the case of *one-pass aeration tilling*, just before receiving the sludge, the soil is tilled. It is generally performed by a specific mechanical system that applies the sludge close to the ground, immediately following the passage of rolling tines which affect aerator-type tillage of the soil.	Lapen et al., 2008a,b
Subsurface injections		10–13 cm (typical application depth, as reported in the cited studies)	*Subsurface injection* consists of the injection of biosolids within 50 cm of the top of the soil surface. Generally, injection can be used for LMB application or DMBs that have a low solid content and can be easily shovelled	Edwards et al., 2009; Topp et al., 2008b

each compound, together with the minimum, maximum, average and standard deviation values for MECs and where possible, the minimum and maximum for background concentration values in case of surface runoff.

It emerges that variability ranges vary from 1 to 3 orders of magnitude and concentrations were found between <LOD and 1477 ng/L (ibuprofen according to Topp et al., 2008b). Average values varied between <LOD and 707 ng/L (ibuprofen). The highest concentrations (ibuprofen, tonalide, and gemfibrozil) were found in the investigation by Topp et al. (2008b) in which a liquid anaerobically digested sludge was applied on silt loam soil (point 12 in Fig. 2).

The intervals emerging from Fig. 3 also depend on the fact that some studies presented measured concentrations of PPCPs in different events after the sludge had been applied. As remarked by several authors (among them Topp et al., 2008b; and Sabourin et al., 2009), the temporal patterns of runoff exports (aqueous + particulate) were different from the investigated compounds. Concentrations varied, and for some compounds (triclosan, atenolol, acetaminophen, and sulfamethoxazole) the highest values correspond to the first rainfall after sludge application, for others they may occur during subsequent events (for naproxen during the second event, on the third day following application; for triclocarban, carbamazepine and caffeine after seven days, during the third rain event).

With regard to background concentrations (the red stars in Fig. 3), these were found to be <LOD for all compounds with the sole exceptions of estrone, androstenedione (with 2.2 ng/L and 1.54 ng/L respectively, Yang et al., 2012), caffeine and triclosan (21, 27, 49 ng/L and 35, 37, 47 ng/L respectively, Sabourin et al., 2009). All studies reported that no previous sludge application on soil had occurred before their investigation. The occurrence of compounds in soil could thus be due to other sources, for instance irrigation with surface water containing residues of the compounds under study (Ma et al., 2018). In this context, Table S5 shows the measured concentrations for the selected compounds in surface water.

With regard to tile drainage samples, 46 compounds were analyzed, but for 13 hormones (17-α-estradiol, 17-β-estradiol, α-dihydroequilin, androstenedione, equilenin, equilin, estradiol benzoate, estriol, mestranol, norethindrone, norgestrel, progesterone and testosterone), Gottschall et al. (2013) only reported background concentrations (always below the corresponding LOD, as shown by the red stars in Fig. 4) and this is the reason why these are not included in Fig. 4, which shows the remaining 33.

Among all these PPCPs, the most studied belong to different classes: the antiseptic triclosan (90 values), the stimulant cotinine (85 values), and the psychiatric drug carbamazepine (80 values). For 8 compounds, the collected data vary between 33 and 75, and for the other compounds the available data are <5. Measured concentrations varied between 1.5 and 4117 ng/L and, for each compound, the range of variability was equal to 2 or 3 orders of magnitude, with the exception of sulfapyridine, estrone and fluoxetine whose range was of 1 order of magnitude.

The highest values (>1000 ng/L) are related to ibuprofen (4117 ng/L), naproxen (1045 ng/L), triclosan (3676 ng/L), gemfibrozil (1040 ng/L), and carbamazepine (1136 ng/L). All these values were collected by Lapen et al. (2008b), whose investigation dealt with the application of liquid anaerobically digested sludge on silty clay loam (number 15 in Fig. 2).

Average concentrations varied between 1.5 ng/L (ofloxacin and miconazole) and 551 ng/L (triclosan).

The background concentrations were investigated for all the compounds (Lapen et al., 2008b; Edwards et al., 2009; Giudice and Young, 2011; and Gottschall et al., 2013) and they resulted below LOD or below LOQ, with four exceptions. As reported in Fig. 4, these refer to naproxen (35 ng/L), triclosan (95 ng/L), atenolol (135 ng/L) and cotinine (5 ng/L) and were reported by Edwards et al. (2009), who investigated the application of dewatered anaerobically digested sludge on loam soil (number 13 in Fig. 2), which had previously received sludge.

Further details regarding these collected data are reported in Table S6, in particular the number of data for each compound, together with the minimum, maximum, and average values and standard deviation (SD) for MECs and, where possible, the minimum and maximum for background concentration values in the case of tile drainage.

Observed concentrations for the selected compounds in both runoff and tile drainage are generally lower than the values found both in the municipal WWTP secondary effluent, in surface water as well as in runoff in case of reclaimed water reuse. The corresponding range of concentrations found in literature (Verlicchi et al., 2012; Ben et al., 2018; Chalew and Halden, 2009; Metcalfe et al., 2003; and Pedersen et al., 2005) are reported in Tables S5 and S6.

Moreover, the observed concentrations for hormones are unlikely to result in any significant pulse environmental exposure impact, from a pure tile effluent concentration perspective (Gottschall et al., 2013). In addition, once released into surface water, the dilution effect and photodegradation of microcontaminants due to UV exposure contribute to a further reduction of their concentrations. Sabourin et al. (2009) remarked that the runoff concentrations of the selected PPCPs were lower than the reported acute toxicological endpoints, and for most compounds a thousand times lower.

3.2. Range of concentrations for the bacteria included in the review

Few papers tackle the monitoring of bacteria in surface runoff and tile drainage after sludge disposal. Four studies have investigated surface runoff (Dunigan and Dick, 1980; Atalay et al., 2007; Eldridge et al., 2009; Peyton et al., 2016; Wallace et al., 2014) and two tile drainage (Gottschall et al., 2013; Lapen et al., 2008a). Moreover, different units of measures were sometimes used and thus analyses are not easy to perform.

Fig. 5 reports the collected concentrations and background concentration for bacteria species with >12 reported measures, with the exception of Fecal coliforms as they are in another unit of measurement. A descriptive statistical analysis was carried out for all of the bacteria (Table 4).

Investigations by Peyton et al. (2016) with 5 differently treated sludges applied on rural land showed that there is no correlation between sludge treatment and runoff concentration of Fecal and Total coliforms.

The profile of bacteria content in runoff after three different simulated rain events (1 day, 2 days and 15 days after application; same rain intensity) strictly depends on the sludge type (differently treated) applied on the soil (Peyton et al., 2016) and the maximum concentrations vary in different cases. This could be explained by the fact that once sludge is applied on the soil, UV light and desiccation are responsible for the decay of occurring bacteria (Lang et al., 2007), but in some cases, bacteria could also find soil conditions which favor their development.

A comparison with TC and FC concentrations in runoff in the case of dairy cattle slurry application shows that they were always 1 order of magnitude higher than in the case of treated sludge application (Peyton et al., 2016).

It emerges that investigations report a variable number of values - from 3 (Atalay et al., 2007) to 46 (Gottschall et al., 2013); background concentration was measured once in the studies by Atalay et al. (2007), Eldridge et al. (2009) and Wallace et al. (2014) for surface runoff and Gottschall et al. (2013) for tile drainage.

Wallace et al. (2014) highlighted the low content of FC in control plots (116 CFU/100 mL) and a wide variability in the case of sludge application on soil with or without a vegetative filter, from 4880 to 35,720 CFU/100 mL. These values are higher than the maximum amount allowed in Missouri (where the investigation took place) for whole body contact recreation use (maximum of 206 CFU/100 mL).

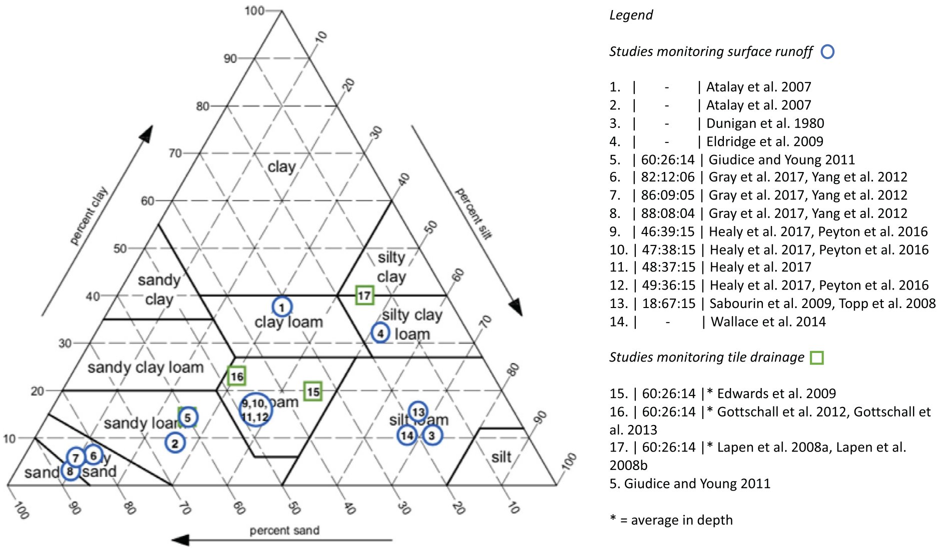


Fig. 2. Characteristics of the soil texture in the investigations included in this review in the soil textural classification triangle.

Sludge disposal always led to an increment in the bacteria concentrations in surface runoff and tile drainage with concentration ranges as wide as 3–5 orders of magnitude depending on many factors, as will be discussed later.

The investigation by Eldridge et al. (2009) was the only scenario in which this phenomenon did not occur. They found a background concentration for *E. coli* equal to 251,188 MPN/100 mL and after sludge application, MECs in surface runoff were always lower, between 25,000 and 31,000 MPN/100 mL.

Measured concentrations are strictly correlated to the sampling procedure followed in the investigations. As reported in Table S1, water samples were instantaneous or (time or volume) composite, and in some cases (Yang et al., 2012) water samples were related to early, middle and late rain events corresponding to the mix of different collected samples. Lapen et al. (2008a) found that LMB application-induced contamination starts some minutes after sludge application. *E. coli* and *C. perfringens* were still high 24 h after application while over a study season basis, *E. coli* showed a significant decline in mass loads, whereas *C. perfringens* presented some peaks during the observation periods and did not follow a similar pattern of decay.

Unfortunately, it is not possible to compare the observed concentration ranges for surface runoff and tile drainage, as data are reported in different units of measurement.

3.3. Influence of the main factors affecting runoff and tile drainage quality – PPCPs

Once in the soil, a micropollutant may be subject to different phenomena: it may be sequestered, sorbed on the soil matrix (depending on its organic carbon content, CEC, pH, etc.), biodegraded (due to microorganisms present in the soil as well as in the applied sludge), photodegraded (due to light exposure), it may take part in chemical reactions with other compounds, or it may be transported away by a liquid phase. Prediction of its behaviour is complex, as environmental and matrix conditions may rapidly change, and intense rain events may enhance its vertical mobility through soil pores. The following sections will discuss the main factors affecting the occurrence of microcontaminants and bacteria in runoff and tile drainage in sludge-amended soil.

3.3.1. Influence of K_{ow}

The partitioning between solid and liquid phases was not always addressed by the authors, as it requires lengthy investigations, as shown in Yang et al. (2012). More frequently, coefficients derived from literature were used. In this context, the octanol-water partition coefficient K_{ow}, describing the distribution between the octanol and water of a compound, is commonly used to assess the sorption potential on a solid phase by a compound. As a rule of thumb, if LogK_{ow} > 4 it could be expected that the compound is highly adsorbed due to hydrophobic partitioning, while if LogK_{ow} < 2.5 the compound has a low level of sorption (Verlicchi and Zambello, 2015). The graphs shown in Figs. 6 and 7 report MECs in runoff and tile drainage vs. LogK_{ow} for the investigated compounds. For quick reference, Table S7 compiles LogK_{ow} values for the compounds investigated in surface runoff or tile drainage analysis.

In Figs. 6 and 7, values are dispersed in a wide cloud and it emerges that no clear correlation exists between the sorption potential of a compound and its measured concentration in runoff or in tile drainage. It is important to bear in mind that the reported data refer to different investigations, in terms of soil characteristics, rain fall intensity, sludge application rate, and concentration for the different compounds in the applied sludge and the MECs depend on these factors.

Limiting attention to single investigations where soil characteristics, sludge properties and rainfall intensity are defined and are the same for all the compounds, it seems that LogK_{ow} may be correlated with the pharmaceutical mass load (or concentration) in the runoff. Sabourin et al. (2009) found that chemicals with LogK_{ow} < 2.45 (atenolol, carbamazepine, cotinine, caffeine and acetaminophen) were rapidly transported in runoff and those with LogK_{ow} > 3.18 tended to be retained in the soil (gemfibrozil, naproxen and ibuprofen). The same behaviour is confirmed by the investigations and results obtained by Gottschall et al. (2012) referring to fluoxetine, miconazole, tetracyclines and fluoroquinolones (with LogK_{ow} > 4). These compounds were not found in tile drainage and they may have been more strongly bound to DMB, which can also explain their long-term detection in incorporated DMB.

Sabourin et al. (2009) also found that although sulfamethoxazole had a LogK_{ow} equal to 0.89, it was not largely exported in runoff (only 0.51% was found on a mass basis). Triclosan and triclocarban have similar K_{ow}, but in the study by Sabourin et al. (2009), it was found that about 40-times more triclosan was exported than triclocarban, and

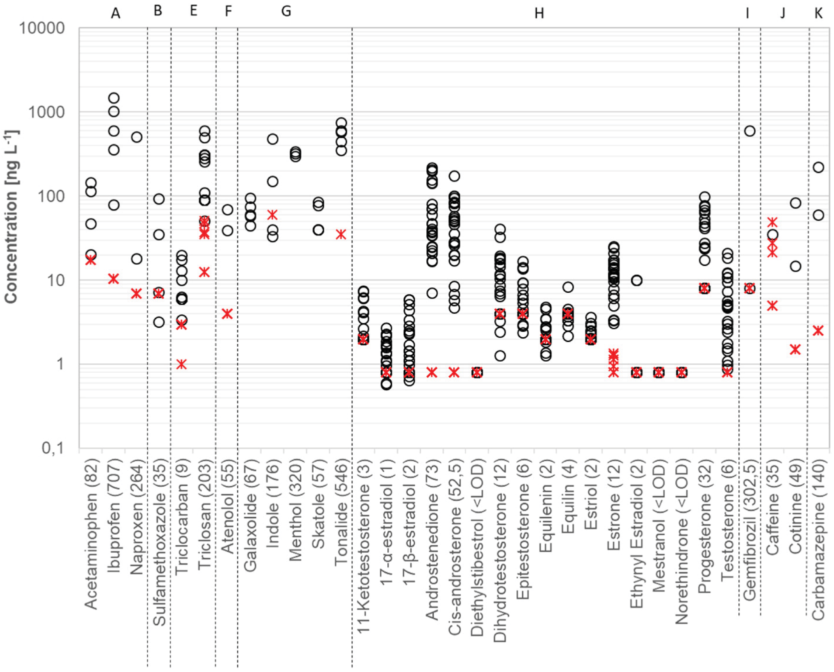


Fig. 3. Measured concentrations of selected compounds in land runoff with (MEC, ○) or without (background concentration, *) sludge application on soil.
Data from: Giudice and Young (2011), Gray et al. (2017), Healy et al. (2017), Peyton et al. (2016), Sabourin et al. (2009), Topp et al. (2008b), and Yang et al. (2012).

that even their concentrations in the sludge applied on the soil were similar (around 7000 and 8000 ng/g dm, respectively). This different behaviour could be explained by the different values of pK_a (the dissociation constant, reported in Table 1) of the two antiseptics (8.1 for triclosan and 12.7 for triclocarban) and the ionic forms at the pH of the soil/runoff, as discussed in Giudice and Young (2011). They also

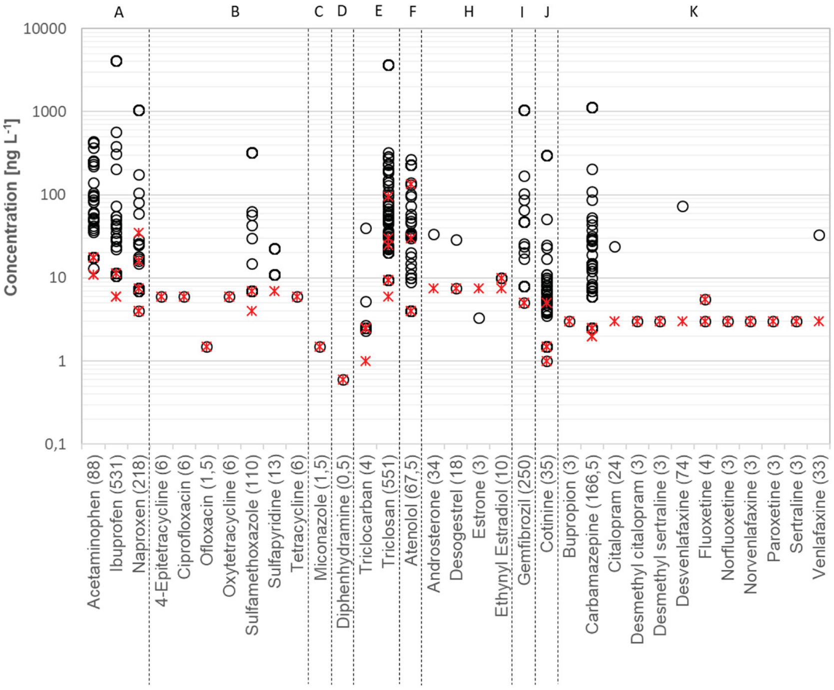


Fig. 4. Range of concentrations for a selection of compounds in drainage samples with (MEC, ○) or without (background concentration, *) sludge application on soil.
Data from: Edwards et al. (2009), Giudice and Young (2011), Gottschall et al. (2012), and Lapen et al. (2008a).

remarked that in their study conditions (soil pH at 8 and runoff pH variable between 7.8 and 8) half of the triclosan present in the sludge is ionized and as a result, it is much more prone to leachate and to be exported in the runoff than the neutral molecules of triclocarban.

The study by Yang et al. (2012) thoroughly investigated the fractioning between dissolved and suspended-particle bound phases for a wide spectrum of steroid hormones and found that LogK_{ow} values were not correlated with estrogen sorption to colloids.

According to Cunningham (2008), the octanol-water *distribution* coefficient D_{ow} is more adequate in studying microcontaminants, as it is pK_a-dependent at environmental pH.

D_{ow} is defined by Eq. (1), and, according to Schwarzenbach et al. (2003), evaluated through Eq. (2) for acidic compounds and Eq. (3) for basic ones:

$$D_{ow} \equiv \frac{\text{concentration in } n-\text{octanol}}{\text{concentration in water}} \tag{1}$$

$$\text{Log}D_{ow} = \text{Log}K_{ow} + \text{Log}\frac{1}{1+10^{pH-pK_a}} \qquad \text{(acidic compound)} \tag{2}$$

$$\text{Log}D_{ow} = \text{Log}K_{ow} + \text{Log}\frac{1}{1+10^{pK_a-pH}} \qquad \text{(basic compound)} \tag{3}$$

where $\text{Log}D_{ow} = \log_{10} D_{ow}$.

In the case of neutral moieties, the two previous correlations result in Eq. (4):

$$\text{Log}D_{ow} = \text{Log}K_{ow} \tag{4}$$

The rule of thumb when using LogD_{ow} to predict PPCP behaviour in aquatic compartments is good sorption if Log$D_{ow} > 3$ and low sorption if Log$D_{ow} < 1$.

3.3.2. The influence of soil characteristics

On the basis of the lab investigations by Topp et al. (2008b), Cha and Cupples (2009), and Al-Rajab et al. (2010a,b, 2015), soil characteristics (soil texture, pH, moisture content and temperature) seem to have a relevant influence on the quality of the runoff/tile drainage of studied microcontaminants.

As expected, runoff and tile drainage flows depend on the level of soil saturation. In loose and uncompacted soil, percolation is higher than land runoff, whereas when the soil becomes compact, percolation is lower than surface runoff (Giudice and Young, 2011).

Moreover, all studies agree with the consideration that moisture content and temperature in the soil mostly influence the fate of microcontaminants (sorption, degradation, and mineralization) and soil texture does not seem to influence leaching (that is the passage in the liquid phase due to rain events). In particular, Topp et al. (2008a), Cha and Cupples (2009), and Al-Rajab et al. (2010a,b, 2015) mainly refer to triclosan, triclocarban and naproxen, and in-depth investigations on the fate of all the compounds under review are not available for different soil characteristics.

Macropores, due to worm burrows, soil cracks and abandoned root channels, can favor the rapid gravity flow of contaminant-laden material in the vadose zone towards tile and shallow ground water depth (Lapen et al., 2008a). The pore size and distribution of the soil also affects the mobility of PPCPs within the medium. Hormones in a soil characterized by macropores rapidly move downwards (and may be collected in tile drainage), whereas in the case of soil with micropores, they move slowly and to a lesser extent (Gottschall et al., 2013).

It is important to highlight that at the modest slope of the investigations under review, surface runoff generally occurs when the soil is saturated, otherwise rain drops enter the sludge-amended soil and percolates. During their passage through the solid phase, rain drops meet compounds (including microcontaminants) sorbed on the soil which may be transported to the liquid phase (leaching) according to the compound-specific equilibrium conditions at the occurring environmental conditions (temperature, pH, etc.).

From a microscopic view point, the soil environment is continuously changing and surface runoff/tile drainage following two different rain events may have different characteristics. This is confirmed by Gray et al. (2017), who analyzed and compared the surface runoff in the same plot 1, 8 and 35 days after sludge application and the surface runoff in another plot 1 and 35 days after sludge deposition, in order to evaluate the fate of a wide spectrum of microcontaminants (triclosan, menthole, indole, skatole, galaxolide and tonalide) under different rainfall patterns, and Sabourin et al. (2009) who analyzed the surface runoff quality in five different plot sets that received rainfall after 1, 3, 7, 21 and 36 days, respectively.

As already highlighted in Section 3.3.1 for triclosan and triclocarban, soil pH influences the ionization of a compound and thus its partition between soil and liquid (runoff/leachate).

3.3.3. Influence of sludge treatment

Liquid or dewatered sludge has different behaviour once applied on a soil. Topp et al. (2008a,b) and Sabourin et al. (2009) thoroughly investigated these issues and found in particular that LMB applied to unsaturated (also called uncompacted) soil behaves as a liquid, filling the available pore space. In this way, the exposure of PPCPs to soil microorganisms is favored and their biodegradation promoted. In contrast, in the case of dewatered sludge, the diffusion of oxygen into DMB aggregates, as well as the diffusion of PPCPs out of the DMB aggregates into the surrounding soil matrix are limited, resulting in greater persistence of PPCPs.

If an LMB is applied on a soil, PPCPs have a greater potential to transport within and over the soil at the time of application. Dewatered biosolids require an external source (rainfall or irrigation water) to favor the mobilization of pollutants within the soil/biosolids.

The highest concentrations of PPCPs in tile drainage following the application of biosolid slurry (i.e. liquid municipal biosolids) were detected immediately after application (Lapen et al., 2008b). In contrast, large cohesive DMB aggregates remain at the point of deposition on the soil. The concentrations of PPCPs in tile drainage following the application of dewatered biosolids were generally lower than those following LMB application, and, in contrast to LMB applications, the highest concentrations were only measured some time after application (Edwards et al., 2009).

Presumably, the slower release of residues from the DMB aggregates, and drying and physical deterioration of the aggregates was extending the period of availability for transport in runoff, compared to LMB where the PPCPs were applied in a more uniform matrix.

It is important to highlight that in the case of tile drainage in LMB-amended soil, the time the LMB took to reach the tiles (around 80 cm below) ranged between 3 and 39 min in unsaturated soil (Lapen et al., 2008b), whereas in the case of DMB-amended soil, contaminants due to dewatered sludge application reached the tile at least 8 days after sludge application (Edwards et al., 2009).

The maximum concentrations of the selected PPCPs in tile drainage were higher in the case of application of LMB than that of DMB.

Anoxic conditions in soils (vadose zone) do not favor the degradation of contaminants, in particular persistent ones, and this could have contributed to the persistence of some LMB-derived PPCPs in the tile drainage in the soils investigated by Edwards et al. (2009) in 2006, which had previously been used for another investigation by Lapen et al. (2008b), who applied LMB on the same plots.

3.3.4. Influence of sludge application rate and of pharmaceutical load

In the different investigations, the applied sludge ranged between 2683 kg/ha (Healy et al., 2017) and 29,536 kg/ha (Peyton et al., 2016) (see Table S1). Some investigations used extremely high quantities of sludge on soil in order to enhance the behaviour of the microcontaminants under study (Giudice and Young, 2011 and

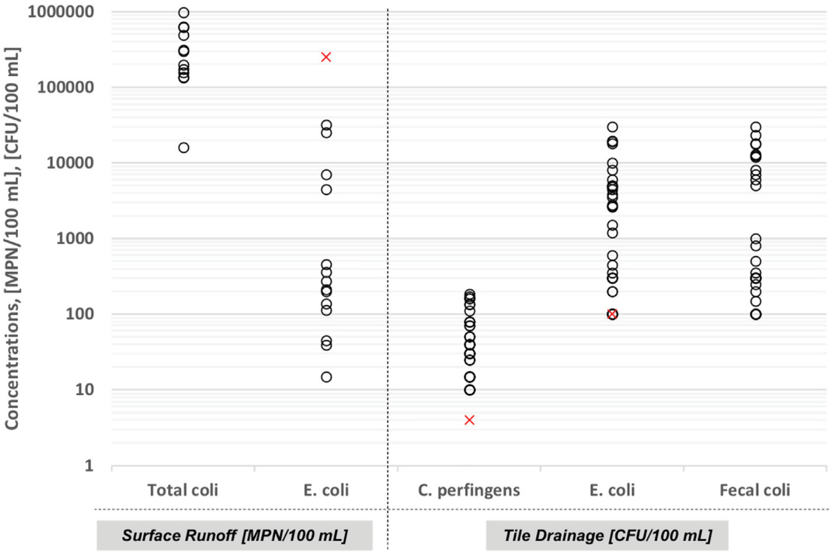


Fig. 5. Collected data for bacteria in surface runoff and tile drainage.
Data from Eldridge et al. (2009), Gottschall et al. (2013), and Peyton et al. (2016).

Gottschall et al., 2012). Current regulations allow a maximum rate which can be expressed in terms of kg/ha year or kg/ha over a longer period, for instance 3 years in Italy. Sometimes the maximum sludge rate to apply depends on the N and P maximum applied rate, as reported in Table S3. Tables 5 and 6 reports PPCPs concentrations in runoff and tile drainage after sludge application. Attempts to correlate the available data were carried out, but no clear correlation was found for the studied compounds between the applied load and resulting concentrations.

A factor which seems to affect runoff concentration is the solid content of the applied sludge. Sabourin et al. (2009) used dewatered sludge (the so-called solid sludge, with a solid content of >18%) on the same soil where Topp et al. (2008b) carried out their studies with liquid biosolids. They found that with dewatered sludge, the concentrations of microcontaminants were in general lower than in the case of liquid biosolid application and only some days after application they may be found. This delay in the release of PPCPs may be explained by the fact that in dewatered sludge, PPCPs are more retained in the aggregates.

3.3.5. *Influence of the sludge application method*

Edwards et al. (2009) remarked that the investigated application mode (direct injection, surface spreading and tilling) may influence the quality of tile drainage and surface runoff, due to the resulting different capacities of breaking dewatered sludge solids/aggregates apart and the fact that the microcontaminants and microorganisms have different types of exposure to the atmosphere (mainly UV and oxygen).

Moreover, the moisture content of the applied sludge may also greatly influence the behaviour of PPCPs, as remarked by Edwards et al. (2009). In the case of dewatered sludge, microcontaminants present a more modest level of mobility and potential to biodegrade than in the case of liquid sludge, and differences may be more likely to occur in PPCPs associated with aggregate interiors rather than exteriors. According to the cited study, there is no significant difference in the concentrations of PPCPs in tile drainage in the case of surface spreading and direct injection of dewatered sludge.

Surface spreading allows a faster degradation of the most degradable PPCPs.

Topp et al. (2008b) found that through the injection of liquid sludge into the soil, surface runoff presents a lower concentration of selected microcontaminants in a short time as direct injection promotes pollutant sequestration and reduces the surface mobility of pollutants. Analysis of surface runoff after a prolonged period showed that the release of microcontaminants may occur later for the most persistent compounds retained within the soil, whereas for those which are more biodegradable, degradation processes take place and their concentration is low or they are not detected (Al-Rajab et al., 2015).

In their investigation comparing sludge application approaches (surface spreading and subsurface spreading) on the quality of tile drainage in terms of a selection of different PPCPs, Lapen et al. (2008b) remarked that microcontaminants rapidly move downwards, reaching the tile drains in a few minutes, regardless of the application method: the larger the soil macropores, the shorter their transport time. Applied sludge fluidity also influences the gravity-based flow of occurring pollutants: liquid municipal biosolids can be considered similar to diluted

Table 4
Main findings in monitoring bacteria occurrence in surface runoff and tile drainage.

	Microorganism (u.o.m.)	Background concentr.	Min	Max	Mean	SD	# data
Surface runoff	Total coliforms [CFU/mL]	0	63.1	79.4	71.3	11.5	3
	E. coli [CFU/mL]	0	1.8	2.4	2.1	0.4	3
	Fecal coliforms [CFU/100 mL]	100	5000	36,000	16,167	17,222	4
	Fecal coliforms [MPN/mL]	0–70	60	55,000			12
	Total coliforms [MPN/100 mL]	–	15,858	980,600	344,622	280,808	12
	E. coli [MPN/100 mL]	251,188	15	31,622	5009	10,191	15
Tile drainage	*C. perfringens* [cts/100 mL]	4.0	10	185	50	49	46
	E. coli [cts/100 mL]	100	100	30,000	3504	6363	46
	Fecal coliforms [cts/100 mL]	–	100	30,000	3568	6963	45

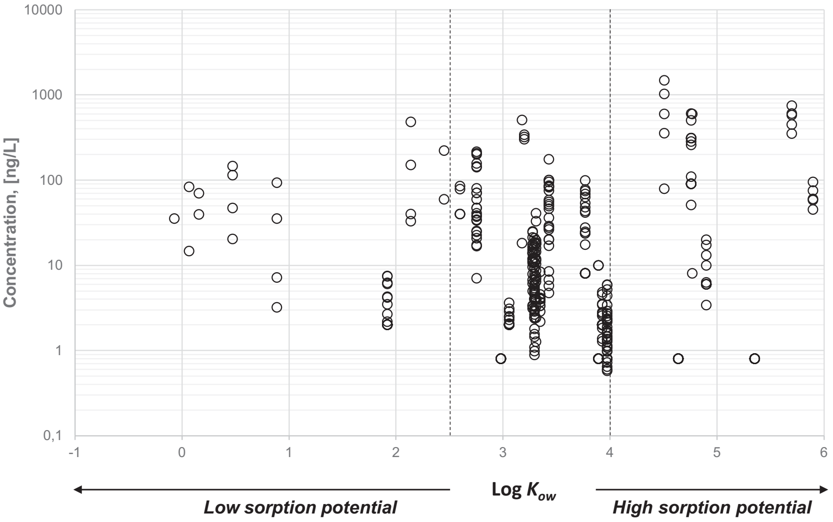


Fig. 6. Runoff concentrations versus LogK_{ow} for the investigated compounds.
Data from: Giudice and Young (2011), Gray et al. (2017), Healy et al. (2017), Peyton et al. (2016), Sabourin et al. (2009), Topp et al. (2008b), and Yang et al. (2012).

wastewaters and thus they rapidly move downwards. In this context, the sorption to the macropore walls is limited, as the rapid vertical motion of the leaching stream is the dominant process. The sludge is subsurface spread by means of an applicator which applies slurry close to the ground immediately following the passage of rolling tines that affect aerator-type tillage of the soil (Lapen et al., 2008a,b). This application method leads to the reduction of soil macropore size and fosters the sorption of microcontaminants and liquid biosolids in the tilled surface layers.

3.3.6. Influence of rainfall intensity, frequency and volume

Giudice and Young (2011) analyzed the composition of the surface runoff during three storm events of the same intensity occurring on the same plot (2 m^2) 3, 9 and 24 days after sludge application. Data reported on triclosan and triclocarban show that for each rain event, micropollutant concentration in surface runoff remains constant in the first 4, 8, 12, 16, 20 and 24 L. They also found that in the three rain events, measured concentrations varied. This could be attributed to the sequestration of the compounds, and their scarce biodegradation within the soil rather than to the increasing water volume applied to the plot in the three events.

In Yang et al. (2012) and Gray et al. (2017) the concentration of a selection of anthropogenic waste indicators (fragrances and hormones) in runoff due to three replicated 100-year rainfall events was investigated. After 35 days the runoff concentration decreased with respect to previous rain events (1 and 8 days after sludge application) but remained at comparable values. This suggests that hormones may persist in the soil even if leaching and, possibly, onsite transformation may occur. That study compared the runoff quality in the case of frequent heavy rain events and after only one intense rainstorm, 35 days after sludge application. It was found that onsite attenuation was minimal and that natural weathering processes may make some compounds more easily leachable from the sludge amended soil even in the case of dry periods. In addition, they also found that once the soil becomes saturated, an increase in runoff rate leads to an increment in hormone load.

In tile drainage, the concentrations of investigated compounds decreased in the rain events occurring many days after sludge application on the soil (Lapen et al., 2008b) compared to the events nearly immediately after the application.

3.4. Influence of the main factors on the concentrations of microorganisms in runoff and tile drainage

3.4.1. Sludge treatment

No specific investigation compared the quality of runoff and tile drainage in terms of microorganisms in the case of LMB and DMB application. It can be expected that a "liquid" biosolid could lead to a higher concentration in tile drainage than a dewatered one, as it quickly crosses the layer through the macropores and reaches the tile drain (Lapen et al., 2008a,b).

Referring to surface runoff, similarly to PPCPs, there could be a slower release of microorganisms adherent to DMB aggregates, and they become available to the runoff over a longer period (Sabourin et al., 2009).

Thermal drying, gamma irradiation and disinfection greatly reduce microorganism content in sludge and thus, in the case of land reuse, the risk of microbial contamination of the receiving waters due to surface runoff or tile drainage is reduced.

3.4.2. Application methods

If the sludge is spread on the surface, the major removal mechanisms are due to desiccation and ultraviolet light (Lu et al., 2012; Gondim-Porto et al., 2016), whereas if the biosolid is incorporated into the soil, microorganism survival is strictly related to soil pH, organic matter content, texture, temperature, and competitive organisms developed in the soil.

In tile drainage, investigations by Lapen et al. (2008a) showed that one-pass aeration tilling mode (Table 3) enhances contaminant sorption/retention in the soil as it disrupts surface macropores and increases sorptivity. In their investigation, they reported that the average mass load ratios between tilling mode and one-pass aeration tilling application were 6 for *E. coli* and 12 for *C. perfringens*. Unlike the case of PPCPs investigated by Edwards et al. (2009) (see Section 3.3.5), Gottschall et al. (2013) found differences in the concentrations of bacteria in tile drainage in the case of surface spreading and direct injection of dewatered sludge: concentrations of *E. coli*, Enterococci, *C. perfringens* were higher in the case of direct injection.

3.4.3. Soil characteristics

Interesting findings were reported by Atalay et al. (2007) in their investigation of the content of *E. coli* and Total coliform in runoff and percolate in two different sludge-amended soils - sandy loam and clay loam. They found that in the case of clay loamy soil the concentrations of *E. coli* were higher than in the sandy loam. With regard to the Total coliforms, no significant differences were found when the sludge was amended to the two soils. They also found that sludge incorporated into the first 5 cm of soil instead of the first 10 cm may lead to a higher occurrence of microbes in the surface runoff.

3.4.4. Rainfall

Unfortunately, little data is available for evaluating the effect of rainfall on microbial occurrence in surface runoff. According to Peyton et al. (2016), there could be a regrowth of Fecal coliform after a rain event on dry soil. Earlier studies (Dunigan and Dick, 1980) found that Fecal coliform indicator bacteria counts in surface runoff waters from sewage-treated plots were very high during the first days of sludge application, but they rapidly decreased as the soil became drier.

4. Lessons learned from past investigations

4.1.1. Importance of sludge type (liquid or dewatered) on the runoff and tile drainage quality and quantity

Soon after land application, liquid municipal sludge moves more rapidly through the available pores in unsaturated soil, ensuring a greater exposure of contaminants to soil microorganisms, which may accelerate degradation processes. On the other hand, in the case of application of dewatered sludge, the degradation of contaminants is hampered by the modest diffusion of oxygen into the aggregates and of microcontaminants out of the aggregates towards the soil environment. In addition, when liquid sludge is applied, microcontaminant concentrations in surface runoff and tile drainage are higher than in the case of DMB (Sabourin et al., 2009).

4.1.2. Phenomena occurring within the soil

They are quite complex and affected by many parameters (Gray et al., 2017; Gottschall et al., 2013; Topp et al., 2008a; Healy et al., 2017; Lapen et al., 2008a). Different scenarios have been highlighted:

- the mass of microcontaminants which leachates after biosolid application is small with regard to the quantity present in the soil due to sludge application, and runoff concentrations are generally low and similar after different rain events;
- the release of microcontaminants could be minimal and occur in many successive events;
- rain events tend to wash down biosolid particulates and the microcontaminants are associated with the suspended solids rather than being leached in the dissolved phase (and chemical analysis does not detect them);
- microcontaminants are not released in the dissolved phase but persist in the soil and their mass reduction is only due to soil erosion, washed out by heavy rain events via solid aggregates,
- natural weathering processes may make some compounds more easily leachable from the sludge-amended soil even in the case of prolonged dry periods.
- referring to microorganisms, due to the unfavorable soil conditions for microorganisms, in the sludge-amended soil, they are immediately subject to desiccation, irradiation, competition with other species already present, etc. Their concentration in runoff and tile drainage is also affected by rain event characteristics: rain duration, intensity and frequency.

4.1.3. Persistence of microcontaminants in the soil

Some compounds persist in the soil environment within sludge aggregates for a long time: up to 1 year after sludge application. Their runoff concentrations generally decrease, following first order kinetics but, sometimes they may also remain constant. This means that even for long periods of time, these compounds do not seem to deplete within the soil. The reason could be due to the fact that sludge is the end product of a series of mechanical and above all biological treatments under different conditions (aerobic, anoxic, and anaerobic) within wastewater treatment plants. Macro- and micro-contaminants still present in the treated sludge are thus resistant to the different environments and for these reasons are still present even after many weeks or months.

Some hormones can interconvert via microbial activities within the soil. As a result, their runoff concentration may increase after multiple rainfall events and exposure to environmental conditions due to the biodegradation of a related compound.

4.1.4. Environmental risk posed by the release of microcontaminants via surface runoff and tile drainage

Based on current findings limited to specific compounds, it seems unlikely that a significant environmental risk could be due to the release of PPCPs by tile drainage and surface runoff. However, neither mixture

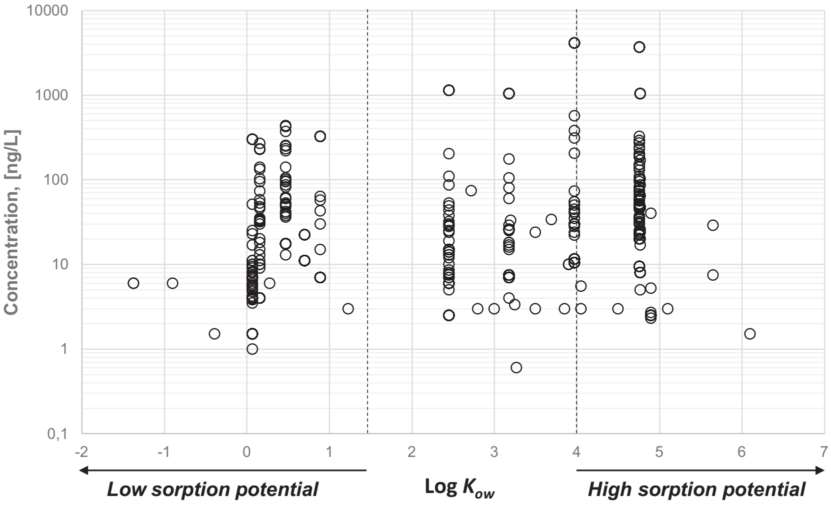


Fig. 7. Tile drainage concentrations versus LogK_{ow} for the investigated compounds.
Data from: Edwards et al. (2009), Giudice and Young (2011), Gottschall et al. (2012), and Lapen et al. (2008b).

Table 5
Runoff concentration as a function of the applied load of pharmaceuticals.

	Sludge concentration	Application rate	Load [mg/ha]	Runoff concentration [ng/L]	Reference
Acetaminophen	28.6 ng/g	8000 kg/ha	229	20.3	Sabourin et al., 2009
	100,000 ng/L	93.5 m^3/ha	9350	47; 114; 146	Topp et al., 2008b
Naproxen	394 ng/g	8000 kg/ha	3152	18.1	Sabourin et al., 2009
	10,000 ng/L	93.5 m^3/ha	935	509	Topp et al., 2008b
Sulfamethoxazole	12.4 ng/g	8000 kg/ha	99	3.2	Sabourin et al., 2009
	10,000 ng/L	93.5 m^3/ha	935	7.15[c]; 35.4; 93	Topp et al., 2008b
Triclocarban	50 ng/g	2683.3 kg/ha	134	6[b]; 10	Healy et al., 2017
	8194 ng/g	8000 kg/ha	65,552	3.4	Sabourin et al., 2009
	17,600 ng/g	22,500 kg/ha	396,000	6.3; 13.1; 17.3	Giudice and Young, 2011
Triclosan	270 ng/g	6751.4 kg/ha	1823	90[b]	Healy et al., 2017
	4900 ng/g	2683.3 kg/ha	13,148	90[b]	Healy et al., 2017
	7066 ng/g	8000 kg/ha	56,528	109.7	Sabourin et al., 2009
	9140 ng/g	3500 kg/ha	31,990	310[a,b]; 500[a]; 600[a]	Gray et al., 2017
	15,900 ng/g	22,500 kg/ha	357,750	51[b]; 282.1; 309.6	Giudice and Young, 2011
Atenolol	1.6 ng/g	8000 kg/ha	13	39.6	Sabourin et al., 2009
	10,000 ng/L	93.5 m^3/ha	935	70	Topp et al., 2008b
Gemfibrozil	31 ng/g	8000 kg/ha	248	8	Sabourin et al., 2009
	10,000 ng/L	93.5 m^3/ha	935	597	Topp et al., 2008b

[a] Values graphically estimated.
[b] <LOD.
[c] <LOQ.

effects nor the chronic effects on different aquatic species have as yet been investigated.

4.1.5. Good practices for sludge disposal

Correct sludge disposal on rural land should favor the retention of pollutants and microcontaminants in the rooting zone away from groundwater, artificial drainage and surface water sources. Bearing this in mind, good practices include (but are not limited to): (i) proper sludge rates of application, (ii) pre-application tillage of the soil to foster the disruption of continuous macropore networks and improve surface soil sorptivity, (iii) land application when soil macroporosity is reduced and when the soil sorption capacity is higher, (iv) controlling product placement and using application equipment that decreases the absolute amount of amendment available for local infiltration/surface runoff; (v) installation of a deeper tile drainage system, and application on soil characterized by a lighter texture, as it may enhance the sorption and sequestration of microcontaminants and finally, (vi) lowering temperatures, which may reduce the decomposition of DMB and the release of active ingredients of microcontaminants.

5. Main areas lacking in investigations, further research and perspectives

In many investigations, the main scope was the occurrence of target compounds in runoff or tile drainage, and data referring to rainfall events were often incomplete. In particular, the volume of rain entering and exiting the system is not always provided and thus, neither a water mass balance nor a pollutant mass balance is possible. A complete hydrologic description of the plot where the investigation takes place is necessary because the transport of PPCPs in soil is strongly influenced by the soil saturation ratio.

As remarked by Gray et al. (2017) and Yang et al. (2012), even if intense rain events are considered in the investigations, longer monitoring periods (>40 days) should be adopted, as losses of microcontaminants may occur many days after application, especially in the case of dewatered sludge applied in the soil.

Table 6
Tile drainage concentrations as a function of the applied load of pharmaceuticals.

Compound	Concentration [ng/g]	Application rate [kg/ha]	Load [mg/ha]	Tile drainage concentration [ng/L]	reference
Acetaminophen	18.7	22,000	411.4	13	Gottschall et al., 2012
	24	8000	192	17.5[a,c]; 36[a], 38[a], 40[a], 42[a], 48[a], 51[a], 53[a], 60[a], 62[a], 87[a], 105[a], 220[a], 233[b]	Edwards et al., 2009
Ibuprofen	63.6	22,000	1399.2	24	Gottschall et al., 2012
	750	8000	6000	11.5[a,c]; 22[a]; 28[a]; 30[a]; 5[a]; 73	Edwards et al., 2009
Naproxen	6	22,000	132	4[b]	Gottschall et al., 2012
	470	8000	3760	7.5[a]; 18; 29	Edwards et al., 2009
Triclocarban	4940	22,000	108,680	40	Gottschall et al., 2012
	8000	8000	64,000	2.5[a]	Edwards et al., 2009
Triclosan	10,900	22,000	239,800	73	Gottschall et al., 2012
	14,000	8000	112,000	9.5[a,c]; 20[a]; 22[a]; 22.5[a]; 23[a]; 24[a]; 25[a]; 30[a]; 31[a]; 32[a]; 34[a]; 36[a]; 37[a]; 45[a]; 46[a]; 48[a]; 52[a]; 56[a]; 57[a]; 59[a]; 63[a]; 68[a]; 75[a]; 82[a]; 95[a]; 101[a]; 140[a]; 185[a]; 190[a]; 230[a]; 240[a]	Edwards et al., 2009
Gemfibrozil	24	8000	192	8[c]	Edwards et al., 2009
	57	22,000	1254	5[b]	Gottschall et al., 2012
Cotinine	1.3	8000	10.4	1.5[a,c]; 3.9[a]; 4.8[a]; 5.5[a]; 5.7[a]; 6.5[a]; 8.2[a]; 9.5[a]; 11	Edwards et al., 2009
	9.4	22,000	206.8	1[b]	Gottschall et al., 2012
Carbamazepine	9	8000	72	2.5[a,c]; 6[a]; 7[a]; 7.5[a]; 7.6[a]; 8.5[a]; 13[a]; 14[a]; 19[a]; 24[a]; 30[a]; 32[a]; 38[a]; 44[a]; 49[a]	Edwards et al., 2009
	183.6	22,000	4039.2	13; 5	Gottschall et al., 2012

[a] Values graphically estimated.
[b] <LOD.
[c] <LOQ.

Sampling strategies adopted in the investigations are not always well reported and it is not clear exactly how and with what frequency the sampling occurred. In order to have reliable data and reproducible tests, all details should be provided and analyses should be performed according to standard methodologies. In addition, concentrations should be provided in tables, as in figures they are not easily readable. In any case, a descriptive statistical analysis of the collected data should be reported in terms of the minimum, maximum and standard deviations.

Environmental conditions are essential in defining the main removal mechanisms of pollutants. These parameters are rarely provided but soil temperature or at least ambient temperature should be made available.

Further research should address the effect of different soil compositions and different agricultural management practices (including application methods, sludge application rate and frequency, and irrigation application rate) on the quality of surface runoff and tile drainage.

Investigations should consist of a comparative analysis of the results achieved by applying different types of sludge, different application rates, different application methods, and different irrigation frequencies on different soils in order to evaluate the most influential parameters on surface runoff. A common observation is that PPCPs tend to accumulate in soil, mainly in the case of soil characterized by micropores which do not favor the downward mobility of contaminants. Long-term investigations should also address the probability of the release of these accumulated compounds in the case of intense rain events, or drought/wet periods which could greatly influence the mobility of the contaminants by creating preferential paths within the soil.

Further studies should focus on the occurrence of microbial indicators in surface runoff and in tile drainage during different rainfall events, in particular different frequencies and in different soil types. The occurrence of antibiotic-resistant genes and bacteria in sludge amended soil should also be addressed, as highlighted by Urra et al. (2019).

In the Supplementary materials section, tables and figures provide further details of the reviewed studies (Table S1 in an Excel file and Fig. S1 and Tables S2–S7 in a pdf file). Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.11.249.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Occurrence of Micropollutants in Wastewater and Evaluation of Their Removal Efficiency in Treatment Trains: The Influence of the Adopted Sampling Mode

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Abstract: The monitoring of micropollutants in water compartments, in particular pharmaceuticals and personal care products, has become an issue of increasing concern over the last decade. Their occurrence in surface and groundwater, raw wastewater and treated effluents, along with the removal efficiency achieved by different technologies, have been the subjects of many studies published recently. The concentrations of these contaminants may vary widely over a given time period (day, week, month, or year). In this context, this paper investigates the average concentration and removal efficiency obtained by adopting four different sampling modes: grab sampling, 24-h time proportional, flow proportional and volume proportional composite sampling. This analysis is carried out by considering three ideal micropollutants presenting different concentration curves versus time (day). It compares the percentage deviations between the ideal concentration (and removal efficiencies) and the differently measured concentrations (removal efficiencies) and provides hints as to the best sampling mode to adopt when planning a monitoring campaign depending on the substances under study. It concludes that the flow proportional composite sampling mode is, in general, the approach which leads to the most reliable measurement of concentrations and removal efficiencies even though, in specific cases, the other modes can also be correctly adopted.

Keywords: average daily concentration; mass loading; micropollutants; removal efficiency; sampling mode; uncertainties

1. Introduction

In planning a monitoring campaign, difficulties may arise in defining the sampling strategy, namely the mode and frequency of sample withdrawal in order to collect a number of samples which can be considered representative of the environment, the phenomenon or the process under study. Limiting attention to the water environment (namely raw wastewater, treated effluent, surface water and groundwater), different sampling modes may be utilized: water samples can be instantaneous (grab samples) or composite. In the second case, the resulting composite samples may be time proportional, flow proportional or volume proportional. Moreover, the reference interval for each composite sample could be 24 h or a fraction of the day (12 h, 4 h, or 3 h) [1]. With regard to withdrawal frequency, it is important to plan the sampling in order to pinpoint the (expected or potential) different behaviors in the occurrence of the compounds under study over a period of time [2,3].

In the case of monitoring campaigns tackling compounds occurring at very low concentrations, in the range of ng/L–µg/L—the so-called 'micropollutants'—it is fundamental to adopt an adequate sampling strategy and also to report it in detail along with the collected results [4–6]. Pharmaceuticals and personal care products, flame retardants and parabens are just some of the groups of (micro)pollutants of emerging concern. There has been a sudden increase in studies and publications dealing with the occurrence of these (micro)pollutants in different water environments, and relative removal technologies, from conventional treatments to the most promising technologies and different treatment trains. Most of them are still unregulated compounds (thus their limits in the case of discharge of a treated effluent into a surface water body have not yet been defined), but attention to their potential effects on the environment and human health is increasing and studies are in progress in many parts of the world [7–9].

Micropollutants can also be present in industrial wastewater. For instance, a petrochemical wastewater treatment plant may receive raw wastewater from different production wards within the industrial pole, characterized by a wide spectrum of pollutants. Cattaneo et al. [10] report the case of the petrochemical site of Porto Marghera, near Venice in Italy, where the purpose-built wastewater treatment plant must adhere to (strict) authorized limits for the occurrence of macropollutants (among them: suspended solids, biological oxygen demand, total Kjeldahl nitrogen, and nitrates) and ten micropollutants (the so-called "ten forbidden substances": cyanides, arsenic, cadmium, mercury, lead, organic chloride pesticides, hexachlorobenzene, tributyltin, polychlorinated biphenyls (PCB), dioxins and polycyclic aromatic hydrocarbons (PAH)) in the treated effluent. Sometimes, regulations may also require that the wastewater treatment plant guarantees removal for a selection of (micro)pollutants, in order to demonstrate that it acts as an efficient barrier against them. It is important to underline that in all these situations, a correct sampling mode must be adopted and clearly reported in detail with the results in order to be able to evaluate how representative and reliable the collected measured concentrations are.

Investigations into the occurrence of micropollutants in wastewater have highlighted that many of them may exhibit a substantial variation in concentration over the day (e.g., sulfamethoxazole and ciprofloxacin, [11–13]), the week (e.g., fluoruracil, diatrizoate, iomeprol and iohexol [2]), and the month (e.g., cefazolin and carbamazepine, [3]). Others have drawn attention to the temporal variation and distribution of selected pharmaceuticals in surface water bodies (among them [14,15]).

The issue of the influence of the sampling mode adopted in monitoring micropollutants has been addressed by many researchers in the last 10 years. Only in a few studies has this issue has been addressed with great detail (among them [2,4–6,16,17]); more often the issue is remarked on but not well discussed [1]. Particularly interesting are the sophisticated studies carried out by Ort and colleagues in [5,6,16,17] regarding the occurrence of pharmaceuticals and diagnostic agents in raw (municipal and hospital) wastewater and treated effluents, as well as in surface water, leading to suggestions for monitoring campaigns of micropollutants on the basis of the number of pulses containing the substance of interest (i.e., the number of toilet flushes at the sampling location) for a catchment area.

The current paper focuses on this issue following another approach: it faces the question by presenting and discussing numerical examples referring to some (representative) micropollutants characterized by different concentrations versus time curves.

In particular, it refers to three substances presenting very different profiles of concentration over the day (a highly variable compound, a randomly variable compound and a compound with low variability), and for each of them it evaluates: (i) the average daily concentration in the case of grab sampling, 24-h time proportional, 24-h volume proportional and 24-h flow proportional composite sampling; and (ii) the daily mass loading based on the estimated average concentrations and the provided flow rate. Finally, it assesses (iii) the removal efficiency for one of the three substances based on the different values of average concentrations found by applying the different sampling modes. This study ends with the evaluation of the (percentage) deviations between the "measured" concentration obtained by adopting a specific sampling mode and the "ideal" average concentration of each representative compound, as well as the (percentage) deviation between the evaluated removal efficiency and the ideal one.

2. Materials and Methods

This study refers to a “theoretical” case study regarding the occurrence of three micropollutants characterized by a different concentration profile versus time (over the day). The simulated substances do not correspond to three specific compounds, but each of them is representative of a group of compounds with a similar concentration trend versus time (see Section 2.1). In this context, the investigations by [11,12,18,19] clearly show the variations in the concentration of micropollutants in municipal raw wastewaters and hospital effluents over a typical day. These experimental values provide us useful insights into the different possible profiles of concentration of micropollutants and allow us to define theoretical ad hoc curves of concentrations versus time for three different representative scenarios.

As to flow rate, the study refers to a small urban settlement, which, according to the technical literature, is characterized by enhanced variations at well-known day hours [20]. A very similar flow pattern was found for the effluent of a medium-large hospital [12,21,22]. In this context, an ad hoc curve of flow rates versus time (during a typical day) was defined on the basis of literature data and evidences [20,21] (see Section 2.2).

It is important to keep in mind that, in the following, attention has to be paid to the variations in concentrations and flow rate over the day and not to the specific (absolute) values reported in the graphs. This means that considerations and results developed in this study can be applied to a small urban settlement as well as a medium to large hospital characterized by similar concentration profiles but different (maximum and minimum) concentration values (often higher in the hospital effluent, [3,21].

2.1. Definition of Representative Compounds

Three key compounds were considered for the study:

- a substance whose concentration in wastewater presents few but evident variations over the day, such as the diagnostic agents gadolinium and iopamidol [18], the cytostatic agent 5-fluoruracil [2] or the diuretic furosemide and the antibiotic sulphamethoxazole [13]. Such a substance is called a ‘high variability substance’, HV_Sub. During the night, its concentration decreases even lower than the corresponding limit of detection (Lod) for some hours;
- a substance whose concentration in wastewater presents a modest variation over the day, and is also detectable during the night, such as the anti-inflammatory ketoprofen [19], the antiseptic triclosan and the anticonvulsant agent phenytoin [13], and the antibiotic trimethoprim [13,23]. This is called the ‘low variability substance’, LV_Sub. It may happen that during the night its concentration decreases to values below its limit of detection, but only for very short periods;
- a substance whose concentration “randomly” varies over the day, such as the antibiotics ciprofloxacin [12] lincomycin [23], the anti-inflammatories diclofenac [13], and 4-tert octylfenol (a degradation product of a surfactant). This substance is called a ‘random variability substance’, RV_Sub. Its profile pattern is not easily predictable.

Based on literature data and in particular on the observed temporal variations in concentrations reported for the cited compounds in wastewater [2,4,11–13,18,19,23], 24 values of concentrations were set (one for each hour of a day) for the three key compounds (Table S1). Based on them, a nonlinear regression curve was carried out for each substance, by means of the software MATLAB R2018b. The corresponding polynomial functions are reported in Equations (1)–(3) (where concentration is in ng/L and time in min). In this way, the concentration c versus time t curves were set as continuous functions $c(t)$ (Figure 1).

$$c_{HV\_Sub}\,(t) = 0.015t^8 - 0.16t^7 + 6.58t^6 - 141.33t^5 + 1660.2t^4 - 10367t^3 + 30900t^2 - 32689t + 1289.9 \quad (1)$$

$$c_{RV\_Sub}\,(t) = -0.27t^5 + 14.93t^4 - 280.73t^3 - 1977.7t^2 - 2162.1t - 14009 \quad (2)$$

$$c_{LV\_Sub}\,(t) = +0.008t^9 + 0.2t^8 - 3.07t^7 + 26.93t^6 - 126.64t^5 + 304.67t^4 - 612.86t^3 + 1502.8t^2 \quad (3)$$

These curves may represent the occurrence in the influent wastewater of a treatment step of three compounds whose characteristics are reported above.

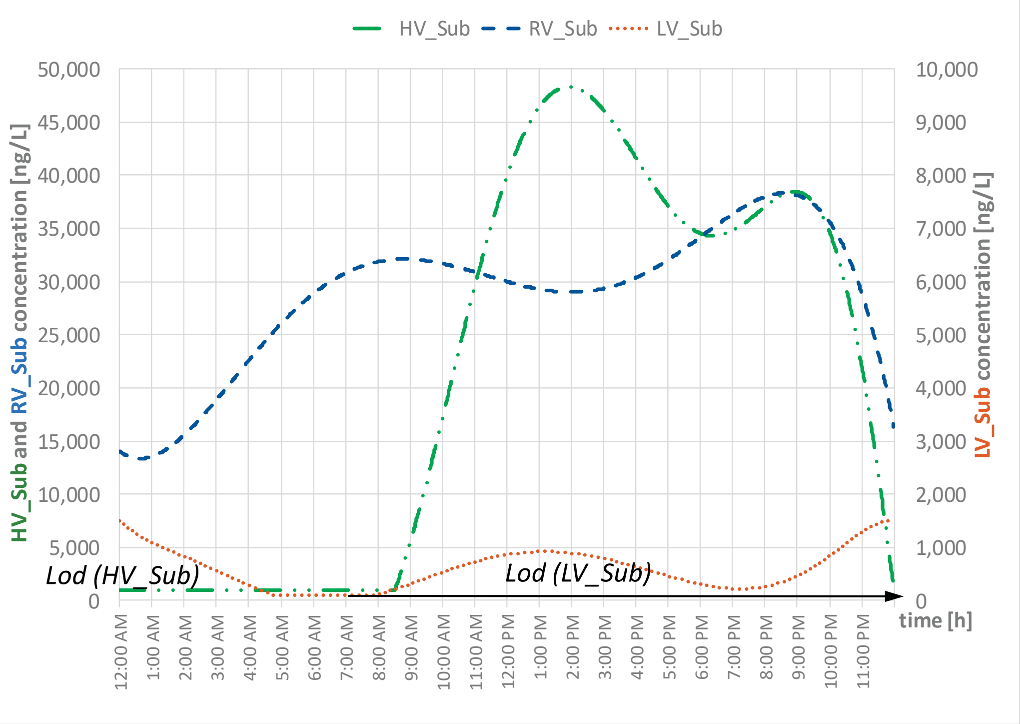

Figure 1. Concentrations versus time for the three key compounds considered in the study. Note that the Y-axis for the low variability substance (LV_Sub) is on the right and the Y-axis for the high variability substance (HV_Sub) and the random variability substance (RV_Sub) is on the left.

2.2. Flow Rate Curves Versus Time

It was assumed that the flow rate refers to the wastewater generated by a small catchment area (around 3500 inhabitants characterized by an individual water consumption of 200 L/(inhabitant day)) or a medium-large hospital (characterized by around 900 beds with a patient water consumption of 700 L/(patient day), according to literature [21]).

The selection of this size of wastewater source (small urban settlement or medium-large hospital) was in order to obtain more frequent and enhanced variations with regard to a larger urban settlement, as clearly shown by data provided in literature [12,22]. The flow rate referring to the whole day Q_{daily} is 634.5 m^3/d. Based on literature studies on curves of flow rate versus time (day) in settlement/hospital of this size [12,20,22], 24 values of flow rate were set (Table S1) and by software MATLAB R2018b a nonlinear regression was carried out leading to Equation (4) (Q is in m^3/h and time t in min). It is reported in Figure 2.

$$Q(t) = +0.01t^{11} - 0.11t^{10} + 0.78t^{\,9} - 3.20t^8 + 7.12t^7 - 7.77t^6 + 5.10t^5 + 16.19t^4 \quad (4)$$

The wastewater volume flowing as a function of the time $V(t)$ is obtained by the integration of Equation (4):

$$V(t) = \int_{t=0}^{1440} Q(t)dt = 0.0001t^{12} - 0.01\,t^{11} + 0.078\,t^{10} - 0.35\,t^9 + 0.89\,t^8 - 1.11\,t^7 + 0.85\,t^6 + 3.24\,t^5 \quad (5)$$

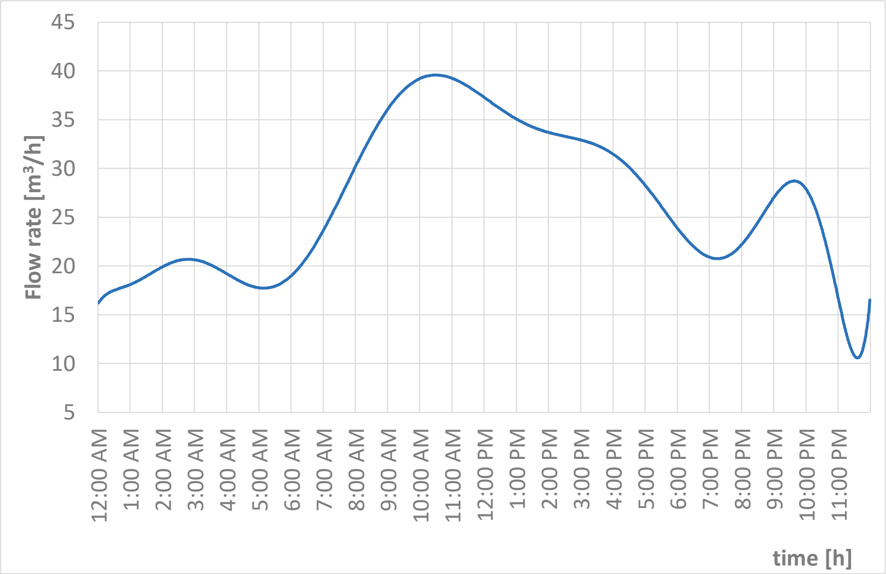

Figure 2. Flow rate versus time for the case study considered.

2.3. The Sampling Modes Adopted and Compared

The sampling modes compared in this study are those defined in Table 1:

Table 1. Description of the sampling modes adopted and compared in this study for average concentrations of the different compound.

Sampling	Description	Water Volume Sampled	Sampling Time, (Number of Samples)
Grab	The sampling consists of instantaneous (grab) wastewater withdrawal(s). The monitoring may include either one grab sample or a number of grab samples. The sampling time is defined by the investigation (monitoring protocol).	The requested wastewater volume for analysis	8 a.m. (1) 8 a.m. + 5 p.m. (2) 8 a.m. + 12 p.m. + 5 p.m. (3) 8 a.m. + 12 p.m. + 4 p.m. + 11 p.m. (4)
24-h time proportional composite	The sampling is performed at constant time intervals. It is the most common sampling mode. This is also called constant time, constant volume (CTCV)	A constant volume V_{sample} taken at each sampling instant	Every hour (24) Every 2 h (12) Every 4 h (6) Every 8 h (3)
24-h flow proportional composite	The sampling is performed at constant time intervals. The volume of wastewater taken is proportional to the flow rate flowing at each instant of sampling. This is also called constant time, variable volume (CTVV)	A linear interpolation curve is defined between the minimum and maximum wastewater flow and wastewater sampled over the whole observed range of variability of the wastewater flow (see Figure 3)	Every hour (24) Every 2 h (12) Every 4 h (6) Every 8 h (3)
24-h volume proportional composite	The sampling takes the same wastewater volume at variable time intervals, after a defined volume of wastewater has passed the sampling point. This is also called constant volume, variable time (CVVT)	A constant volume V_{sample} is taken at each defined sampling time	Frequency: Three times a day (3) Six times a day (6) Twelve times a day (12) Twenty-four times a day (24)

With regard to the flow proportional sampling mode, in order to define the direct proportionality curve between wastewater to be sampled and the flowing wastewater flow rate, the expected range of variability of the flow rate has to be known. In the case study, the observed range varied between 17.3 m^3/h and 38 m^3/h but, for the sake of caution, it was supposed that it might vary between 10 m^3/h and 50 m^3/h. It was then supposed that in the case of a flow rate of 10 m^3/h, the volume to sample would be equal to 20 mL, and in the case of 50 m^3/h, the volume to sample would be 100 mL, resulting in the linear relationship between volume to sample (y) and flow rate (x) y = 2x (Figure 3). Other direct proportional curves could be assumed for different cases.

In order to complete the analysis and the comparison among the available sampling strategies, the Supplementary Material contains Figures S1–S4 showing some details of the different sampling modes. Each graph remarks on the number and volume of samples withdrawn and the instant at which wastewater samples are taken in order to have all the information necessary to obtain the average concentration of the compound under study according to the adopted sampling approach. The flow rate curve versus time is also drawn in order to remark how variations in the flow rate may affect the evaluation of the micropollutant average concentration.

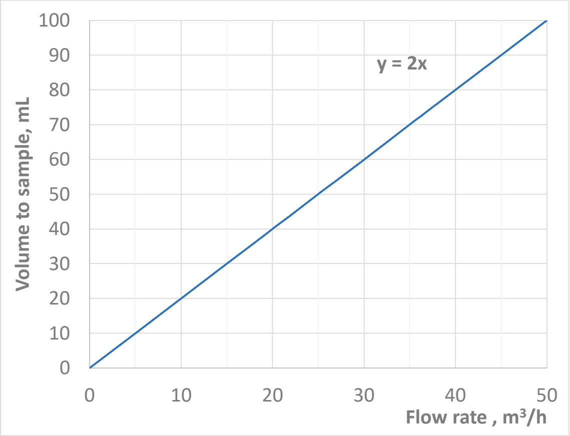

Figure 3. Relationship (direct proportionality) between volume to sample and flow rate for the flow proportional sampling mode.

2.4. *Daily Average Concentration Evaluation*

The ideal (true) obtainable concentration c_{ideal} for each compound was evaluated by means of Equation (6):

$$c_{ideal} = \frac{\sum_{i=1}^{1440} c_i\, Q_i}{\sum_{i=1}^{1440} Q_i} \tag{6}$$

where c_i is the concentration (ng/L) at minute i (in total 60 × 24 min = 1440 min) and Q_i is the flow rate (L/min) at the same minute i. Note that Q_i is numerically equal to the volume flowing during the minute i (V_i). The concentration value can be considered an accurate value (on a minute measurement basis) of the concentration of the compound. A shorter time interval could also be assumed, for instance the second, and in this case i varies up to 86,400.

The average concentrations of the key compounds were evaluated by assuming the different sampling modes. Note that, with regard to Figure 1, for the substances HV_Sub and LV_Sub, during the night their concentrations decrease below the corresponding Lod (according to the adopted analytical methods, but this issue is beyond the current study). For the sake of caution, it was assumed that the concentration was equal to the Lod (respectively 1000 ng/L and 100 ng/L). In addition, their corresponding limits of quantification (Loq) were assumed equal to 2500 ng/L and 250 ng/L: when their concentration was below the corresponding Loq, it was set equal to 0.5 Loq, according to [24].

In the case of grab sampling, the daily average concentration $\bar{c}_{grab}$ (ng/L) of a compound in the wastewater is based on the number n of the water grab samples withdrawn (Equation (7)). They were assumed to be 1, 2, 3 or 4 (as described in Table 1):

$$\bar{c}_{grab} = \frac{\sum_{i=1}^{n} c_i}{n},\ n = 1,\ 2,\ 3,\ 4 \tag{7}$$

where c_i is the concentration of the key compound in sample i in ng/L.

In the case of 24-h time proportional composite sampling, the daily average concentration of the key substance $\bar{c}_{time\ prop}$ was evaluated according to Equation (8):

$$\bar{c}_{time\ prop} = \frac{\sum_{i=1}^{k} c_i V_{sample}}{k\ V_{sample}} = V_{sample}\frac{\sum_{i=1}^{k} c_i}{k\ V_{sample}} = \frac{\sum_{i=1}^{k} c_i}{k},\ \ k = 24,\ 12,\ 6,\ 3 \tag{8}$$

where c_i is the concentration (ng/L) of the key compound in sample i, V_{sample} is the wastewater volume sampled (mL) at each withdrawal (always the same) and k is the number of samples taken according to the defined monitoring protocol (Table 1).

In the case of 24-h flow proportional composite sampling, the daily average concentration of the key substance $\bar{c}_{flow\ prop}$ (ng/L) was evaluated according to Equation (9):

$$\bar{c}_{flow\ prop} = \frac{\sum_{i=1}^{k} c_i\ \alpha\ Q_i}{\sum_{i=1}^{k} \alpha\ Q_i},\ \ k = 24,\ 12,\ 6,\ 3 \tag{9}$$

where c_i is the concentration of the key compound in sample i, in ng/L, $\alpha\ Q_i$ is the withdrawn wastewater volume (mL), α being the coefficient of direct proportionality (equal to 2) between the flow rate Q_i flowing at the sampling point at that instant and the volume to be sampled (see graph in Figure 2).

In the case of 24-h volume proportional composite sampling, the daily average concentration of the key substance $\bar{c}_{volume\ prop}$ (ng/L) was evaluated according to Equation (10):

$$\bar{c}_{volume\ prop} = \frac{\sum_{i=1}^{k} c_i\ V_{sample}}{k\ V_{sample}} = V_{sample}\frac{\sum_{i=1}^{k} c_i}{k\ V_{sample}} = \frac{\sum_{i=1}^{k} c_i}{k},\ \ k = 24,\ 12,\ 6,\ 3 \tag{10}$$

where c_i is the concentration of the key compound in sample i, in ng/L, and V_{sample} the wastewater volume (mL) sampled exactly after that the defined fraction $\frac{1}{k}$ of the daily volume of wastewater produced (V_{daily}) is flowed. Note that numerically, V_{daily} corresponds to Q_{daily}.

For the sake of clarity, it is here reported the sequence of steps necessary to obtain the average concentrations resulting from applying the different sampling modes described in Table 1. For grab sampling, time proportional and flow proportional composite sampling modes, the steps are:

1. definition of the sampling times according to Table 1;
2. calculation of the values of concentrations at each sampling time defined in the last column of Table 1 for the representative compound under study by the corresponding curve (Equations (1)–(3));
3. evaluation of the average daily concentration by applying the equation corresponding to the selected sampling mode (Equations (7)–(9)).

For the volume proportional composite sampling mode, the steps are:

1. definition of the frequency of sampling (k samples), according to the last column of Table 1 and the wastewater volume V_{vp} ($=V_{daily}/k$) which has to flow before collecting a water sample;
2. evaluation of the k sampling instants t_n, by means of the $V(t)$ curve (Equation (5)) posing $V(t_n) = n\ V_{vp}$ with $n = 1, \ldots, k$;
3. calculation of the values of concentrations at each sampling time t_n by the corresponding curve (Equations (1)–(3));
4. evaluation of the average daily concentration by applying Equation (10).

2.5. Mass Load Evaluation

The daily mass load ML (ng/d) of each substance can be evaluated as the product of the average concentration of the compound of interest $\bar{c}$ (ng/L) according to the different sampling modes

(Equations (7)–(9)) and the daily flow rate Q_{daily} (L/d). It is clear that this is directly proportional to the average concentrations through the daily flow rate (=634.5 m^3/d).

$$ML = \bar{c}\, Q_{daily} \tag{11}$$

2.6. *Removal Efficiency Evaluation of a Micropollutant: Considerations and Remarks*

As discussed in [25], with regard to a generic wastewater treatment step (Figure 4), the percentage efficiency μ in removing a specific contaminant j is defined on the basis of the mass loading (corresponding to the product: concentration × flow rate) in its influent (stream number 1) and effluents (stream numbers 2 and 3) at a set time interval, in accordance with Equation (12):

$$\mu_{total,\ j} = \frac{c_{1,j}\, Q_1 - \left(c_{2,j}\, Q_2 + c_{3,j}\, Q_3\right)}{c_{1,j}\, Q_1} \times 100 \tag{12}$$

As reported in the caption of Figure 4, the step could produce two different effluents (as in a conventional activated sludge system or in a membrane bioreactor: the clarified effluent or the permeate and the excess sludge). Quite often, the equation used for the evaluation of removal efficiency in an activated sludge system does not consider the occurrence of the (micro)pollutant in the excess sludge (this assumes $c_{3,j}$ = 0) and, as reported in [25], this leads to an "apparent" removal efficiency, generally higher than the total removal efficiency.

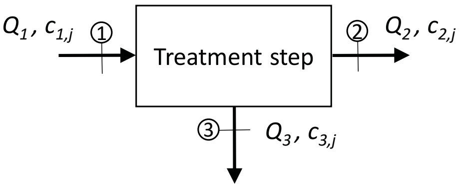

Figure 4. Representation of a generic wastewater treatment step, for instance an activated sludge system with the two effluents: a liquid phase (the clarified effluent, stream number 2) and the solid phase that is the excess sludge (stream number 3). In the case of a treatment step with only one effluent stream, stream number 3 does not appear.

In this study we have evaluated removal efficiency in the case of a treatment step with only one effluent stream (namely a polishing treatment by constructed wetlands, lagoons, and rapid filtration). Moreover, the time interval assumed for its evaluation is the day, hence the micropollutant concentrations c_1 and c_2 (referring to the influent and the effluent) considered are the daily average concentrations obtained by following the different sampling modes described in Table 1, Q_1 = Q_2, and they are numerically equal to V_{daily}.

The ideal removal efficiency μ_{ideal} was evaluated by means of Equation (13):

$$\mu_{ideal} = \frac{\sum_{i=1}^{1440} c_{1,i}\, Q_{1,i} - \sum_{i=1}^{1440} c_{2,i}\, Q_{2,i}}{\sum_{i=1}^{1440} c_{1,i}\, Q_{1,i}} \tag{13}$$

where $c_{1,i}$ and $c_{2,i}$ are the micropollutant concentrations (ng/L) at minute i in the influent and effluent respectively, $Q_{1,i}$ and $Q_{2,i}$ are the flow rates (L/min) at minute i in the influent and effluent ($Q_{1,i}$ = $Q_{2,i}$).

Case Study for the Evaluation of Removal Efficiency

The analysis of the removal efficiency evaluation refers to the data reported in Figure 5, which represents the profile of a randomly variable compound, as described in Section 2.1 for the influent and effluent of a small wastewater treatment plant, characterized by a hydraulic retention time (HRT) of 12 h.

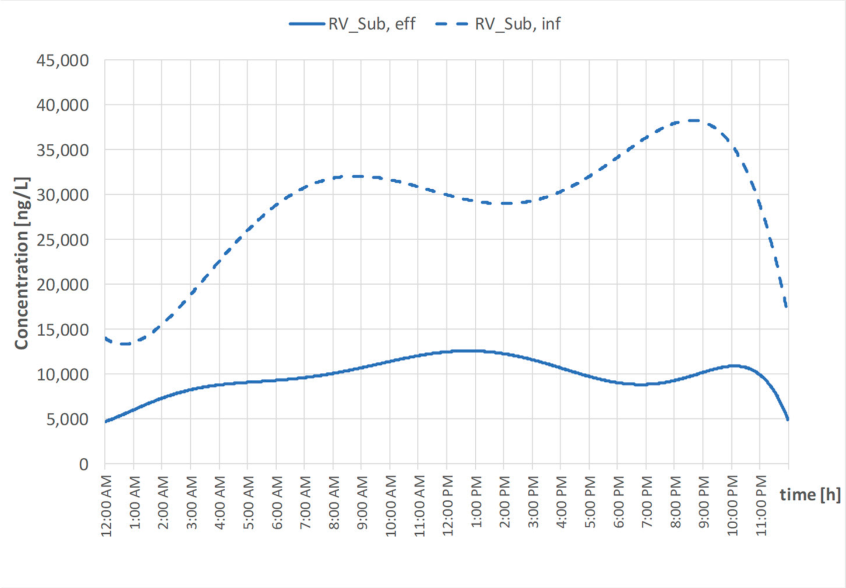

Figure 5. Occurrence of the same compound in a small wastewater treatment plant influent (dashed line) and effluent (continuous line).

The correlation between the concentration of the key compound and time (min) in the influent corresponds to Equation (2) and for the effluent, to Equation (14). This curve is obtained following the same procedure adopted for Equations (1)–(3) and it is based on the 24 raw data compiled in Table S1:

$$c_{\mathrm{RV\_Sub,\,eff}}\,(t) = +0.09t^7 - 2.59t^6 + 34.09t^5 - 206.33t^4 - 408.29t^3 - 1082.9t^2 + 4736.7t^1 \quad (14)$$

where t is in minutes and $c_{\mathrm{RV\_Sub,\,eff}}$ in ng/L.

The removal efficiency for the key compound was evaluated according to the different sampling modes defined in Table 2.

Table 2. Description of the sampling modes adopted and compared in this study for the removal efficiency evaluation of RV_Sub.

Sampling	Sampling Time for Influent and Effluent (Number of Samples)	Some Remarks and Number of Estimated Values of Removal Efficiencies in Brackets
Grab	Every hour (24), hydraulic retention time (HRT) not considered Every hour (24), HRT considered	Removal evaluated each hour (24 values)
	8 a.m.; 5 p.m. (2) HRT not considered 8 a.m.; 5 p.m. (2) HRT not considered 8 a.m.; 12 p.m.; 5 p.m. (3) HRT not considered 8 a.m.; 12 p.m.; 5 p.m. (3) HRT considered 8 a.m.; 12 p.m.; 4 p.m.; 11 p.m. (4) HRT not considered 8 a.m.; 12 p.m.; 4 p.m.; 11 p.m. (4) HRT considered	Removal based on average values for influent and effluent (one value)
Time proportional	24-h time proportional composite sample, time interval between two consecutive withdrawals equal to 1 h (1)	(One value)
Flow proportional	24-h flow proportional composite sample, time interval between two consecutive withdrawals equal to 1 h (1)	(One value)
Volume proportional	24-h volume proportional composite sample. Twenty-four samples a day mixed for the composite sample as reported in Table 1 (1)	(One value)

In addition, the removal efficiency of RV_Sub was also estimated, assuming that concentrations were known with a frequency equal to 1 min. This is considered the "ideally obtainable" value of removal efficiency. The collection of this amount of concentrations for many micropollutants is completely unrealistic, due to the high costs and time requested for their analytical determination.

3. Results

3.1. Average Concentration of the Key Compounds

The ideally obtainable daily average concentrations of the three representative compounds were found by applying Equation (6) and are reported in Table 3.

Table 3. Ideal average concentrations c_{ideal} for the three substances and corresponding standard deviation (SD) (c_{ideal} ± SD).

HV_Sub, ng/L	LV_Sub, ng/L	RV_Sub, ng/L
24,561 ± 18,305	586 ± 377	29,609 ± 6674

These values are compared here with the average concentrations resulting from applying the different sampling modes described in Table 1, according to the procedure described in Section 2.4. Details of the application of this procedure is reported in Tables S2–S4 with regard only to RV_Sub. For all the substances, the evaluated average concentrations are here reported in tables: Table 4 refers to the case of a different number of grab samples, Table 5 to 24-h time proportional composite sampling, Table 6 to flow proportional composite samples and finally, Table 7 to volume proportional composite samples.

Table 4. Average concentrations of the three substances in the case of grab samples (with the different number of samples collected).

Number (#) of Grab Samples	HV_Sub, ng/L	LV_Sub, ng/L	RV_Sub, ng/L
1	1000	112	31,852
2	19,041	287	31,954
3	26,014	478	31,301
4	26,117	724	30,263

Table 5. Average concentrations of the three substances in the case of time proportional sampling (with the different number of samples collected).

Interval (h), (#of Samples)	HV_Sub, ng/L	LV_Sub, ng/L	RV_Sub, ng/L
1 (24)	21,751	590	28,664
2 (12)	21,518	595	28,472
4 (6)	20,270	608	27,799
8 (3)	14,535	750	25,409

Table 6. Average concentrations of the three substances in the case of flow proportional sampling (with the different number of samples collected).

Interval (h), (#of Samples)	HV_Sub, ng/L	LV_Sub, ng/L	RV_Sub, ng/L
1 (24)	24,477	590	29,525
2 (12)	24,412	596	29,443
4 (6)	23,550	581	29,000
8 (3)	17,406	612	27,543

Table 7. Average concentrations of the three substances in the case of volume proportional sampling.

Frequency (#/d)	HV_Sub, ng/L	LV_Sub, ng/L	RV_Sub, ng/L
3	18,848	888	25,948
6	22,359	644	28,702
12	23,867	602	29,314
24	24,365	590	29,541

It emerges that for all three substances, average concentrations resulting from the grab sampling mode present the widest ranges of variability, whereas the 24-h flow proportional composite sampling show the smallest ranges of variability. Moreover, one grab sample may lead to an enhanced underestimation or overestimation, depending on the time of sampling and the concentration profile. In the case of a substance with a "flat" curve of concentrations versus time, a grab sample could be considered representative of the "average" daily concentration whatever time it is taken. But in all the other situations, a grab sample should be avoided.

An increment in the frequency of withdrawal for the composite sampling mode always leads to an average concentration measurement, which is closer to the ideal value, whatever the concentration profile.

With regard to the HV_Sub average concentrations reported in Tables 4–7, it emerges that the lowest value is 1000 ng/L, and the highest is 26,117 ng/L found with the grab sampling mode. This is due to the fact that this substance presents very low concentrations during the night (between 12:00 a.m. and 9:00 a.m. it was below its limit of detection (Lod) and for the sake of caution, was assumed to be equal to its Lod value) and the lowest value corresponds to one grab sample taken at 8:00 a.m. and the highest to four grab samples taken at 8:00 a.m., 12:00 p.m., 4:00 p.m. and 11:00 p.m., with only one sample collected in the interval in which concentrations are very low, assumed equal to the corresponding Lod (1000 ng/L).

With regard to LV_Sub, the lowest average concentration was found with one grab sample (112 ng/L) and the highest with the 24-h volume proportional sample, with samples taken three times a day (818 ng/L).

Finally, referring to RV_Sub, the highest average concentration was found with the grab sample taken at 8:00 a.m. and the lowest average concentration with the 24-h composite sampling mode (three samples taken every eight hours). It is important to observe that the highest value does not correspond to the maximum concentration of the RV_Sub profile of concentration: 38,298 ng/L occurring at 8:35 p.m.

For each of the three substances, the percentage deviation ($=\frac{c - c_{ideal}}{c_{ideal}} \times 100$) between the ideal average concentration c_{ideal} (see Table 3) and the "measured" average concentrations obtained following a specific sampling mode are reported in the three "target" diagrams in Figure 6. The circumferences refer to percentage deviations (1%, 10%, 40% and 100%) on a logarithmic scale. Full symbols represent situations in which the average measured concentration is higher than the corresponding ideal concentration (overestimation) and empty symbols to situations in which the average measured concentration is lower than the corresponding ideal concentration (underestimation).

It emerges that for all three compounds, the sampling mode and frequency which lead to the best estimation of the average concentration are always the 24-h flow proportional composite sampling with samples taken every hour and the 24-h volume proportional composite sampling with 24 samples per day. Moreover, the sampling mode with the smallest deviation is flow proportional: the deviation always remains below 10% with only one exception (HV_Sub with samples taken every eight hours).

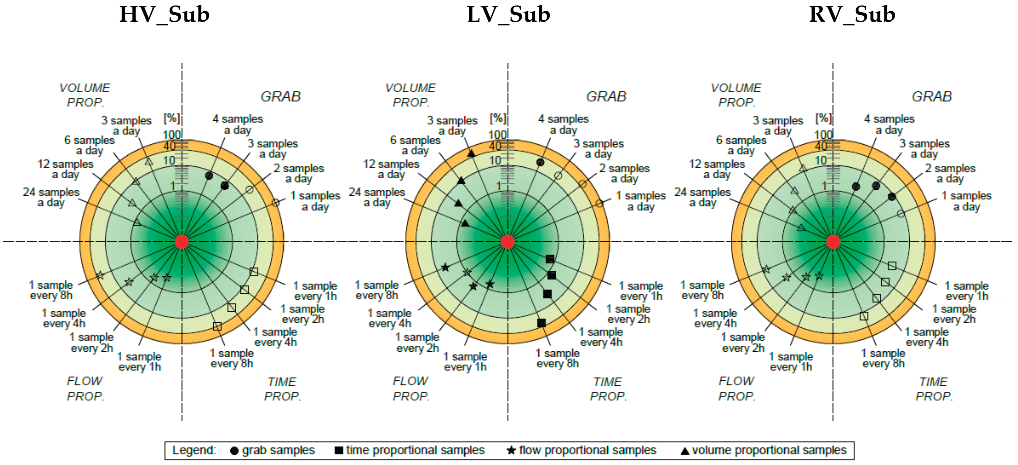

Figure 6. Percentage deviations between the ideal concentration of each substance (red dot) and the measured average concentrations found following the different sampling modes, defined in Table 1. Circumferences in the three graphs refer to the different values of percentage deviation on a log scale. Full symbols correspond to an overestimation and empty symbols to an underestimation.

It is interesting to observe that the "measured" average concentration is only overestimated (full symbol) for LV_Sub, whereas for HV_Sub and RV_Sub measured average concentrations are underestimated (empty symbols), with just a few exceptions. This fact can be explained by the different concentration profiles versus time of the compounds. Figure 1 shows that LV_Sub is the only compound with night concentrations even higher than diurnal ones and, in the case of time and volume proportional composite samplings (which do not consider the weight of the flow rate) this leads to an overestimation. The ideal average concentration, as shown by the definition in equation 6, weights the concentration with the flow rate, which is lower during the night (Figure 2).

These considerations provide a good explanation as to why time proportional composite sampling could be a good mode for RV_Sub. For this substance, the range of percentage deviations is the smallest in comparison to the range of the other two compounds.

The analysis of the different average concentration values for the three compounds highlights that the selection of the sampling mode which is best suited to the aim of the monitoring campaign depends on the type of substance and on its expected concentration profiles, if known. It could be of interest to know the average concentration of the compound in order to design a treatment train capable of removing it. It could also be of interest to know the highest concentration during the day in case an environmental risk assessment should be carried out (in this case, the European Guidelines [26–28] suggest taking the maximum concentration of a compound in order to consider the worst-case scenario). In fact, if the substance has very low concentrations during the night or in well-known daytime intervals, monitoring planning could avoid this period.

3.2. Mass Load Evaluated for Each Substance

The ideal mass loading of each substance was evaluated by Equation (11) and is reported in Table 8. As highlighted in Section 2.5, the percentage deviations with respect to the ideal value of the mass loading of each substance is the same as those found for the average concentrations with regard to the same sampling mode and (obviously) substance.

Table 8. Evaluation of the mass load for the three substances.

HV_Sub, g/d	LV_Sub, g/d	RV_Sub, g/d
15.6	0.37	18.8

3.3. Average Removal Efficiency for RV-Sub

The ideally obtainable daily removal efficiency was obtained by applying Equation (13) and is equal to 67.8%. On the basis of the average daily concentrations in the influent and effluent obtained by the different sampling modes (Table 2), the corresponding removal efficiencies were evaluated.

In the first case, a grab sample mode is followed; the flow rate at the entrance and exit of the treatment step is assumed to be the same and samples are taken at the same time (HRT of the treatment step is not considered). The removal efficiency based on only one grab sample during a day and varies between 53% and 76% depending on the sampling time. If samples are taken considering the HRT of the plant (12 h), the removal efficiency varies between 7% and 84%, always depending on the sampling times at the two points. Figure 7 reports the values in both scenarios.

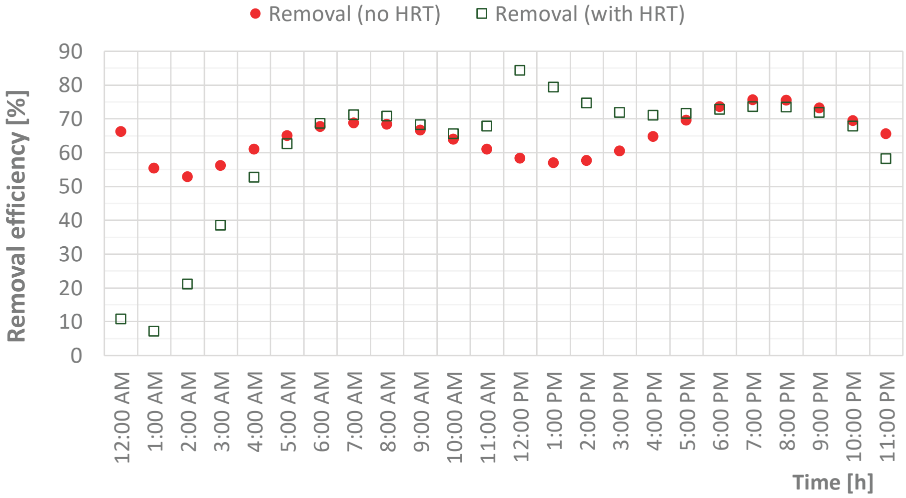

Figure 7. Evaluated removal efficiency of the RV_Sub on the basis of single grab samples taken at the influent and effluent at the same time (colored circle) and considering the hydraulic retention time (HRT) of the treatment step (void square).

Table 9 reports the RV_Sub removal efficiencies in the case of a different numbers (2, 3, 4) of grab samples taken in the influent and effluent, at the same time (case 1) and considering the HRT of the plant (case 2). It is important to underline that the removal efficiency is evaluated on the basis of the average values in the influent and the effluent of the n grab samples taken as remarked in the last column of Table 2.

Table 9. Removal efficiency of RV_Sub in the case of grab samples in different scenarios.

Number of Grab Samples	Case 1: HRT not Considered	Case 2: HRT Considered
2	68.9	71.2
3	65.9	75.3
4	64.3	71.1

It emerges that when HRT is considered, the removal efficiency is always higher than when it is neglected, and it is also higher than the ideally obtainable removal efficiency (equal to 67.8%).

In the case of 24-h time proportional composite sampling, the daily removal efficiency was equal to 65.8%; in the case of 24-h flow proportional composite sampling, the daily removal efficiency was equal to 67.8%, and in the case of 24-h volume proportional composite sampling, the efficiency was 65.4%, all of which are very close to the ideal removal efficiency (67.8%).

The target graph in Figure 8 reports and compares the percentage deviations ($=\frac{\mu-\mu_{ideal}}{\mu_{ideal}} \times 100$) between the ideal removal efficiency μ_{ideal} (67.8%, corresponding to the red circle in the center) and the values μ found following the different sampling modes.

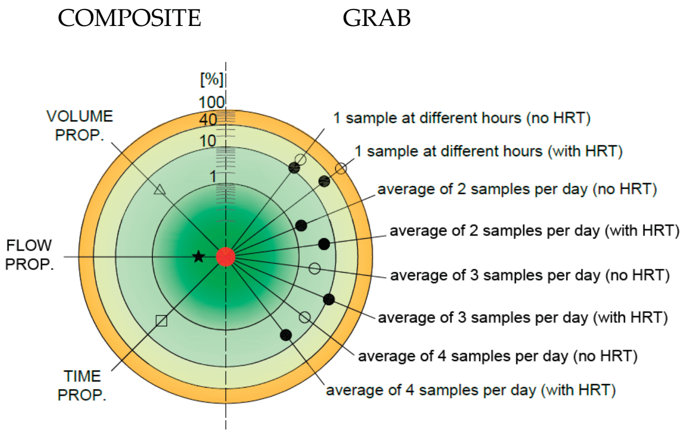

Figure 8. Percentage deviations between ideal removal efficiency for RV_Sub (red dot) and the evaluated removal efficiency found following the different sampling modes, defined in Table 1. Circumferences in the three graphs refer to different values of percentage deviation on a log scale. Full symbols correspond to an overestimation and empty symbols to an underestimation.

It emerges that, in the case of removal efficiency based on one grab sample, the ranges of percentage deviations vary between (−22%; +11.5%) without considering HRT and between (−89.6%; +24.2%) if HRT is considered. In case of more grab samples taken in a day, (considering or not considering the HRT), the percentage deviation remains between −5.0% and 11.1%. The flow proportional composite sampling mode leads to the most accurate evaluation (0.06%), compared to time proportional (−2.9%) and volume proportional (−3.5%) modes.

4. Discussion and Final Remarks

This study highlights the influence of the sampling mode on the collected measured concentrations of micropollutants which present different concentration profiles versus time (over the day). It also compares the removal efficiencies achieved in an ideal treatment step when influent and effluent concentrations are collected following different sampling modes. Unfortunately, this is not always reported and described in published papers, as highlighted by [1,5]. In particular in [1], a review dealing with the removal of pharmaceuticals from wastewater by different constructed wetlands, an analysis of the information regarding the adopted sampling modes allows the reader to "weigh up/assess" the reliability of the collected data presented. The most adopted mode in the case of monitoring campaigns regarding micropollutants in different water environments is that of 24-h time proportional composite sampling, whatever the micropollutant and its occurrence profile.

The three "ideal" substances considered in the current study are representative of three different cases and give some insights into the expected scenarios a researcher could find in investigating campaigns in terms of monitoring the occurrence and evaluating the removal of micropollutants. The analysis reported and discussed here provides some figures regarding the expected deviations with regard to ideal values, also called the 'true' concentrations and the "true" removal efficiency of a micropollutant. It was found that the flow proportional composite sampling mode leads to the best evaluation of the average concentration of a micropollutant (whatever the concentration profile is) and also of its removal efficiency. It is followed by the volume proportional composite sampling mode, and then by the time proportional one. The grab sample can be adopted if the number of collected samples is able to catch the main (expected) variations of concentrations over the day, in particular when the concentration curve versus time is flat, or when the aim of the monitoring campaign is to find the maximum concentration during the day in case of environmental risk assessment and it is known when it may occur. As most of the micropollutants are unregulated compounds, guidelines for sampling campaigns dealing with them are not available.

To complete the discussion on reliability of collected (measured data) it is important to spend some words on the issue of the uncertainties associated with the direct measurements of concentrations in the water environment. In the current study, it was found that the average evaluated concentrations obtained by applying Equations (1)–(3) and (12) lead to an uncertainty varying in the range between <1% and 30% for 24-h flow proportional composite sampling, between <1% and 40% for 24-h time proportional composite sampling, between <1% and up to 51% for 24-h volume proportional composite sampling and even up to 95% in case of one grab sample in a day.

These values are in agreement with other studies which found uncertainties varying from 10% in the case of 24-h flow proportional composite sampling [17,29] to 25% (even 100%) if time proportional composite sampling is adopted [30]. Regarding uncertainties associated with chemical analysis, literature studies found that they are lower than those for sampling; they may vary between 4% and 16% [31]. Finally, uncertainties in flow rate measurement may vary between 6% according to [17] to 20% according to [32].

These considerations underline the importance of properly defining a sampling mode in order to provide highly reliable data regarding the occurrence and also removal of micropollutants from wastewater.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/11/6/1152/s1, Table S1: Concentrations of the three representative compounds and values of flow rates used for defining the corresponding profile of concentrations and flow rate over the day (Figures 1, 2 and 5 in the manuscript); Table S2–S4: Evaluation of the average concentrations of the three representative compounds following the different sampling modes; Figure S1: Flow rate profile (dashes) and withdrawn volume (full circles) for each grab sample. Note the volume is always the same at the defined instants of time in case of four grab samples (i.e., 8:00 a.m.; 12:00 p.m.; 5:00 p.m. and 11:00 p.m.); Figure S2: Flow rate profile (dashes) and volume withdrawn (full circles) for the 12 water samples. Also in this case, the sample volume is constant. Samples are taken every 2 h; Figure S3: Flow rate profile (dashes) and volume withdrawn (full circles) for the 12 water samples. The volume taken for the different samples is proportional to the flow rate at the sampling time. Samples are taken every 2 h; Figure S4. Flow rate (dashes) profile and volume withdrawn (full circles) for the 12 water samples. The volume taken for the different samples is constant. Samples are taken when $\frac{1}{12}Q_{daily}$ is passed at the sampling point.

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Review

A review of the occurrence of selected micropollutants and microorganisms in different raw and treated manure – Environmental risk due to antibiotics after application to soil

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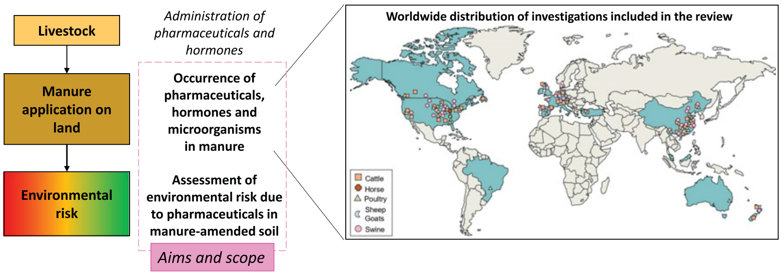
HIGHLIGHTS

- Cattle, swine, poultry and horse manures were included in the current study
- Concentrations of antibiotics and hormones in different manures were reviewed
- Concentrations of antibiotics in swine manure-amended soil were predicted
- Environmental risk assessment was carried out in case of swine manure application
- Sulfamethazine, chlortetracycline and doxycycline are the most critical compounds

GRAPHICAL ABSTRACT



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ABSTRACT

This study consists of a review based on 104 papers published between 1980 and 2019, which dealt with the occurrence of pharmaceuticals, hormones and a selection of microorganisms in raw and treated manure from different types of animal farms. The selected pharmaceuticals and hormones are those regularly administered to livestock for treating and preventing diseases. Worldwide, manure is commonly spread on soil as a fertilizer due to its nutrient content. However, this practice also represents a potential pathway for micropollutant release into the environment. In this context, this study evaluates the predicted concentrations of some antibiotics in soil after the application of swine slurry on soil and compares them with corresponding measured concentrations found in the literature. Enrofloxacin, oxytetracycline and chlortetracycline were the antibiotics with the highest concentrations that were found in raw and treated manure and that showed a high risk together with sulfamethazine. Future research should focus on monitoring other pathogens, parent compounds and their main metabolites in raw and treated manure, studying the spread and development of antibiotic resistance genes in the environment due to residues of antibiotics in manure applied to soil, and evaluating predicted no effect concentrations of pharmaceuticals and hormones commonly administered to livestock with regard to terrestrial organisms.

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Contents

1. Introduction

Livestock is one of the main economic activities in many countries worldwide and farm management can differ from country to country, as well as the management of the waste produced by the activities themselves, in particular zootechnical effluent (Verlicchi et al., 2019).

The distribution maps of various livestock provided by FAO (Gilbert et al., 2018) show that the most common types of animal are cattle, followed by sheep and goats, swine, poultry and finally horses. The highest densities of cattle (number of heads/km^2) are in America (mainly South America), India, and some European countries; sheep are distributed as leopard spots across Europe, Asia and Africa; poultry farming takes place in many European and Asian countries; pig farms are common in Europe and China; and horse farms are present at a very lower density in America and other parts of the world. One problem related to livestock farms is the management of the manure and other types of zootechnical effluents generated at the farms.

In China, the production of livestock manure was up to 3.26 billion tons in 2009 (Zhang et al., 2009), while in the US and Canada, the annual estimated quantities of livestock manure were 132 million tons and 178 million tons, respectively (Dolliver and Gupta, 2008; Hofmann and Beaulieu, 2006). More specifically, the ranges of amounts of manure produced by the 1000 heads registered for the different animals are 17,400–26,100 kg/d for cattle, 21,000–25,000 kg/d for swine, 1200–1800 kg/d for sheep, 1000–1600 kg/d for goats, and 45–58 kg/d for poultry (MLA, 2003; Sims and Maguire, 2005). These figures underline the consistent daily amount of animal waste, even in small-medium livestock, which requires proper management (accumulation and/or treatment) and disposal (recovery, cotreatment with other wastewater and with the organic fraction of solid wastes, etc.).

Due to their nutrient contents, raw and treated zootechnical effluents may be considered as amendments (Combalbert et al., 2012) and applied to the soil for agricultural needs. This practice was and still is commonly followed in many countries, but there is an ongoing debate on the potential risks caused by the emission of the residues of contaminants that have not been properly retained or that cannot be removed during the treatment process. Over the last few years, increasing attention has been paid to the occurrence of (unregulated) contaminants of emerging interest (the so called micropollutants, in particular pharmaceuticals and personal care products) in any environmental compartment and to the main pathways which allow their introduction/release in the environment (Verlicchi et al., 2012; Ghirardini and Verlicchi, 2019; Al Aukidy and Verlicchi, 2017; Kuppusamy et al., 2018; Tasho and Cho, 2016).

With regard to the zootechnical sector, based on the technical literature, it was found that different classes of pharmaceuticals are (regularly or as needed) administered to farm animals for the treatment and prevention of bacteria, parasite diseases, and fungal and yeast infections; the control of gastrointestinal worms, liver flukes and lung worms; the control of hormonal activities and growth promotion (Boxall et al., 2004; Sarmah et al., 2006a; Pan et al., 2011).

The main groups of veterinary medicines administered in the UK and in the Netherlands, as reported in a study by Boxall et al. (2003), include: antimicrobials, endectocides, coccidiostats and antiprotozoals, antifungals, aquaculture treatment drugs, hormones, growth promoters, anaesthetics, euthanasia products, tranquillizers, nonsteroidal anti-inflammatories agents, and enteric bloat preparations. A more detailed list of the classes and main agents is compiled in the European Commission report (Tavazzi et al., 2018).

About 11 million kg of antibiotics are sold for disease control (therapeutic), prevention and growth promotion (sub-therapeutic) purposes in the US alone (USFDA, 2018) and >90% of the cattle in the US receives steroid hormone treatments for growth promotion through implants behind the ears or as feed additives (USDA, 2013). Sweden banned the use of antibiotics as livestock growth promoters in 1986, Switzerland in 1999 (Haller et al., 2002) and the European Commission has banned the marketing and use of antibiotics as growth promoters in animal feed since January 2006 (EC, 2003). This practice is still commonly adopted in many other parts of the world (Pikkemaat et al., 2016).

In addition to pharmaceuticals and hormones, other groups of micropollutants may be found in the different manures. This is the case of the plasticizer bisphenol A (released from the food containers or manure storage tanks) and the parabens methylparaben and propylparaben (Aznar et al., 2018; Zhang et al., 2014). Another category among contaminants to consider in order to better characterize manure includes microorganisms, both indicator microorganisms as well as pathogen microorganisms.

Being this said, the disposal of manure in soil for agricultural needs represents an opportunity but at the same time a threat. This review intends to provide a snapshot of the concentrations of the main contaminants of emerging interest and of selected microorganisms in different types of raw and treated manure produced by different animals. In addition, it presents and discusses a method for the prediction of the manure-amended soil concentrations for a selection of compounds and compares predicted concentrations with measured concentrations (found in the literature). The review ends with a focus on an environmental risk assessment in swine manure-amended soil, by means of the risk quotient approach, for a selection of antibiotics commonly administered to pigs. The collection of data presented and discussed herein aims to represent the baseline for further studies in order to evaluate the impact of the spreading of manure on soil and its contribution to the immission of residues of micropollutants in the environment via soil and the subsequent percolation/runoff. In addition, the adopted approach for an environmental risk assessment may also be applied to other types of manure if applied to land.

2. Definition and types of manure included in this review

Manure characteristics are strongly related to the animal producing it (namely animal type, weight, growing stage, sex, age), to the environment and conditions in which the livestock live, to the farm (in terms of type, size and management). Table 1 reports the principal groups of raw manure, based on manure constituents and phases, the types considered in this study, the corresponding percentage content of nutrients and dry matter, and the main types of treated manure used in land amending with their main chemical characteristics.

We will call manure in which concentrations of selected pollutants are generally expressed in terms of ng/L "liquid manure": this will refer to urine, flushing materials, lagoon effluent, lagooning sludge and anaerobically digested manure. We will refer to "semiliquid manure" in case of slurry or liquid fraction of manure. In a few cases, the authors have provided concentrations in lagooning sludge and anaerobically digested manure in ng/(g dry matter (dm)). It is thus useful to note that manure bulk density may be assumed equal to 500–780 kg/m^3 for bedding manure, and around 1000 kg/m^3 for raw liquid manure and slurry (BUR Regolamento Regionale ER, 2017; EC-TGD, 2003).

Collected values of concentrations of micropollutants and microorganisms will be reported according to this classification. We tried to relate micropolluatnt concentrations to other types of classification, such as stall type and size or stall management. Due to an incomplete description of the farms and of their management in the reviewed papers, these attempts did not lead to consistent results and we preferred to maintain the classification reported in Table 1. The only further analysis we carried out referred to a comparison between micropollutant concentrations in dairy and beef cattle raw manure.

3. Framework of the study

The study is based on 104 published papers on peer reviewed journals between 1980 and 2019. They were selected from Scopus, assuming the following keywords "Pollutants AND manure", "Microorganisms AND manure", "Pharmaceuticals AND manure", "Antibiotics AND manure", "Hormones AND manure".

A paper was included in the review if it referred to *real* concentrations of veterinary pharmaceuticals, hormones and microorganisms, and clearly stated the animal producing the manure and the type of raw or treated manure.

The studies were carried out in 20 countries all over the world (mainly in the US, China, Canada, Spain and Germany) and refer to manure produced (in descending order) by pigs, cattle, poultry and, to a lesser extent, sheep and horses. Fig. S1 reports the types of manure investigated in the different countries and gives the corresponding references.

Some studies included more than one investigation (different manure, different treatment before application, etc.), therefore, based on the 104 papers that were included in the review, 241 investigations were identified.

Table S1 reports the details of the 241 experimental campaigns carried out in the 104 papers, as well as the principal features addressed in each of them (e.g. seasonal variation of micropollutant content in manure, environmental risk assessment after spreading, measured or predicted concentration of micropollutants in manure-amended soil, study of the fate of micropollutants once spread on rural land, factors influencing pollutant mobility in soil, etc.).

The pollutants monitored in the reviewed studies were: 145 chemical compounds (including parent compounds, some metabolites and transformation products) belonging to 11 therapeutic classes and 16 microorganisms. Table 2 summarises the compounds according to their classes, whereas Table S2 includes the main properties of the compounds and the references for the occurrence data included in this review.

First of all, the study briefly addresses manure management (stockpiling or onsite treatment) and common practices applied in different countries across the world. It gives a quick overview of the main characteristics of legislation in force and then presents the ranges of concentrations found in both raw and treated manure.

Concentrations in raw manure are generally expressed in terms of ng/g of dry matter (dm) for all the types described in Table 1. For manures with a dry matter content of <10%, micropollutant concentrations may also be reported in ng/L (this is the case for investigations referring to slurry, liquid fractions of manure due to percolation, flushing materials, and urine). With regard to treated manure, data referring to lagoon effluent are only given in ng/L while data regarding lagooning sludge and anaerobically digested manure are expressed in terms of both ng/L and ng/g dm. Finally, data referring to composting and alum treated manure are given in ng/g dm. Tables S3–S5 provide information about the observed excretion factors of pharmaceuticals and hormones administered to the different animals as well as their half-life in manure and manure-amended soil.

Collected concentrations of microcontaminants in raw manure and treated manure produced by different animals are reported in Figs. 1–10 and 11–16, respectively. A comparison of the concentrations in raw and treated manure is reported in Figs. S2–S9. Concentration values and the highest ranges of variability of the selected micropollutants are discussed (Tables 3 and 4) and an attempt to correlate them with the main factors characterizing the farm type, activity and management operations was carried out. In particular the influence of the type of cattle farm (dairy or beef cattle farm) on the concentrations of the selected micropollutants in raw manure is presented in Figs. S10 and S11.

Concentrations of microorganisms in raw manure are shown in Fig. 17 and concentrations in treated manure in Fig. 18. Tables S6–S16 report the descriptive statistical analysis for each type of manure in terms of number of data, minimum, maximum, average values, standard deviation and the 75th percentile.

Then an estimation of the soil concentration (predicted environmental concentration [PEC]) for a selection of 10 antibiotics in cases of swine slurry-amended soil was carried out following the model described in the European Technical Guidance Document on risk assessment (EC-TGD, 2003) (Table 5). These values are compared with the measured concentrations found in the literature (Table 5 and also Table S18 with many details). Based on the PECs, an environmental risk assessment was carried out by means of the risk quotient approach (Fig. 19). The study is completed with a discussion of the most critical compounds which can enter into the environment via manure disposal and the main risks for the environment due to manure spreading on the soil.

3.1. Quality assurance of literature data

As reported by the EC Technical Guidance Document on risk assessment (EC-TGD, 2003) and as stated by many Authors (among them Liebig et al., 2006; Ternes and Joss, 2006, Verlicchi et al., 2012), it is vital that the quality of literature data is assured. In this context, to be included in the current review, studies had to provide an in-depth description of the animal producing the manure, the type of manure to be sampled and analysed, its treatment (if present), a description of the analytical methodology used for the assessment of measured concentrations of microcontaminants and microorganisms, and the quality assurance programme adopted for sampling, analysis and processing. In particular, with regard to microcontaminants, the following information had to be provided: list of analytes, solvents and chemicals used; details of sampling, transport and storage in addition to sample volume; analytical methods adopted, including pH adjustment, filtration and filter material; extraction and solvent evaporation techniques; derivatization and detection method; surrogate and/or instrumental standards used; methods and limits of quantification, recovery measurements, procedural and instrumental blanks used; sampling conditions, location, sampling frequency and mode (in particular spatial distribution of sampling to produce a representative manure sample).

If reported values of concentrations referred to spiked concentrations, or the type of manure was not well described, the paper was rejected.

In a few cases, studies were included even if the sampling procedure was not exhaustively reported but the analytical methods were clearly reported and the data collected allowed us to complete the chemical and bacteriological characterisation of the wide spectrum of manure types reviewed (see Table 1) in terms of micropollutants and microorganisms.

It was found that studies published more recently were more accurate than older ones in the description of Materials and Methods. Those published before 2000 provide fewer details. These studies were included because they reported the concentrations of microorganisms and selected micropollutants in different manures, but their main aims were to analyse the land runoff once the manure was distributed on the soil (Busheé et al., 1998; Nichols et al., 1997, 1998) or the effects on nutrient mineralisation on soil and plant growth (Patten et al., 1980). In Table S1, the level of accuracy of each study is highlighted as well as the main issues addressed in the reviewed papers.

The collected data reported in the graphs and tables in the manuscript and supplementary material come not only from tables, but also from graphs. In this case, the uncertainties associated with the values add to the sampling and analysis uncertainties (as discussed in Verlicchi and Zambello, 2016 and, following the same approach, reported in Ghirardini and Verlicchi, 2019), even in cases where the data reading was quite accurate. If a literature value was reported below its limit of detection (LOD), in this review it was assumed equal to the corresponding LOD and if it was reported below its limit of quantification (LOQ), it was assumed to be half its LOQ value.

4. Manure generation, management, treatment, disposal and available legal requirements

Pharmaceuticals may be administered to animals for different periods of time, sometimes for prevention, other times for specific treatments. For instance, in swine livestock, the antibiotics chlortetracycline and tylosin are regularly administered for treatment, prevention and growth promotion with treatment lengths ranging from 27 days (respiratory diseases), 41 days (enteric diseases) and 62 days for promoting growth and weight gain (APHIS, 2012). Further details of the administration of specific pharmaceuticals are reported in Table S1 lines 15 and 16, see for instance Ray et al. (2017), Sura et al. (2015) and Van Donk et al. (2013).

Once administrated, the compound is only partially assimilated, and the remaining fraction is excreted via animal urine and faeces. Available values of excretion factors are reported in Table S3 for a group of the selected compounds. Management operations of the farms vary depending on the animal type, the size and the country. Unfortunately, they are not always reported. On the basis of the description provided by Arikan et al. (2007), Chen et al. (2018), Derby et al. (2011), Hoese et al. (2009), Joy et al. (2014) Ray et al. (2017), Sarmah et al. (2006b), Watanabe et al. (2010), Zhang et al. (2013), Zhou et al. (2013a, 2013b) it is possible to have an idea of how different they may be.

When excreted, manure is generally stored in pits and lagoons if it is semiliquid or liquid (Combalbert et al., 2012; Ben et al., 2013; Gadd et al., 2010) or in heaps in the case of bedding or solid manure (Derby et al., 2011; Kelley et al., 1994). As reported in Raman et al. (2004), manure storage pits and holding ponds are designed to store the volume of a given time period (6–12 months), after which the stored manure can be land applied. In dairy cattle farms, different units may be present: calf hutches, hospital pens, milking barns, heifer freestall. These units are characterized by specific management operations leading to the generation of different manure types which may be mixed or destinated to different ways of disposal (Watanabe et al., 2010). During storage, organic matter may be subjected to degradation processes the kinetics of which is strictly related to environmental conditions (namely oxygen concentration, temperature and rainfall) (Tavazzi et al., 2018). At the same time, there could also be a reduction in the content of the different microorganisms due to unfavourable conditions. Sometimes, as is the case for dairy manure collected by flushing freestalls, it is first necessary to separate the liquid fraction from the solid fraction by using different techniques (settling basin sedimentation or mechanical screening) (Hafner et al., 2017). If the manure is subjected to composting, degradation of the different compounds may occur. This is strictly related to the adopted temperature, pH, microbial enzymes present and microorganisms developed in the system (Ramaswamy et al., 2010; Ezzariai et al., 2018; Spielmeyer, 2018).

To have an idea of the degradation kinetics of the compounds in the stockpiled manure under investigation, Table S4 reports the half-life times and corresponding kinetic model for a group of pharmaceuticals according to the different animals. Most of these data refer to batch tests and not to real and prolonged investigations. The interesting investigations carried out by Berendsen et al. (2018) state that degradation of antibiotics is mainly due to abiotic processes and varies considerably mainly depending on the manure type and, to a lesser extent, the type of animal producing it. Of all the antibiotic classes, sulphonamides dissipate quickly in all manure types, presenting a half-life between 0.2 and 30 days, whereas, tetracyclines, quinolones, macrolides and lincomicides are more persistent (much higher than 30 days). The fate and transport of microcontaminants are correlated with the manure type in soil, for instance if it is spread as a solid phase or a liquid phase (Wallace and Aga, 2016; Tasho and Cho, 2016; Gros et al., 2019).

According to Zhang et al. (2013), lagoon effluent is often pumped for irrigation at least once a year, lagooning sludge instead is removed every 5–20 years and then spread on soil.

Once disposed on soil, the behaviour of the residues of micropollutants depends on their nature (Chen et al., 2018) and different biotic and abiotic processes may occur (Tasho and Cho, 2016). An interesting analysis is carried out by Solliec et al. (2016) and Gros et al. (2019), who tried to explain the attenuation of antibiotics in soil amended with (swine) manure over a prolonged period, correlating it with the properties of the compound and the soil characteristics. According to Hutchison et al. (2005), once manure is spread on soil, commonly microorganisms (*E. coli*, *Salmonella*, *Listeria*) decline rapidly: 1 log unit reduction was observed within 1.5–2.5 days for all these species. *E. coli* was detected up to 32 days, Salmonella up to 63 days and Listeria up to 128 days.

Land disposal of manure has to fulfil the legal limits set in the various countries regarding maximum quantity of manure of different origins as well as specific periods and weather conditions (rainy periods must be avoided) in which manure can be applied. An in depth analysis of the legal requirements set in the different countries is beyond the aims of this review. In general, a common parameter is organic nitrogen content, which defines the maximum quantity of manure, which can be spread (Aga et al., 2005). The limit has been established to avoid the risk of contaminating aquifers due to percolation. At EU level, Directive 91/676/EEC (EU, 1991), establishes the value at 170 kg organic N/(ha year) in areas at risk of nitrate contamination (vulnerable zones). In the Po Valley in Northern Italy, according to a regional regulation (Regione Emilia-Romagna, 2017), there is another limit in addition to the above limit for areas that are not at risk of eutrophication which corresponds to 340 kg organic N/(ha year). Other maximum values may also be adopted. For instance, the annual application rate in Australia corresponds to 240 kg N/ha year (Eldridge et al., 2009).

Table 1
Definition of the different types of raw and treated manure considered in this review, their content of macronutrients and the corresponding references.

Manure categories	Manure types	Description [unit of measurement for micropollutant concentrations]	Dry matter [%]	C_{tot} [%] N_{tot} [%] P_{tot} [%] K_{tot} [%]	References
Raw manure					
Bedding manure	Cattle, horse, sheep or pig bedding manure	Mixture of faeces, urine and bedding material (including straw, wood shavings and sawdust) and other dry adsorbents, low-cost material. [ng/g dm]	20.9–69.9	11.8–12.9 0.4–2.2 0.2–4.0 0.9–4.0	Arikan et al., 2009; Derby et al., 2011; Hutchison et al., 2004; Patten et al., 1980
	Poultry litter	Mixture of faeces, urine, spilled feed, animal waste (feathers, blood, etc.) and bedding material. Generally deriving from indoor ground breeding of broiler chickens. [ng/g dm]	33.3–78.5	12.6–50.4 1.1–5.9 1.1–3.2 2.0–3.3	Aznar et al., 2018; Arikan et al., 2016; Dutta et al., 2010; Jenkins et al., 2006; Leal et al., 2012; Nichols et al., 1997
Solid manure	Cattle and horse solid manure	Manure with medium-high dry matter content that could be scraped from stalls (mostly faeces, but may contain urine), or solid fraction of slurry obtained with separation processes.	24.4–65.0	10.4–48.1 0.6–4.6 0.1–2.5 0.1–3.2	Amarakoon et al., 2014; Arikan et al., 2016; Aust et al., 2008; Karci and Balcioğlu, 2009; Ray et al., 2017; Wallace and Aga, 2016; Wallace et al., 2018
	Pig solid manure	[ng/g dm]	28.0–29.0	35.3–41.0 1.3–2.7 1.5–3.2 0.7	Bao et al., 2009; Gros et al., 2019; Zhang et al., 2019
	Poultry manure	Mixture of faeces, urine and, to a lesser extent, animal waste (feathers, blood, etc.). Bedding material is absent. Generally obtained from shallow scrape of alley in egg production facility (e.g. from laying hens in battery cages). [micropollutants in ng/g dm]	33.0–79.4	24.9–46.2 1.7–7.1 0.7–6.7 1.9–5.0	Bao et al., 2009; Conde-Cid et al., 2018; Delgado et al., 2018; Dutta et al., 2010; Ho et al., 2014; Karci and Balcioğlu, 2009
Semi-liquid manure	Cattle slurry	Faeces and urine (often accumulated from slatted floor) accumulated in slurry pit. [ng/g dm and also ng/L]	0.5–8.3	17.5–36.5 0.2–2.8 0.04–0.1 0.4–0.5	Conde-Cid et al., 2018; Khan and Lee, 2012; Peyton et al., 2016; Wallace et al., 2018
	Pig slurry		0.3–8.3	16.3–41.4 0.1–3.4 0.01–3.1 0.1–2.5	Blackwell et al., 2009; Conde-Cid et al., 2018; Gros et al., 2019; Hutchison et al., 2004; Jacobsen and Halling-Sørensen, 2006; Joy et al., 2014; Kjær et al., 2007; Lamshöft et al., 2010
	Cattle and horse liquid (fraction) manure	Liquid fraction of manure, obtained through percolation, centrifugation or other separation practices. [micropollutants in ng/g dm and also ng/L]	4.9	NA NA 0.05 0.2	Wallace and Aga, 2016; Wallace et al., 2018
	Pig liquid (fraction) manure		<1–1.6	NA 0.1 1.0 NA	Combalbert et al., 2012; Gros et al., 2019
Liquid manure	Cattle, horse and pig urine	Liquid waste generated by any animal species. [ng/L]	NA	NA 0.1–1.7 NA NA	Hoogendoorn et al., 2010
	Cattle shed flushing material	Dirty water composed of faeces, urine, wash water from stalls and, if collecting tank is outdoors, rainwater. [ng/g dm and also ng/L]	<2	NA NA NA NA	Hutchison et al., 2004
	Pig house flushing material		<2	NA 0.6[a] 0.1[a] 0.4[a]	Edwards and Daniel, 1994; Hutchison et al., 2004
	Poultry house flushing material		<2	NA NA NA NA	Hutchison et al., 2004
Types of treated manure and brief description of treatment					
Lagooning sludge		Sludge accumulated in 1–5 m deep open air or covered ponds. Generally removed from 5 to 20 years and applied on soil as amendment (Hamilton et al., 2006). [ng/g dm and also ng/L]	3.2(33)–25(34)	NA 0.5 0.06 0.4	Frey et al., 2013; Kuchta and Cessna, 2009; Wallace et al., 2018
Lagoon effluent		Water collected from upper part of lagoon receiving manure (water phase). Residence time generally varies from 2 to 6 months. Often used for irrigation purposes (Bodman, 1996). [ng/L]		NA 0.04–0.15 0.03–0.14 0.02–0.04	Khan and Lee, 2012
Compost		Mixture of manure and organic material (e.g. hay, straw or decomposed leaves) that results from aerobic	33.5–79.0	10.1–48.8 0.8–3.6	Aznar et al., 2018; Biswas et al., 2017; Cessna et al., 2011; Derby et al., 2011; Larney et al., 2003; Liu et al., 2015;

(continued on next page)

Table 1 (*continued*)

Manure categories	Manure types	Description [unit of measurement for micropollutant concentrations]	Dry matter [%]	C_{tot} [%] N_{tot} [%] P_{tot} [%] K_{tot} [%]	References
		composting process favoured by regular turning and controlling of moisture and temperature. [micropollutants in ng/L]		0.2–3.7 1.4–3.2	Ray et al., 2017
Digested manure		Mixture of manure and organic material (e.g. hay, straw or decomposed leaves) that results from anaerobic digestion process generally occurring at least at 40 °C for up to 6 months. [ng/g dm and also ng/L]	4.3	NA 0.3 0.02 0.1	Wallace et al., 2018
Pellet manure		Extremely dense and low moisture content manure granules made by compression of dung at high temperature (at last 100 °C). [ng/g dm]	78–94	NA 2–4.5 1.6–1.8 NA	Dutta et al., 2010; Haggard et al., 2005; McMullen et al., 2005
Alum treated manure		Manure in which $Al_2(SO_4)_3$ is added to reduce water extractable constituents between flocks. [ng/g dm]	75.0–78.5	NA 5.2 1.3 NA	Haggard et al., 2005; Nichols et al., 1997

[a] Estimated assuming a bulk density of 1000 kg m^{-3}.

To have an idea of the required area where the produced manure may be applied, we can consider a farm with 100 dairy cows. Assuming that the manure produced corresponds to 1700 kg/d (MLA, 2003) and a percentage of nitrogen varying between 0.6%–4.6%, the daily amount of nitrogen produced results equal to 10 and 78 kg N/d and on an annual basis to 3700 and 28,500 kg N/year. This quantity would require an arable area between 21 and 168 ha to respect the limit of 170 kg N/ha year set down by some of the regulations mentioned above.

5. Results

As reported above, the investigations included in this review refer to cattle, poultry, swine and, to a lesser extent, horses, sheep and goats. They were carried out in different countries worldwide, mainly in Canada and the US, Europe and (East) China. The map in Fig. S1 shows the various locations and corresponding references. It emerges that most of the investigations in North America dealt with three main types of manure (cattle, swine and poultry), swine and cattle manure in Europe, and swine and poultry manure in China. Horse manure was investigated by Busheé et al. (1998) in North America; manure produced by sheep and goats was investigated by Sarmah et al. (2006b) in New Zealand; and by Hutchison et al. (2004) in the United Kingdom.

As a whole, with regard to the 241 investigations (see Table S1), 37% referred to cattle manure, 34% to swine manure, 27% to poultry manure and 1% (each) to sheep/goat and horse manure.

As mentioned above, although the use of antibiotics as growth promoters in animal feed has been banned in some countries, this practice is still followed worldwide. Data presented in the graphs may also include investigations in countries where antibiotics are still used as feed additives. The snapshot provided by this overview aims to show the observed ranges of variability for the different pharmaceuticals investigated.

5.1. Occurrence of selected micropollutants in different raw manures

Figs. 1–10 below report the concentrations of selected micropollutants grouped according to their class in the different types of raw manure under review (see Table 1).

In Figs. 1–6, concentrations are given in ng/g dm, whereas in Figs. 7–10 they are given in ng/L as they refer to manure with a content of solids <10% (known as liquid or semiliquid manure), namely: slurry, liquid (fraction) manure, flushing material and urine. In some cases, as mentioned in Table 1, the concentrations referring to these kinds of manure are in both units (ng/g dm and ng/L).

With regard to raw cattle manure (Figs. 1 and 2), the collected data refer to 98 compounds belonging to 6 different groups: analgesics/antinflammatories, anticonvulsants, antihelmintics, antimicrobials, hormones and plasticizers, as well as four types of raw manure: bedding manure, liquid fraction manure, slurry and solid manure. As a whole, the concentrations varied between 0.02 ng/g dm (the hormone trendione in slurry) and 225,000 ng/g dm (the antibiotic oxytetracycline in bedding manure).

It emerges that there was highest number of collected data for sulfamethazine and tylosin (69), chlortetracycline (68), oxytetracycline (62), tetracycline (56), sulfadimethoxine (42) and epi-tetracycline (35). The remaining compounds present a lower number of values. Moreover, the widest variability range was found for oxytetracycline (6 orders of magnitude: from 1 10^{-1} to 2.25 10^{5} ng/g dm), followed by chlortetracycline, enrofloxacin and sulfamethazine (5 orders of magnitude each). 11 compounds have a variability range between 1000 and 6000 ng/g dm. When limiting the attention to compounds exhibiting >5 collected values, it emerges that the highest average values (±standard deviation) were found for oxytetracycline (5815 ± 29,452 ng/g dm), monensin (2434 ± 2272 ng/g dm) and enrofloxacin (2318 ± 10,176 ng/g dm). It is interesting to note that as reported in detail in Table S6, the 75th percentile is lower than the corresponding average value for 27 out of the 98 compounds considered. The highest differences were found for oxytetracycline and enrofloxacin: the 75th percentile values were 166 ng/g dm for the first and 33 ng/g dm for the second compound. This is due to the extraordinarily high maximum value of each of compound. In some investigations, the analysis of antibiotics in manure where carried out soon after 5 days of administration to the animals in order to focus on the most critical scenario (Arikan et al., 2007).

With regard to the different raw cattle manure, it emerges that most collected data refer to solid manure (571 values), followed by bedding manure (296) and slurry (116), whereas the liquid fraction was rarely sampled and analysed: only 6 data were collected and all refer to antibiotics.

A rapid glance at Figs. 1 and 2 shows that the maximum values mainly occurred in solid manure (57%) followed by slurry (20%) and then bedding manure (22%).

With regard to poultry manure (Figs. 3 and 4), concentrations are available for 92 compounds belonging to 10 different groups: analgesics/antinflammatories (7 compounds), anticonvulsants (1),

Table 2
Micropollutants and microorganisms included in the review. Micropollutants are grouped according to their therapeutic class. The number in brackets corresponds to the number of compounds or microorganisms included in the group.

Class	Compounds included
Analgesics and anti-inflammatories (8)	Acetaminophen; fenoprofen; flunixin; ibuprofen; ketoprofen; mefenamic acid; naproxen and salicylic acid
Anticonvulsants (1)	Carbamazepine
Antihelminthics (1)	Flubendazole
Antimicrobials (85)	Amoxicillin; amprolium; anhydrochlortetracycline; anhydrotetracycline; atrazine; azithromycin; bacitracin A; benzylpenicillin (or Penicillin G); carbadox; ceftiofur; chloramphenicol; chlortetracycline; ciprofloxacin; clarithromycin; cloxacillin; colistin A; colistin B; cyromazine; danofloxacin; demeclocycline; difloxacin; doxycycline; enrofloxacin; epi-anhydro-tetracycline; epi-chlortetracycline; epi-iso-chlortetracycline; epi-oxytetracycline; epi-tetracycline; erythromycin; erythromycin H_2O; fleroxacin; florfenicol; flumequine; furazolidone; iso-chlortetracycline; lasalocid; leucomycin A5; lincomycin; lomefloxacin; marbofloxacin; metacycline; minocycline; monensin; n^4-acetyl-sulfamethazine; narasin; nicarbazin; norfloxacin; novobiocin; ofloxacin; oleandomycin; ormetoprim; oxytetracycline; pefloxacin; pirlimycin; pristinamycin (or virginiamycin); roxithromycin; salinomycin; sarafloxacin; sulfacetamide; sulfachlorpyridazine; sulfadiazine; sulfadimethoxine; sulfadoxine; sulfaguanidine; sulfamerazine; sulfameter; sulfamethazine; sulfamethizole; sulfamethoxazole; sulfamethoxypyridazine; sulfamonomethoxine; sulfanilamide; sulfapyridine; sulfaquinoxaline; sulfathiazole; sulfisoxazole; tetracycline; thiamphenicol; tiamulin; tilmicosin; toltrazuril; trimethoprim; tylosin; α-apo-oxytetracycline; β-apo-oxytetracycline
Antiseptics (3)	Methyl triclosan; ortho-phenylphenol; triclosan
Beta-blockers (1)	Metoprolol
Hormones (39)	11-Ketotestosterone; 17α-estradiol (E2α or alfatradiol); 17α-estradiol-3-sulfate; 17α-ethynylestradiol (EE2 or ethinyl estradiol); 17α-hydroxyprogesterone; 17α-trenbolone; 17β-estradiol (E2β or estradiol); 17β-estradiol-3, 17-diglucuronide; 17β-estradiol-3, 17-disulfate; 17β-estradiol-3-glucuronide; 17β-estradiol-3-glucuronide-17-sulfate; 17β-estradiol-3-sulfate; 17β-estradiol-3-sulfate-17-glucuronide; 17β-estradiol-17-sulfate; 17β-estradiol-17-glucuronide; 17β-trenbolone; androstadienedione; androstenedione; androsterone; dienestrol; diethylstilbestrol; epiandrosterone (or trans-androsterone); estriol (E3); estriol-3-glucuronide; estriol-3-sulfate; estrone (E1); estrone-3-glucuronide; estrone-3-sulfate; hexestrol; medroxyprogesterone; melengestrol acetate; mestranol; progesterone; testosterone; trendione; α-zearalanol; α-zearalanol; β-zearalanol; β-zearalanol
Inhibitors (xanthine oxidase) (1)	Allopurinol
Lipid regulators (3)	Clofibric acid; fenofibrate; gemfibrozil
Parabens (2)	Methylparaben; propylparaben
Plasticizer (1)	Bisphenol A
Microorganisms (16)	Indicators: *E. coli*; Faecal coliforms; Faecal enterococci; Faecal streptococci; Heterotrophic bacteria; Total coliforms Pathogens: *Aeromonas hydrophila*; *Campylobacter coli*; *Campylobacter jejuni*; *Clostridium perfringens*; *Cryptosporidium parvum*; *Enterococci*; *Giardia intestinalis*; *Listeria*; *Pseudomonas aeruginosa*; *Salmonella*

antimicrobials (53), antiseptics (3), beta-blockers (1), hormones (20), inhibitors (1), lipid regulators (3), parabens (2) and plasticizers (1) and two types of raw manure.

A quick look at the graphs shows that the concentrations varied between 0.03 ng/g dm (the hormone testosterone in poultry litter) and 1.4 10^6 ng/g dm (the antibiotic enrofloxacin in poultry litter produced in a poultry feedlot in China, according to Zhao et al., 2010).

It emerges that the highest numbers of collected data occurred for sulfadiazine (54 values), doxycycline (47), enrofloxacin (44), progesterone (40), chlortetracycline (34), oxytetracycline (33), sulfachlorpyridazine and 17β-estradiol (32), norfloxacin and trimethoprim (31). The remaining compounds present a lower number of collected values. Moreover, the widest variability ranges were found for enrofloxacin (7 orders of magnitude), followed by fleroxacin and oxytetracycline (6 orders of magnitude) and tylosin, sulfadiazine, salinomycin, trimethoprim, erythromycin and difloxacin (5 orders of magnitude). 13 compounds have 4 orders of magnitude variability range and for 8 substances the width of the variability range varied between 1500 and 8500 ng/g dm.

Limiting the attention to compounds exhibiting >5 collected values, it emerges that the highest average values (±standard deviation) were found for enrofloxacin (35,774 ± 213,817 ng/g dm), oxytetracycline (13,769 ± 72,375 ng/g dm), flumequine (11,833 ± 19,581 ng/g dm) and doxycycline (10,935 ± 22,260 ng/g dm). A descriptive statistical analysis of the collected data referring to poultry is reported in Table S7. Based on this, it is interesting to note that for 27 out of the 92 compounds considered, the 75th percentile was less than the corresponding average value. The highest differences were found for enrofloxacin and oxytetracycline (of which the 75th percentiles were 939 ng/g dm and 1600 ng/g dm, respectively) due to the extraordinarily high maximum value of each of them (for details see: Zhao et al., 2010 and Zhang et al., 2015).

With regard to poultry manure, it emerges that only two types of manure are present: poultry manure (exhibiting 699 values) and poultry litter (with 349 values). Details about the definition and characteristics of the two types of manure are reported in Table 1.

A rapid glance at Figs. 3 and 4 shows that maximum values mainly occurred in solid manure (61%) followed by poultry litter (39%).

With regard to swine manure (Figs. 5 and 6 and Table S8), the collected data refer to 77 compounds belonging to 5 different classes: analgesics/antinflammatories (2), antimicrobials (62), antihelmintics (1), hormones (11) and plasticizers (1) and 5 types of raw manure.

As a whole, the micropollutant concentrations varied between 0.05 ng/g dm (the antibiotic trimethoprim in pig slurry) and to 879,600 ng/g dm (this is due to the antibiotic chlortetracycline in solid (fraction) manure, according to Bao et al., 2009). It emerges that the highest number of collected data occurred for antibiotics: doxycycline (100 values), sulfadiazine (93), oxytetracycline (72), chlortetracycline (65), tetracycline and sulfamethazine (59), tylosin (44) and ciprofloxacin (41). Five further antibiotics present a number of values between 30 and 40, another six compounds between 20 and 29, fifteen compounds between 10 and 19. The remaining forty-three substances have 1–9 values.

Moreover, the widest variability ranges were found for oxytetracycline and chlortetracycline (6 orders of magnitude), followed by bacitracin A, doxycycline, tetracycline, lomefloxacin, enrofloxacin, tylosin, sulfamethazine, sulfamonomethoxine, lincomycin and sulfathiazole (5 orders of magnitude). For 19 compounds the variability range width varied between 1000 and 7500 ng/g dm.

Limiting the attention to the 51 compounds exhibiting >5 collected values, it emerges that the highest average values (±standard deviation) were found for chlortetracycline (76,667 ± 176,264 ng/g dm), bacitracin A (28,133 ± 85,165 ng/g dm), chloramphenicol (11,693 ± 28,761 ng/g dm) and oxytetracycline (11,180 ± 43,662 ng/g dm). It is

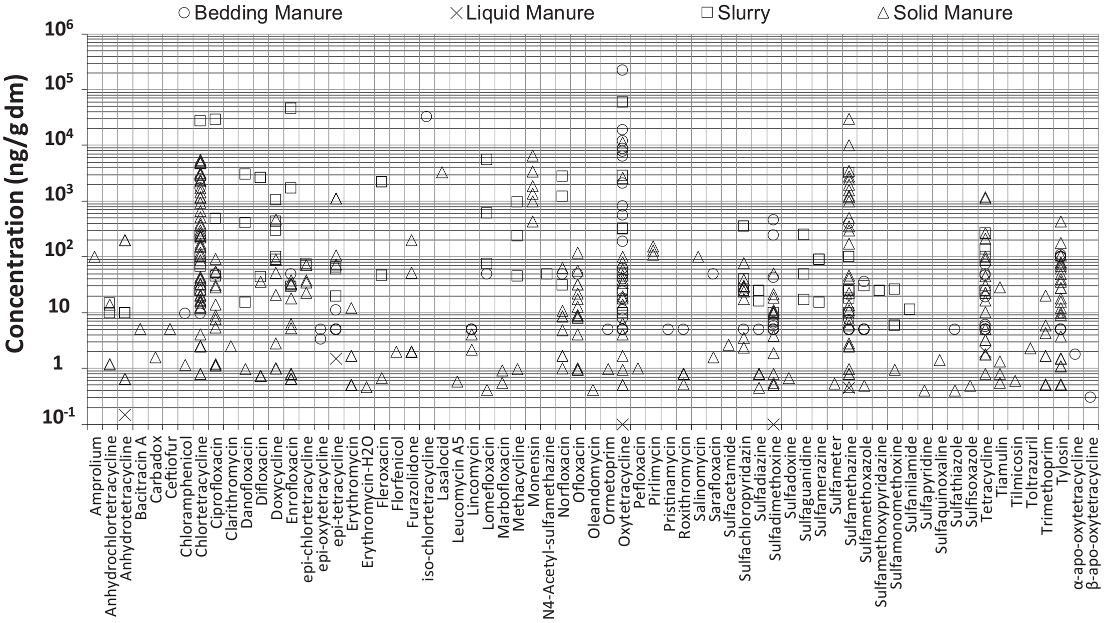

Fig. 1. Occurrence of antibiotics in different types of raw cattle manure. Data from: Aga et al. (2005), Amarakoon et al. (2014), Arikan et al. (2006, 2007, 2009, 2016), Aust et al. (2008), Cessna et al. (2011), Chen et al. (2018), Christian et al. (2003), Conde-Cid et al. (2018), De Liguoro et al. (2003), Dolliver and Gupta (2008), Gros et al. (2019), Hafner et al. (2017), Haller et al. (2002), Hou et al. (2015), Karci and Balcioğlu (2009), Patten et al. (1980), Ray et al. (2017), Sura et al. (2014), Sura et al. (2015), Wallace and Aga (2016), Wallace et al. (2018), Watanabe et al. (2010), Zhang et al. (2015), Zhao et al. (2010), Zhou et al. (2013a).

interesting to note that, as reported in detail in Table S8, and similar to the results found in the previous analysis of cattle and poultry manure, for 36 out of 77 compounds the 75th percentile is lower than the corresponding average. The highest differences (75th percentile – average value) were found for bacitracin A, lomefloxacin and oxytetracycline. This is explained with the extraordinarily high maximum value for the corresponding compound (for details see: Joy et al., 2013; Zhao et al., 2010; Chen et al., 2012).

With regard to the different types of swine manure, it emerges that most of the collected data refers to solid (fraction) manure (780), followed by slurry (327) and the other types presenting <100 data (70 for flushing material, 46 for liquid fraction manure and 15 for bedding manure).

A rapid glance at Figs. 5 and 6 shows that the maximum values mainly occurred in solid (fraction) manure (62%) followed by slurry (32%) and flushing material (6%).

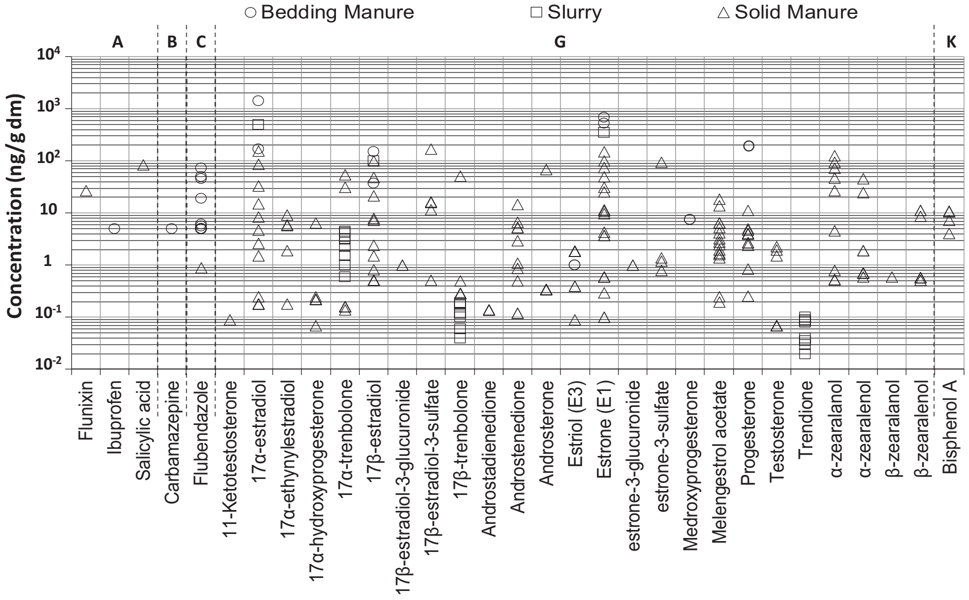

Fig. 2. Occurrence of other micropollutants, belonging to classes A (Analgesics and anti-inflammatories), B (Anticonvulsants), C (Antihelmintics), G (Hormones) and K (Plasticizer) in raw cattle manure. Data from: Bartelt-Hunt et al. (2012, 2013), Biswas et al. (2017), Gall et al. (2014), Gros et al. (2019), Mansell et al. (2011), Raman et al. (2004), Schiffer et al. (2001), Van Donk et al. (2013), Watanabe et al. (2010), Zhang et al. (2014), Zheng et al. (2008).

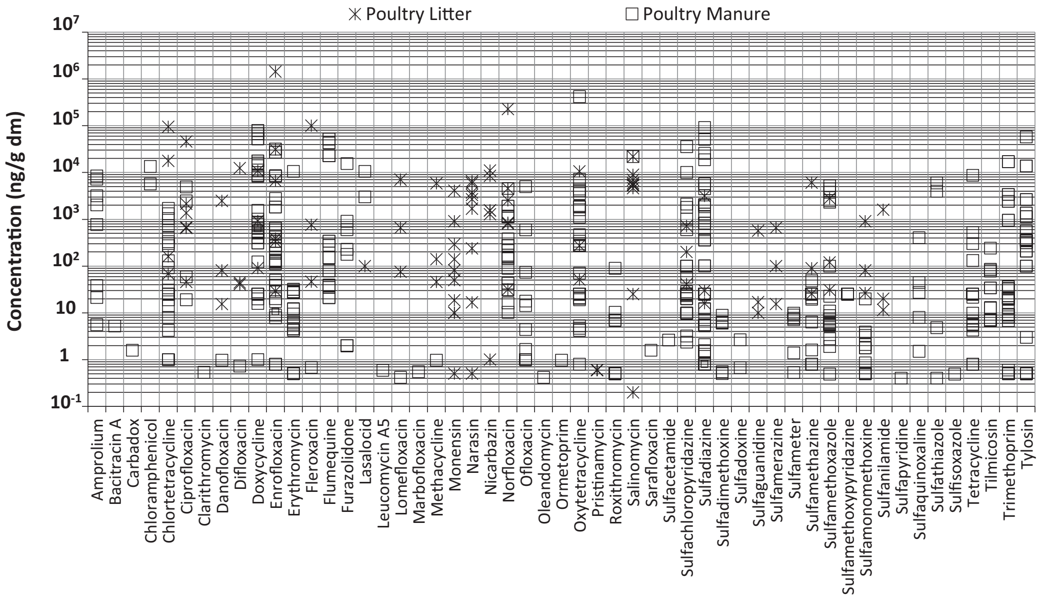

Fig. 3. Occurrence of antibiotics in different types of raw poultry manure. Data from: Arikan et al. (2016), Bao et al. (2009), Conde-Cid et al. (2018), Furtula et al. (2009), Ho et al. (2012, 2013, 2014), Hou et al. (2015), Hu et al. (2008), Karci and Balcioğlu (2009), Leal et al. (2012), Martínez-Carballo et al. (2007), Ramaswamy et al. (2010), Sun et al. (2013), Zhang et al. (2015), Zhang et al. (2019), Zhao et al. (2010), Žižek et al. (2015).

A comparison of the collected data for cattle, poultry and swine manure shows that the maximum values of concentration always occurred in the solid fraction of manure; the antibiotics chlortetracycline and oxytetracycline are the most investigated compounds and they are always the compounds with the highest average values; finally swine manure is the object of the highest number of studies. As to monensin, it was found that it is commonly investigated in cattle manure rather than the other types and it also presented very high concentrations only in cattle manure. According to Łowicki and Huczyński (2013), this can be explained by the fact that it is largely administered for cattle as it may improve food metabolism in the ruminants and it leads to faster growth in cattle. In poultry, it is mainly used for the prevention of Coccidiosis and thus its use is rarer.

With regard to the liquid/semiliquid cattle manure analysis (Figs. 7 and 8, Table S9), it emerges that the collected concentrations refer to 80 compounds belonging to 5 different classes (1 analgesic/antinflammatory, 1 anticonvulsant, 62 antimicrobials, 15 hormones and 1 plasticizer) and to 4 different types of manures: flushing material, liquid manure, slurry and urine. Their concentrations varied between 0.5 ng/L (some sulphonamides in flushing material investigated by

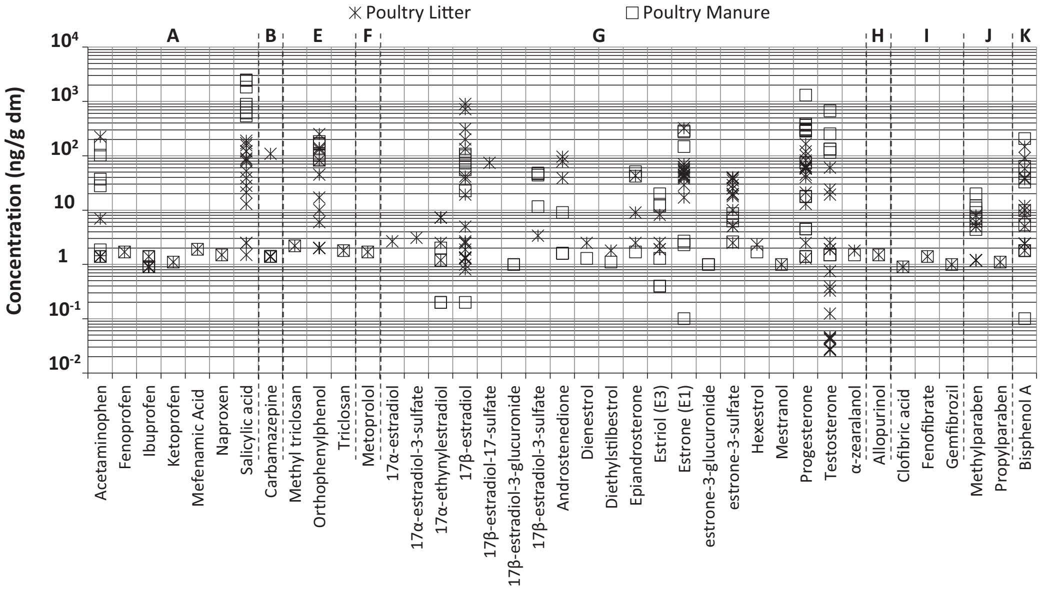

Fig. 4. Occurrence of micropollutants belonging to classes A (Analgesics and anti-inflammatories), B (Anticonvulsants), E (Antiseptics), F (Beta-blockers), G (Hormones), H (Inhibitors), I (Lipid Regulators), J (Parabens) and K (Plasticizer) in raw poultry manure. Data from: Albero et al. (2014), Aznar et al. (2018), Bevacqua et al. (2011), Dutta et al. (2012), Finlay-Moore et al. (2000), Hakk et al. (2005), Ho et al. (2012, 2013, 2014), Jenkins et al. (2006, 2008, 2009), Lu et al. (2014), Nichols et al. (1997, 1998), Shore et al. (1993), Zhang et al. (2014).

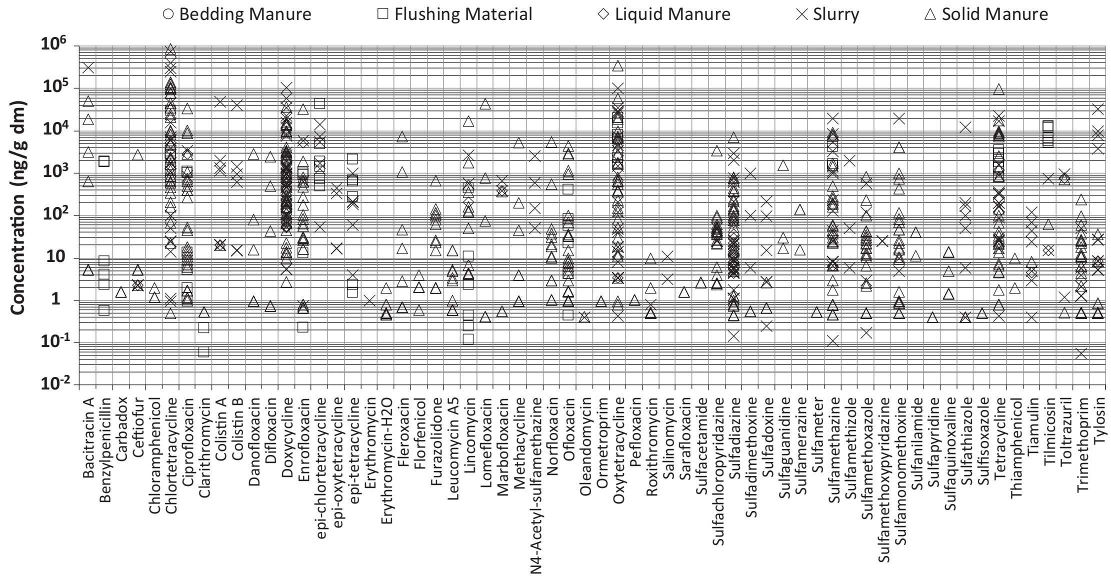

Fig. 5. Occurrence of antibiotics in different types of raw swine manure. Data from: Bao et al. (2009), Chen et al. (2012), Christian et al. (2003), Conde-Cid et al. (2018), Gros et al. (2019), Haller et al. (2002), Hou et al. (2015), Hu et al. (2008), Jacobsen and Halling-Sørensen (2006), Joy et al. (2013, 2014), Martínez-Carballo et al. (2007), Pan et al. (2011), Schlüsener et al. (2003), Tylová et al. (2010), Van den Meersche et al. (2016, 2019), Wang et al. (2019), Zhang et al. (2019), Zhao et al. (2010), Zhou et al. (2012, 2013a, 2013b).

Zhang et al., 2013) and 5.86 10^6 ng/L (chlortetracycline, in slurry, according to Arikan, 2008).

The most analysed compounds were the hormones 17β-estradiol and estrone (34 values) followed by the antibiotics oxytetracycline, sulfamethazine and tetracycline and the hormone 17 α-estradiol (32 values). The widest variability ranges were found for chlortetracycline and epi-chlortetracycline (6 orders of magnitude), followed by iso-chlortetracycline, sulfamethoxazole and sulfamethazine (5 orders of magnitude). 5 compounds have 4 orders of magnitude variability and two compounds variability range of 3 orders of magnitude. The highest values were reported by Arikan (2008) in a dedicated investigation on characteristics of calf manure soon after administration of the antibiotics at the permitted dose of 22 mg/kg body mass per day for 5 days (a standard dose in agricultural practice).

With regard to compounds with at least 5 collected values of concentrations, the highest average concentrations were found for epi-chlortetracycline (766,691 ± 1,649,564 ng/L), followed by chlortetracycline (281,281 ± 1,247,918 ng/L) and iso-chlortetracycline (280,274 ± 687,556 ng/L).

In this case, 25 out of 80 compounds had a 75th percentile lower than the corresponding average value (see Table S9), being the highest differences (75th percentile – average value) for epi-chlortetracycline, chlortetracycline and iso-chlortetracycline. Most of the collected data refer to flushing material (473 values of concentrations) followed by urine (103 values). As for liquid manure, only 36 data are available and refer to antibiotics. Most of the maximum values refer to flushing material.

With regard to raw liquid and semiliquid swine manure (Figs. 9 and 10 and Table S10), the collected data are available for 72 compounds from 5 classes (2 analgesics/antinflammatories, 57 antimicrobials, 1 antihelmintic, 11 hormones and 1 plasticizer) and for 4 types of manures (flushing material, liquid manure, slurry and urine). The collected concentrations vary between 0.1 ng/L (sulfamonomethoxine in flushing material found by Li et al., 2018) and 1.1 10^8 ng/L (chlortetracycline in slurry found by Hoese et al., 2009). A reduced number of papers report concentration data in ng/L, with respect to concentration in ng/g dm. The highest number of values is only 19 for chlortetracycline, 18 for oxytetracycline and sulfamethazine, and 15 for ciprofloxacin and tylosin.

As for the observed variability range, it was found that in some cases it is wider than the previous analysis, referring to ng/g dm, where it was 6 orders of magnitude as a maximum: for chlortetracycline it is 9 orders of magnitude, for sulfamethazine it is 8 orders of magnitude and for sulfamonomethoxine and oxytetracycline it is 7 orders of magnitude. There are 4 compounds with range of 6 orders of magnitude and two with 5 orders of magnitude and the remaining compounds with ranges of 4 orders of magnitude or less.

The highest average concentrations (±standard deviation) was found for chlortetracycline (5.78 10^6 ± 2.5 10^7 ng/L), followed by lincomycin (2.9 10^6 ± 7.7 10^6 ng/L). In Table S10, further details are reported, together with a descriptive statistical analysis of the collected data. An analysis of the 75th percentile values shows that for 11 antimicrobials the 75th percentile is lower than the corresponding average value.

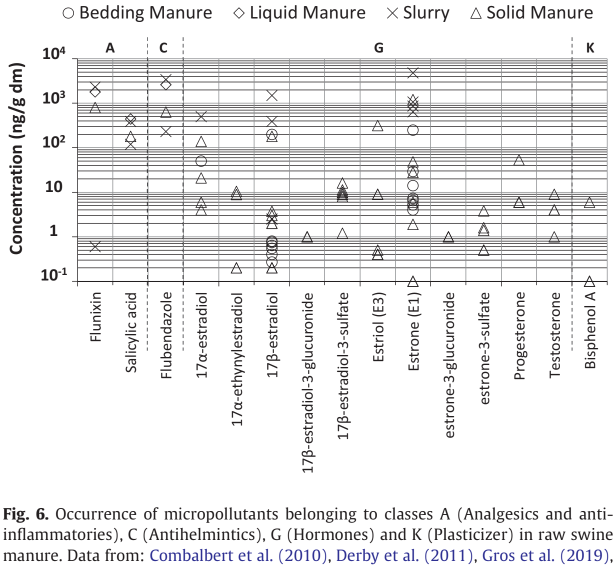

Fig. 6. Occurrence of micropollutants belonging to classes A (Analgesics and anti-inflammatories), C (Antihelmintics), G (Hormones) and K (Plasticizer) in raw swine manure. Data from: Combalbert et al. (2010), Derby et al. (2011), Gros et al. (2019), Kjær et al. (2007), Raman et al. (2004), Zhang et al. (2014).

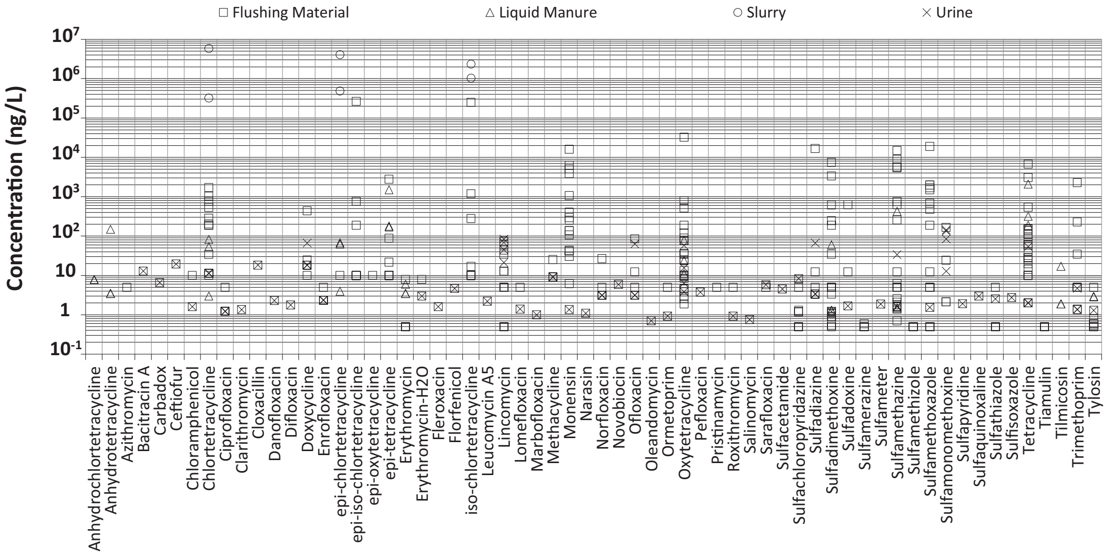

Fig. 7. Occurrence of antibiotics in different types of raw liquid/semiliquid cattle manure. Data from: Arikan (2008), Hafner et al. (2017), Wallace and Aga (2016), Watanabe et al. (2010), Wei et al. (2011), Zhang et al. (2013), Zhou et al. (2013a).

The highest differences were found for chlortetracycline, lincomycin, sulfamethazine, sulfamonomethoxine and tylosin.

Extraordinary high concentrations were found for chlortetracycline and tylosin reported by Hoese et al. (2009). The authors noted that they were higher than those found in other investigations (such as Kumar et al., 2004 and Martínez-Carballo et al., 2007) and were due to the fact that they refer to *fresh* swine manure and not to manure collected in a pit or a lagoon, and that they then sampled and analysed it like in the other investigations.

With regard to the number of values collected per manure type, it emerges that for flushing material there are 188 concentrations, 38 for liquid manure, 53 for slurry, and 31 for urine. The maximum values occurred mainly for slurry (45%), flushing material (34%), urine (11%) and liquid manure (10%).

With regard to poultry house flushing material, data are available only for 8 antibiotics: chlortetracycline, cyromazine, doxycycline, oxytetracycline, sulfadiazine, sulfamethazine, sulfaquinoxaline and tetracycline (Wei et al., 2011). Their highest concentrations vary between 550 ng/L (cyromazine) and 20,700 ng/L (oxytetracycline) and their average concentrations between 90 ng/L (sulfamethazine) and 950 ng/L (doxycycline).

Seasonal variations in antibiotic concentrations may be expected. According to the study carried out by Ben et al. (2013) on the quality of swine wastewater collected in sinks and lagoons in 21 types of livestock in China in winter and summer, it emerges that average concentrations of the monitored antibiotics (5 sulfonamides, 3 tetracycline and 1 macrolide) and their detection frequency are higher in winter than in summer. They explain these seasonal fluctuations by the fact

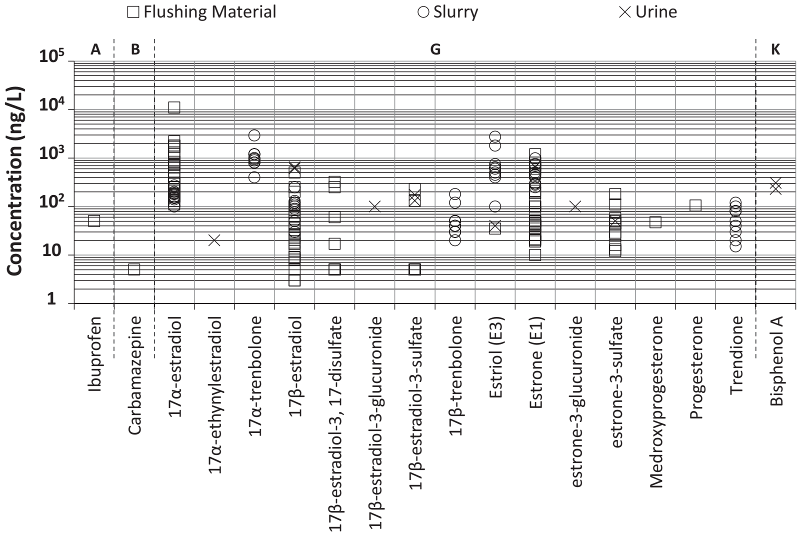

Fig. 8. Occurrence of micropollutants belonging to classes A (Analgesics/anti-inflammatories), B (Anticonvulsants), G (Hormones), and K (Plasticizers) in different types of raw liquid/semiliquid cattle manure. Data from: Gadd et al. (2010), Khan and Lee (2012), Watanabe et al. (2010), Zhang et al. (2014), Zheng et al. (2008).

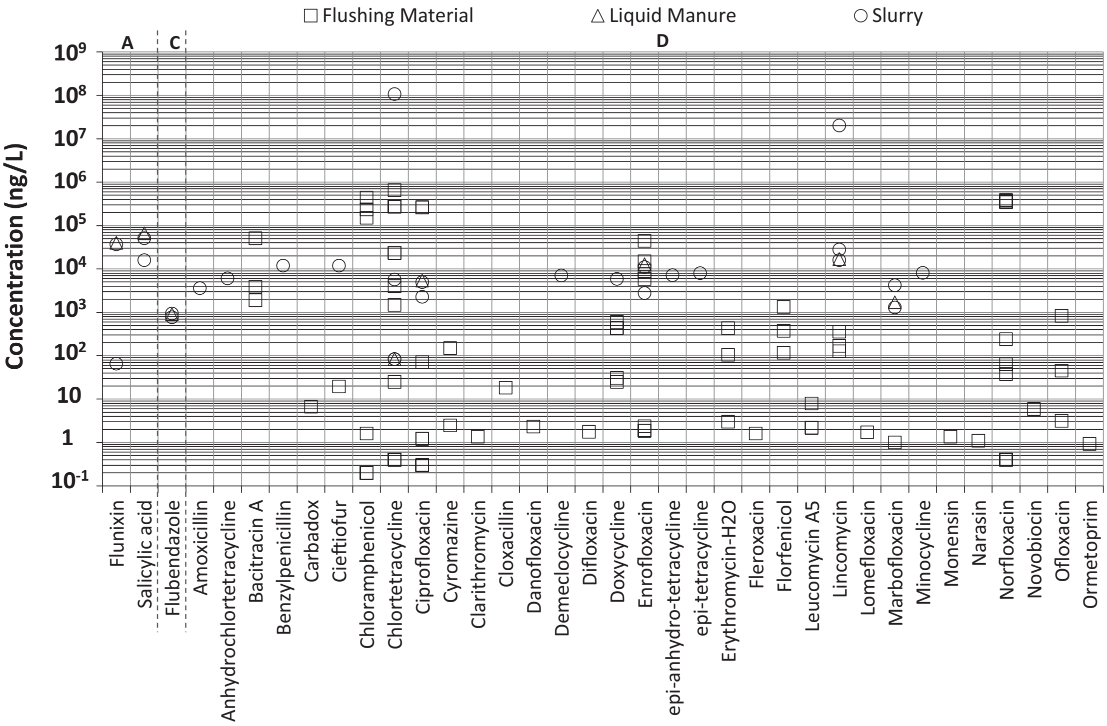

Fig. 9. Occurrence of analgesics/antinflammatories (A), antihelmintics (C) some antibiotics (D) in different types of raw liquid/semiliquid swine manure. Data from: Gros et al. (2019), Hoese et al. (2009), Li et al. (2018), Solliec et al. (2016), Wei et al. (2011), Zhou et al. (2013b).

that, in summer, an enhanced dilution is due to more frequent washing operations and the intensified precipitation events, which are characteristic of the monsoon climate of the area being studied. In addition, they state that, in winter, a higher amount of tetracyclines is administered to animals to prevent flu and other respiratory illnesses which are more frequent in the cold weather. The same conclusions are

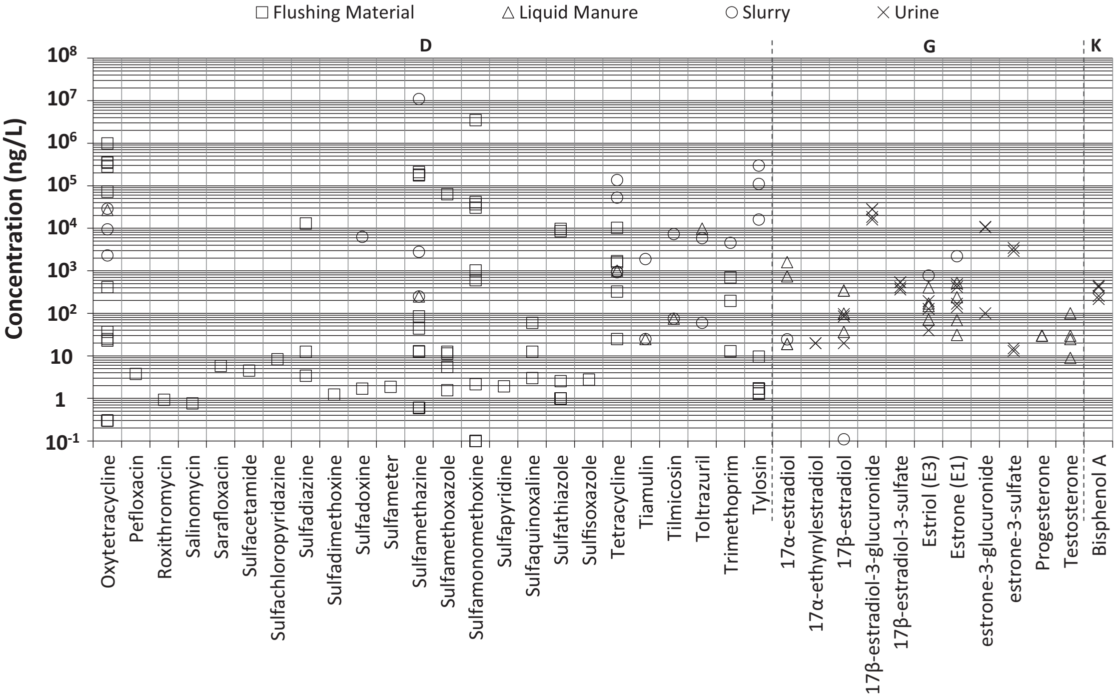

Fig. 10. Occurrence of other antibiotics (D), hormones (G), and plasticizers (K) in different types of raw liquid/semiliquid swine manure. Data from: Burkhardt et al. (2005), Combalbert et al. (2010), Gall et al. (2014), Gros et al. (2019), Hoese et al. (2009), Li et al. (2018), Solliec et al. (2016), Wei et al. (2011), Zhang et al. (2014), Zhou et al. (2013b).

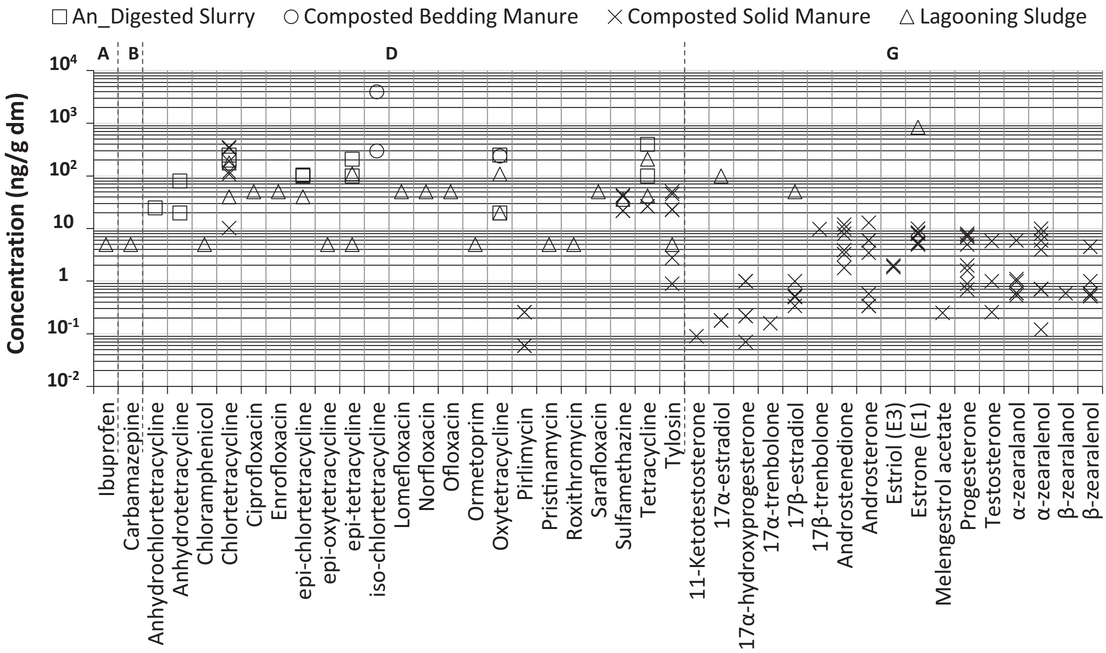

Fig. 11. Occurrence of micropollutants belonging to classes A (Analgesics and anti-inflammatories), B (Anticonvulsants), D (Antimicrobials) and G (Hormones) in treated cattle manure. Data from: Arikan et al. (2007, 2009), Bartelt-Hunt et al. (2013), Biswas et al. (2017), Cessna et al. (2011), Chen et al. (2018), Ray et al. (2017), Raman et al. (2004), Van Donk et al. (2013), Wallace et al. (2018), Watanabe et al. (2010).

confirmed by the investigations by Wang et al. (2019) referring to pig flushing material generated by two swine farms in China. In a previous study (Pan et al., 2011) carried out in the same study area by Ben et al. (2013), it was found that sulphonamides occurred at a higher detection frequency and higher concentrations in summer than in winter (except for sulfamethoxazole) as these antibiotics are generally used to treat a variety of bacterial and protozoal infections which occur more frequently in the hot season. Raman et al. (2004), note that differences between the concentrations referred to in winter and summer could also be due to environmental effects, namely the temperature of the environment which may affect the degradation processes in the case of stored manure.

Wallace et al. (2018) investigated seasonal variations of antibiotics belonging to the class of tetracyclines in solid raw manure from cattle and found that the concentrations of all the investigated compounds (oxytetracycline, chlortetracycline, tetracycline, epi-tetracycline, epi-chlortetracycline and anhydrochlortetracycline) were higher in spring than in winter.

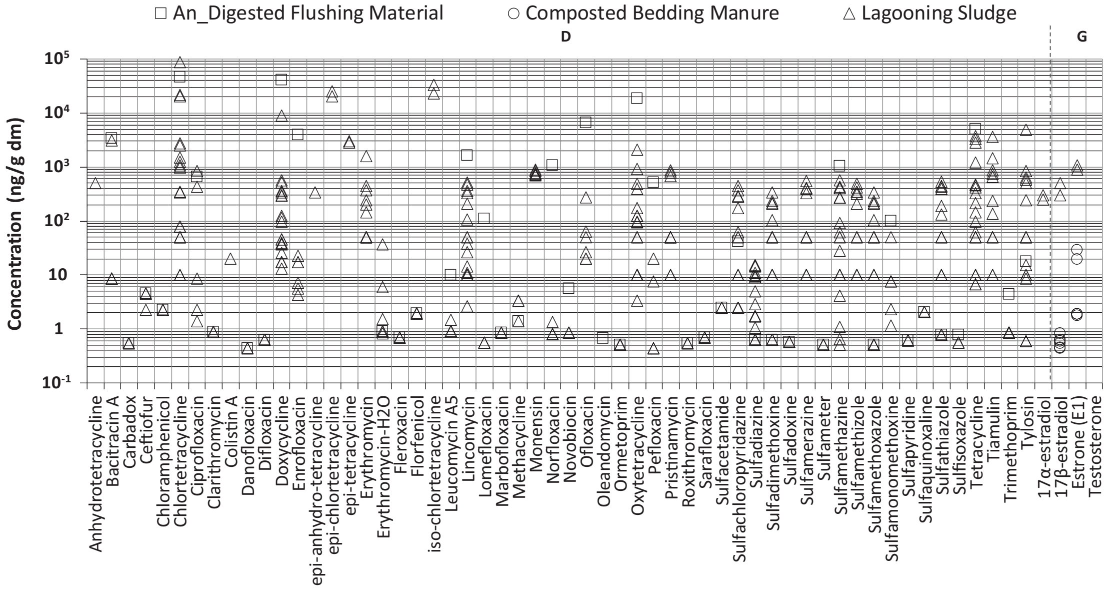

Fig. 12. Occurrence of micropollutants belonging to classes D (Antimicrobials) and G (Hormones) in treated swine manure. Data from: Derby et al. (2011), Frey et al. (2015), Raman et al. (2004), Van den Meersche et al. (2019), Zhang et al. (2013), Zhou et al. (2012, 2013a, 2013b).

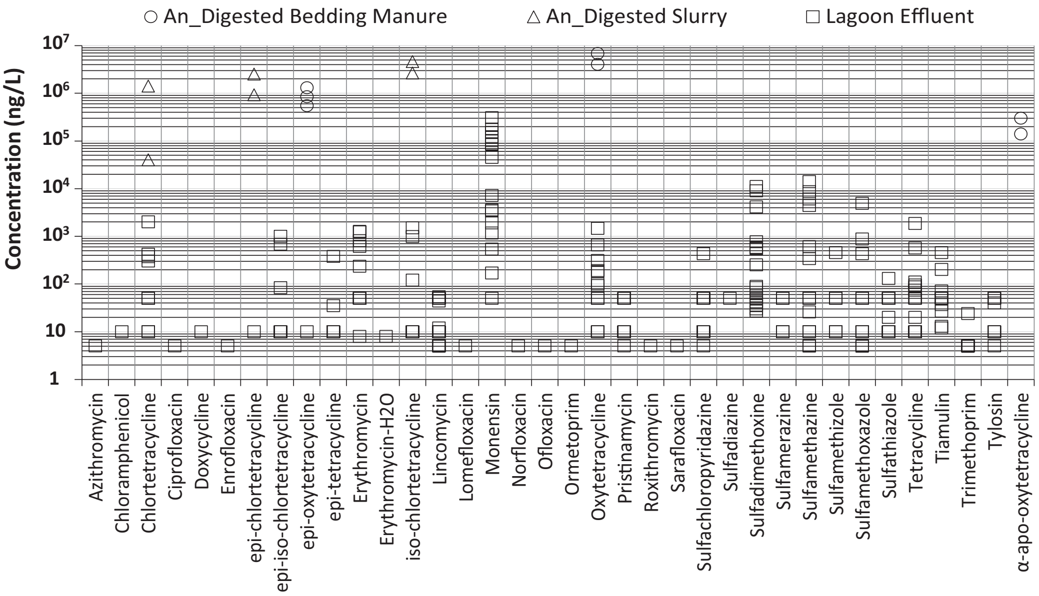

Fig. 13. Occurrence of antibiotics in different types of treated liquid/semiliquid cattle manure. Data from: Arikan (2008), Arikan et al. (2006), Hafner et al. (2017), Watanabe et al. (2010), Zhang et al. (2013).

5.2. Occurrence of selected micropollutants in treated manure

In many cases, manure is stocked and treated before being spread on soil. Common treatments include lagooning, composting, anaerobic digestion, pelletization and alum treatment (Wallace et al., 2018; Cessna et al., 2011, Combalbert et al., 2012). Their main aim is to promote nutrient degradation, liquid-solid separation, dewatering and coagulation.

At the same time, micropollutants may undergo different degradation processes and parent compounds may generate transformation products. The collected data reported in Figs. 11–16 (in Figs. 11 and 12

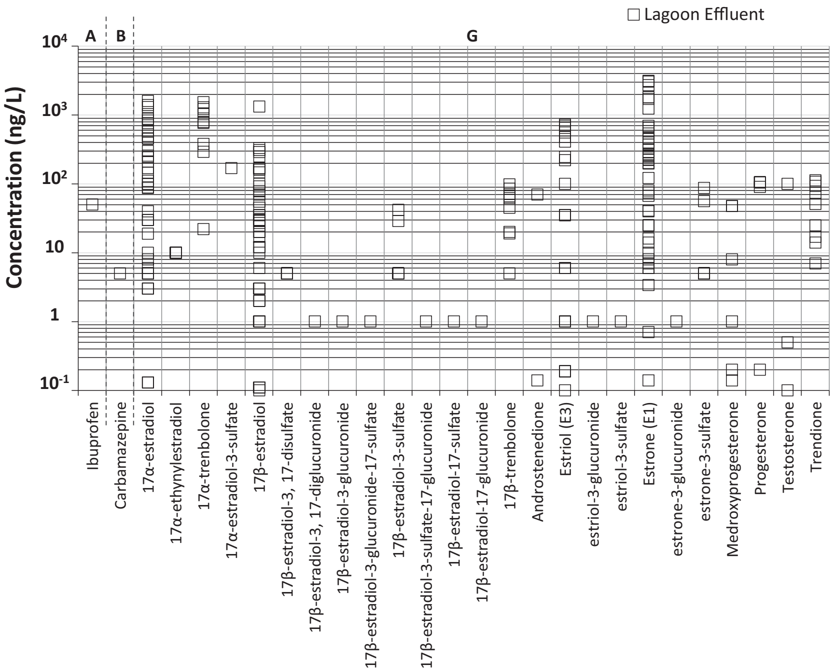

Fig. 14. Occurrence of other micropollutants belonging to classes A (Analgesics and anti-inflammatories), B (Anticonvulsants), and G (Hormones) in different types of treated liquid/semiliquid cattle manure. Data from: Gadd et al. (2010), Gall et al. (2014), Hutchins et al. (2007), Khan and Lee (2012), Kolodziej et al. (2004), Sarmah et al. (2006b), Watanabe et al. (2010), Zhang et al. (2014), Zheng et al. (2008).

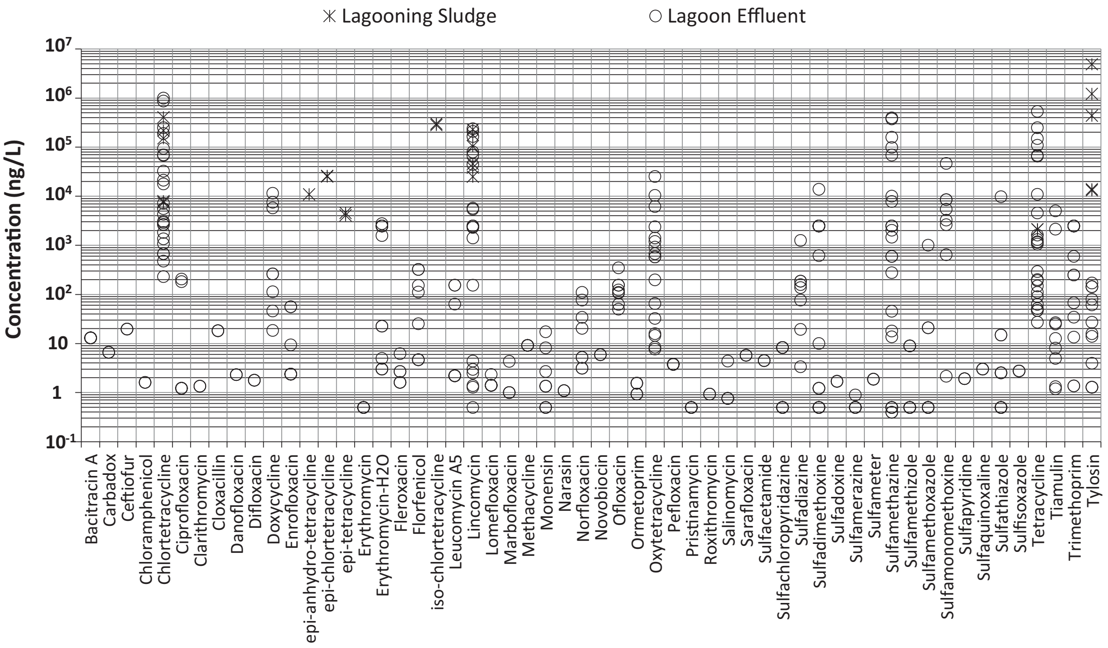

Fig. 15. Occurrence of selected antibiotics in different types of treated liquid/semiliquid swine manure. Data from: Ben et al. (2008), Campagnolo et al. (2002), Dolliver and Gupta (2008), Frey et al. (2015), Kuchta and Cessna (2009), Kuchta et al. (2009), Zhang et al. (2013), Zhou et al. (2012, 2013a, 2013b).

they are given in ng/g dm, and in Figs. 13–16 in ng/L) refer to different types of manure undergoing different treatments. The observed variability ranges of occurrence of selected compounds, average concentrations and the number of data available are discussed here and compared with the corresponding type of raw manure.

Fig. 11 (and Table S11) reports data from treated cattle manure for 41 compounds (1 analgesic/antinflammatory, 1 anticonvulsant, 22 antimicrobials and 17 hormones). 58% of them refers to hormones and 41% to antimicrobials. The most common manure treatment was composting, followed by lagooning and finally anaerobic digestion.

The observed range of concentrations varies between 0.06 ng/g dm (pirlimycin in composted solid manure, Chen et al., 2018) and 4000 ng/g dm (iso-chlortetracycline in composted bedding manure, Arikan et al., 2009). The most investigated compounds were chlortetracycline (10), followed by progesterone, α-zearalanol and estrone (9). The widest variability range was found for 17α-estradiol (3 orders of magnitude). As to compounds with at least 5 values in the graph, the highest average concentrations were found for chlortetracycline (179 ± 114 ng/g dm) and tetracycline (134 ± 148 ng/g dm). 4 compounds out of 41 present the 75th percentile value lower than the corresponding average value: this the case for hormones, 17β-estradiol, estrone, α-zearalanol and β-zearalanol.

Swine manure is commonly subjected to lagooning, composting and anaerobic digestion (Combalbert et al., 2012). Studies investigating occurrence of micropollutants in swine treated manure are summarized in Fig. 12 and Table S12. From these it emerges that 59 compounds were analysed (56 antimicrobials and 3 hormones); the most applied treatment is lagooning (427 values of concentrations included in the review), followed by anaerobic digestion (44 values) and composting (12 values). The observed range of occurrence varies between 0.45 ng/g dm (danofloxacin in lagooning sludge, reported by Zhou et al., 2013b) and 87,900 ng/g dm for chlortetracycline in lagooning sludge (by Zhou et al., 2013b). The most studied compounds were chlortetracycline, doxycycline, oxytetracycline, sulfadiazine and tetracycline with there being 21 values collected for each of them. The highest variability range covers 4 orders of magnitude and refers to oxytetracycline, tylosin, norfloxacin and sulfamethazine. 13 compounds have a range of 3 orders of magnitude. The highest average concentrations were due to iso-chlortetracycline (28,200 ± 6930 ng/g dm), epi-chlortetracycline (22,850 ± 3323 ng/g dm) and chlortetracycline (8985 ± 21,417 ng/g dm).

16 out of 60 compounds present a 75th percentile lower than the corresponding average value. Most of the maximum values were found in lagooning sludge (68%), followed by anaerobically digested flushing material (28%).

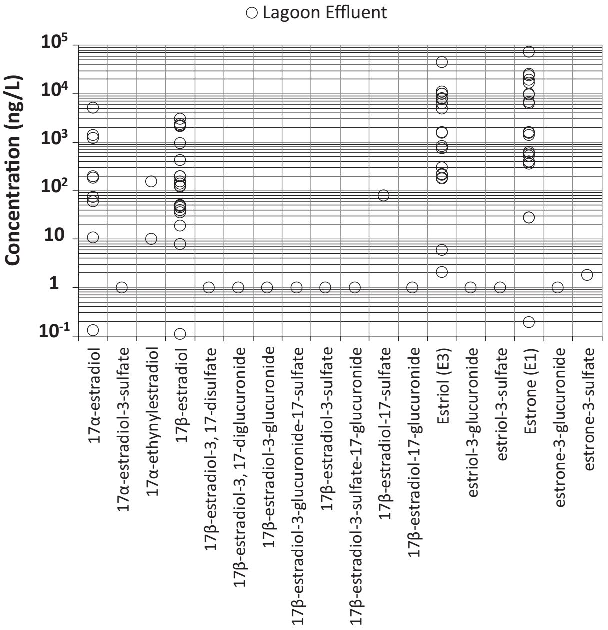

Fig. 16. Occurrence of selected hormones in the effluent of a lagoon receiving swine manure. Data from: Fine et al. (2003), Gall et al. (2014), Hutchins et al. (2007), Sarmah et al. (2006b), Zhang et al. (2014).

Table 3
Analysis of the main characteristics of the raw manure containing the micropollutants with the highest variability range. The colors used in the first column correspond to the animal type. The colored cells regarding "Farm size" and "Dose" correspond to the color cells reported in Table S1 in the supplementary material.

Figure number-Animal type	Compound	Manure type	Range	Order of magnit.	Farm size^1	Animal details	Dose^2	Manure age; other notes	Reference
1 Cattle (ng/g dm)	*Oxytetracycline*	Liquid	0.1 ng/g dm	6	L		n.a.		Wallace et al., 2018
		Bedding	225,000 ng/g dm		VS		D		Arikan et al., 2007
	Chlortetracycline	Solid	0.8 ng/g dm	5	n.a.	Beef	n.a.	Fresh manure	Hou et al., 2015
		Slurry	27,590 ng/g dm		L		n.a.		Zhao et al., 2010
	Enrofloxacin	Solid	0.66 ng/g dm	5	L		R		Zhou et al., 2013a
		Slurry	46,700 ng/g dm		L		n.a.		Zhao et al., 2010
	Sulfamethazine	Liquid	0.45 ng/g dm	5	L		n.a.		Wallace et al., 2018
		Solid	30,250 ng/g dm		S		R		Aust et al., 2008
2. Cattle (ng/g dm)	*17•-estradiol*	Solid	0.18 ng/g dm	4	S		D		Bartelt-Hunt et al., 2012
		Bedding	1416 ng/g dm		L	Dairy	n.a.		Zheng et al., 2008
	Estrone	Solid	0.1 ng/g dm	3	L		n.a.		Zhang et al., 2014
		Bedding	697 ng/g dm		L	Dairy	n.a.	Piled manure 2 weeks	Zheng et al., 2008
	Progesterone	Solid	0.26 ng/g dm	3	S		D		Bartelt-Hunt et al., 2012
		Solid	196 ng/g dm		L	Dairy	n.a.	Piled manure 2 weeks	Zheng et al., 2008
3. Poultry (ng/g dm)	*Enrofloxacin*	Poultry manure	0.8 ng/g dm	7	n.a.		n.a.	Fresh manure	Hou et al., 2015
		Poultry litter	1,420,760 ng/g dm		L		n.a.		Zhao et al., 2010
	Fleroxacin	Poultry manure	0.68 ng/g dm	6	n.a.		n.a.		Zhang et al., 2019
		Poultry litter	99,430 ng/g dm		L		n.a.		Zhao et al., 2010
	Oxytetracycline	Poultry manure	0.8 ng/g dm	6	n.a.		n.a.	Fresh manure	Hou et al., 2015
		Poultry manure	416,750 ng/g dm		n.a.		n.a.		Zhang et al., 2015
	Erythromycin	Poultry manure	0.5 ng/g dm	5	n.a.		n.a.	Fresh manure	Hou et al., 2015
		Poultry manure	12,380 ng/g dm		L		n.a.		Ho et al., 2013
	Difloxacin	Poultry manure	0.73 ng/g dm	5	n.a.		n.a.		Zhang et al., 2019
		Poultry litter	10,910 ng/g dm		L		n.a.		Zhao et al., 2010
	Sulfadiazine	Poultry manure	0.8 ng/g dm	5	n.a.		n.a.	Fresh manure	Hou et al., 2015
		Poultry manure	91,000 ng/g dm		M		n.a.		Martinez-Carballo et al., 2007
	Salinomycin	Poultry litter	0.2 ng/g dm	5	n.a.		n.a.		Furtula et al., 2009
		Poultry manure	22,000 ng/g dm		n.a.		n.a.		Ramasmamy et al., 2010
	Trimethoprim	Poultry manure	0.5 ng/g dm	5	n.a.		n.a.	Fresh manure	Hou et al., 2015
		Poultry manure	17,000 ng/g dm		M		n.a.		Martinez-Carballo et al., 2007
	Tylosin	Poultry manure	0.5 ng/g dm	5	n.a.		n.a.	Fresh manure	Hou et al., 2015
		Poultry manure	57,570 ng/g dm		L		n.a.		Ho et al., 2013
4. Poultry (ng/g dm)	*Testosterone*	Poultry litter	0.03 ng/g dm	4	n.a.		n.a.		Jenkins et al., 2006
		Poultry manure	670 ng/g dm		n.a.	Rooster	n.a.		Shore et al., 1993
	17•-estradiol	Poultry manure	0.2 ng/g dm	3	L	Brood hen			Zhang et al., 2014
		Poultry litter	904 ng/g dm		n.a.		n.a.		Nichols et al., 1998
	Bisphenol A	Poultry manure	0.1 ng/g dm	3	L	Brood hen	n.a.		Zhang et al., 2014
		Poultry manure	207 ng/g dm		n.a.	Indoor broiler	n.a.		Aznar et al., 2018
	Estrone	Poultry manure	0.1 ng/g dm	3	L	Brood hen			Zhang et al., 2014
		Poultry litter	321 ng/g dm		n.a.		n.a.		Albero et al., 2014
	Progesterone	Poultry litter	1.3 ng/g dm	3	n.a.		n.a.		Albero et al., 2014
		Poultry manure	1,310 ng/g dm		L		n.a.		Ho et al., 2013
	Salicylic acid	Poultry litter	1.5 ng/g dm	3	n.a.	Indoor broiler	n.a.		Aznar et al., 2018
		Poultry manure	2,501 ng/g dm		n.a.	Battery cage	n.a.		Aznar et al., 2018
5. Swine (ng/g dm)	*Oxytetracycline*	Slurry	0.43 ng/g dm	6	M		n.a.		Martinez-Carballo et al., 2007
		Solid	354,000 ng/g dm		n.a.	Piglets	n.a.		Chen et al., 2012
	Chlortetracycline	Solid	0.5 ng/g dm (LOD)	6	n.a.		n.a.	Fresh manure	Hou et al., 2015
		Solid	879,600 ng/g dm		n.a.	Hog	n.a.		Bao et al., 2009
	Bacitracin A	Solid	5.22 ng/g dm	5	L	Finishing pigs and sows	n.a.	Collected every day. Feces	Zhou et al., 2013b
		Slurry	320,000 ng/g dm		n.a.		D	Fresh manure	Joy et al., 2013
	Doxycycline	Solid	2.79 ng/g dm	5	L	Finishing pigs	n.a.	Collected every day. Feces	Zhou et al., 2013b
		Slurry	106,000 ng/g dm		n.a.		n.a.		Conde-cid et al., 2018
6. Swine (ng/g dm)	*17•-estradiol*	Solid	0.2 ng/g dm	4	L	Piglets, sow, barrow	n.a.		Zhang et al., 2014
		Slurry	1,500 ng/g dm		n.a.		n.a.		Raman et al., 2004
	Estrone	Solid	0.1 ng/g dm	4	L	Piglets, sow, barrow	n.a.		Zhang et al., 2014
		Slurry	4,800 ng/g dm		n.a.		n.a.		Raman et al., 2004
	Flunixin	Slurry	0.6 ng/g dm	4	n.a.		n.a.		Gros et al., 2019
		Slurry	2,300 ng/g dm		n.a.		n.a.		Gros et al., 2019
	Estriol	Solid	0.4 ng/g dm	3	L		n.a.		Zhang et al., 2014
		Solid	315 ng/g dm		n.a.		n.a.	6 months	Combalbert et al., 2010
7. Cattle (ng/L)	*Chlortetracycline*	Liquid	3 ng/L	6	n.a.		n.a.		Wallace and Aga, 2013
		Slurry	5,860,000 ng/L		VS	Beef calves	D	After 5 days of medication	Arikan, 2008
	epi-chlortetracycline	Liquid	4 ng/L	6	n.a.		n.a.		Wallace and Aga, 2013
		Slurry	4,110,000 ng/L		VS	Beef calves	D	After 5 days of medication	Arikan, 2008
	iso-chlortetracycline	Flushing material	10 ng/L	5	L	Calf hutches	n.a.		Watanabe et al., 2010
		Slurry	2,360,000 ng/L		VS	Beef calves	D	After 5 days of medication	Arikan, 2008
	Sulfamethazine	Flushing material	0.7 ng/L	5	n.a.	Beef cattle	n.a.		Zhang et al., 2013

Figure number-Animal type	Compound	Manure type	Range	Order of magnit.	Farm size[1]	Animal details	Dose[2]	Manure age; other notes	Reference
		Flushing material	15,000 ng/L		L	Calf hutches	n.a.		Watanabe et al., 2010
	Sulfamethoxazole	Flushing material	0.5 ng/L	5	n.a.	Beef cattle	n.a.		Zhang et al., 2013
		Flushing material	19,000 ng/L		L	Calf hutches	n.a.		Watanabe et al., 2010
9. Swine (ng/L)	*Chlortetracycline*	Flushing material	0.4 ng/L	9	n.a.		n.a.		Li et al., 2018
		Slurry	108,000,000 ng/L		VS		D	After 5 days of medication	Hoese et al., 2009
	Chloramphenicol	Flushing material	0.2 ng/L	6	n.a.		n.a.		Li et al., 2018
		Flushing material	441,900 ng/L		n.a.		n.a.		Li et al., 2018
	Ciprofloxacin	Flushing material	0.3 ng/L	6	n.a.		n.a.		Li et al., 2018
		Flushing material	263,100 ng/L		n.a.		n.a.		Li et al., 2018
	Norfloxacin	Flushing material	0.4 ng/L	6	n.a.		n.a.		Li et al., 2018
		Flushing material	389,200 ng/L		n.a.		n.a.		Li et al., 2018
	Lincomycin	Flushing material	126 ng/L	5	L	Piglets	n.a.	Collected every day	Zhou et al., 2013b
		Slurry	20,400,000 ng/L		n.a.		n.a.		Gros et al., 2019
10. Swine (ng/L)	*Sulfamethazine*	Flushing material	0.6 ng/L	8	n.a.		n.a.		Li et al., 2018
		Slurry	11,000,000 ng/L		n.a.		n.a.		Burkhardt et al., 2005
	Sulfamonomethoxine	Flushing material	0.1 ng/L	7	n.a.		n.a.		Li et al., 2018
		Flushing material	3,494,100 ng/L		n.a.		n.a.		Li et al., 2018
	Oxytetracycline	Flushing material	0.3 ng/L	6	n.a.		n.a.		Li et al., 2018
		Slurry	993,800 ng/L		n.a.		n.a.		Li et al., 2018
	Tylosin	Flushing material	1.3 ng/L	5	L	Piglets and sows	n.a.	Collected every day	Zhou et al., 2013b
		Slurry	300,000 ng/L		VS		D	After 5 days of medication	Hoese et al., 2009

[1] Farm size: L = large, M = Medium, S = Small, VS = Very small. See Table S1 for further details.
[2] Dose: R = Rough description in the reference study; D = Detailed description in the reference study.
n.a. = not available.

Regarding hormones, it was found that: aerobic treatments (aerated lagoons and composting) generally promote the reduction of the hormone concentrations, but their effect on reducing the endocrine-disrupting activity is very modest (Combalbert et al., 2012; Derby et al., 2011). Zhang et al. (2014) stated that oxygen and composting time are the main factors affecting the removal efficiency of hormones.

Figs. 13 and 14 refer to concentrations collected for 63 micropollutants in treated liquid-semiliquid cattle manure (further details are also reported in Table S13). The compounds belong to 4 classes: 1 analgesic/anti-inflammatory, 1 anticonvulsant, 36 antimicrobials and 25 hormones. The adopted treatments were lagooning (493 concentrations value), followed by anaerobic digestion (13 values). The observed variability range varies between 0.01 ng/L for estriol (E3) in lagoon effluent (according to Kolodziej et al., 2004), and 6.8 10^6 ng/L for oxytetracycline in anaerobic digested bedding manure (Arikan et al., 2006). The most investigated compounds were hormones (42 values for estrone, 40 for 17β-estradiol and 37 for 17α-estradiol). The widest variability range was of 5 orders of magnitude and were found for 5 antibiotics: oxytetracycline, epi-oxytetracycline, chlortetracycline, and epi- and iso-chlortetracycline.

The highest average values were found for epi-chlortetracycline (1.1 $10^6 \pm 1.3\ 10^6$ ng/L), iso-chlortetracycline (7.3 $10^5 \pm 1.6\ 10^6$ ng/L), epi-oxytetracycline (6.7 $10^5 \pm 5.4\ 10^5$ ng/L) and oxytetracycline (5.4 $10^5 \pm$ 1.7 10^6 ng/L). On the basis of the statistical analysis reported in Table S13, it emerges that for 14 compounds, the 75th percentile is lower than the corresponding average value. The highest differences were found for iso-chlortetracycline (7.2 10^5 ng/L), oxytetracycline (5.4 10^5 ng/L) and chlortetracycline (1.1 10^5 ng/L). The maximum values occurred in lagoon effluent (88%) and anaerobic digested manure (12%).

With regard to treated poultry manure a limited number of data are available and refer to the antibiotic salinomycin (3 values) and the hormones 17β-estradiol (2 values) and testosterone (3 values) (Nichols et al., 1997; Ramaswamy et al., 2010; Hakk et al., 2005; Shore et al., 1993). Limiting the attention to the investigations providing concentrations before and after a specific poultry treatment, it emerges that salinomycin reduces from 22,000 ng/g dm to 76 ng/g dm in the case of composting (Ramaswamy et al., 2010), 17β-estradiol reduces from the initial concentration of 83 ng/g dm to 13 ng/g dm after a composting step (Hakk et al., 2005) and from 133 ng/g dm to 101 ng/g dm if treated with alum (Nichols et al., 1997). The behaviour of testosterone in the case of composting is different: according to Hakk et al. (2005) it reduces from 115 ng/g dm to 11 ng/g dm, whereas according to Shore et al. (1993), it increases from 298 ng/g dm to 525 ng/g dm.

With regard to treated liquid/semiliquid swine manure, Figs. 15 and 16 (and Table S14) report concentration values for 74 compounds (56 antimicrobials and 18 hormones) in lagoon effluent (503 values) and lagooning sludge (25 values). The observed range of variability varies between 0.11 ng/L (17β-estradiol in lagoon effluent, by Gall et al., 2014) and 4.9 10^6 ng/L (tylosin in lagooning sludge by Dolliver and Gupta, 2008).

The most investigated compounds were chlortetracycline (29 values), lincomycin (27 values) and tetracycline (26 values). The widest variability ranges cover 6 orders of magnitude and occurred for sulfamethazine, tylosin and lincomycin. 5 orders of magnitude intervals were found for estrone, sulfadimethoxine. 9 compounds present a range of 4 orders of magnitude.

With regard to compounds with >5 values, the highest average concentrations were found for tylosin (3.9 $10^5 \pm 1.2\ 10^6$ ng/L), chlortetracycline (1.2 $10^5 \pm 2.5\ 10^5$ ng/L) and lincomycin (5.4 $10^4 \pm 7.9\ 10^4$ ng/L).

Based on the data reported in Table S14, 15 compounds present the 75th percentile lower than the corresponding average value. The highest differences were for tylosin (3.8 10^5 ng/L), sulfamethazine (3.9 10^4 ng/L) and tetracycline (3.7 10^4 ng/L). As for the maximum values, they were mainly found in lagoon effluent (88%).

Concentrations of 17 hormones in the effluent of a lagoon receiving poultry house flushing material were provided by the investigations by Gall et al. (2014) and Hutchins et al. (2007). The most analysed compounds were 17α-estradiol, 17β-estradiol, estriol and estrone being seven measures available for each of them. In Gall et al. (2014), the lagoon treatment consisted of three basins in series. The measured concentrations reported referred to each lagoon effluent (influent data are not available) and highlighted that the concentrations of the selected compounds greatly reduced from the first to the second step and even more from the second to the third. For instance, estrone at the exit of the first basin was 2970 ng/L, at the exit of the secondary lagoon 1570 ng/L and after the polishing lagoon 21 ng/L.

Some investigations reported data of concentrations of the same compounds in the raw manure and after its treatment: see for instance Wallace et al. (2018), Arikan et al. (2009) and Ray et al. (2017). Figs. S2–

Table 4
Analysis of the main characteristics of the treated manure containing the micropollutants with the highest variability range. The colors used in the first column correspond to the animal type. The colored cells regarding "Farm size" and "Dose" correspond to the color cells reported in Table S1 in the supplementary material.

Figure number-Treatment type	Compound	Treated manure	Range	Order of magnit	Farm size^1	Animal details	Dose^2	Manure age; other note	Reference
11. Treated cattle manure (ng/g dm)	*17•-estradiol*	Composted solid	0.18 ng/g dm	3	S		D		van Donk et al., 2013
		Lagooning sludge	100 ng/g dm		n.a.		n.a.		Raman et al., 2004
12. Treated swine manure (ng/g dm)	*Norfloxacin*	Lagooning sludge	0.81 ng/g dm	4	L		R	Lagoon feeding= mixture of manure from animals in different stages	Zhou et al., 2013a
		An-digested flushing material	1,080 ng/g dm		L		R	Flushing material from farms housing animals in different stages	Zhou et al., 2013a
	Oxytetracycline	Lagooning sludge	3.4 ng/g dm	4	n.a.		R		Van den Meersche et al., 2019
		An-digested flushing material	19,000 ng/g dm		L		R	Flushing material from farms housing animals in different stages	Zhou et al., 2013a
	Sulfamethazine	Lagooning sludge	0.52 ng/g dm	4	L		n.a.		Zhou et al., 2012
		An-digested flushing material	1060 ng/g dm		L		R	Flushing material from farms housing animals in different stages	Zhou et al., 2013a
	Tylosin	Lagooning sludge	0.61 ng/g dm	4	L		n.a.	Collected every day Lagoon feeding= mixture of manure from animals in different stages	Zhou et al., 2013b
		Lagooning sludge	4,913 ng/g dm		n.a.	Beef cattle	n.a.		Zhang et al., 2013
13. Treated cattle manure (ng/L)	*Chlortetracycline*	Lagoon effluent	10 ng/L	5	L		n.a.		Watanabe et al., 2010
		An-digested slurry	1,400,000 ng/L		VS	Beef calves	D	After 5 days of medication	Arikan, 2008
	epi-chlortetracycline	Lagoon effluent	10 ng/L	5	L		n.a.		Watanabe et al., 2010
		An-digested slurry	2,500,000 ng/L		VS	Beef calves	D	After 5 days of medication	Arikan, 2008
	epi-oxytetracycline	Lagoon effluent	10 ng/L	5	L		n.a.		Watanabe et al., 2010
		An-digested bedding	1,300,000 ng/L		VS	Beef calves	D	After 5 days of medication	Arikan et al., 2006
	iso-chlortetracycline	Lagoon effluent	10 ng/L	5	L		n.a.		Watanabe et al., 2010
		An-digested slurry	4,600,000 ng/L		VS	Beef calves	D	After 5 days of medication	Arikan, 2008
	Oxytetracycline	Lagoon effluent	10 ng/L	5	L		n.a.		Watanabe et al., 2010
		An-digested bedding	6,800,000 ng/L		VS	Beef calves	D	After 5 days of medication	Arikan et al., 2006
14. Treated cattle manure (ng/L)	*17•-estradiol*	Lagoon Effluent	0.13 ng/L	4	n.a.	Dairy and beef cattle	n.a.		Gall et al., 2014
		Lagoon Effluent	1,600 ng/L		n.a.	Dairy cattle	n.a.		Gadd et al., 2010
	17•-estradiol	Lagoon Effluent	0.1 ng/L	4	n.a.	Dairy cattle	n.a.		Kolodziej et al., 2004
		Lagoon Effluent	1,326 ng/L		L		n.a.		Zhang et al., 2014
	Estrone	Lagoon Effluent	0.14 ng/L	4	n.a.	Dairy cattle	n.a.		Kolodziej et al., 2004
		Lagoon Effluent	3,123 ng/L		n.a.		n.a.		Sarmah et al., 2006b
	Estriol	Lagoon Effluent	0.1 ng/L	3	n.a.	Dairy cattle	n.a.		Kolodziej et al., 2004
		Lagoon Effluent	725 ng/L		n.a.		n.a.	Implanted cows	Khan and Lee, 2012
15. Treated swine manure (ng/L)	*Lincomycin*	Lagoon Effluent	0.5 ng/L	6	n.a.		n.a.		Zhang et al., 2013
		Lagoon Effluent	240,000 ng/L		n.a.		n.a.		Campagnolo et al., 2002
	Sulfamethazine	Lagoon Effluent	0.4 ng/L	6	n.a.		n.a.		Zhang et al., 2013
		Lagoon Effluent	400,000 ng/L		n.a.		n.a.		Campagnolo et al., 2002
	Tylosin	Lagoon Effluent	1.3 ng/L	6	L		R	Lagoon feeding= mixture of manure from animals in different stages	Zhou et al., 2013a
		Lagooning sludge	4,924,867 ng/L		n.a.		n.a.		Dolliver and Gupta, 2008
	Sulfadimethoxine	Lagoon Effluent	0.5 ng/L	5	n.a.		n.a.		Zhang et al., 2013
		Lagoon Effluent	14,050 ng/L		n.a.		n.a.		Ben et al., 2008
16. Treated swine manure (ng/L)	*Estrone*	Lagoon Effluent	0.19 ng/L	5	n.a.		n.a.		Gall et al., 2014
		Lagoon Effluent	74,700 ng/L		n.a.		n.a.		Fine et al., 2003
	17•-estradiol	Lagoon Effluent	0.13 ng/L	4	n.a.		n.a.		Gall et al., 2014
		Lagoon Effluent	5,189 ng/L		n.a.		n.a.		Gall et al., 2014
	17•-estradiol	Lagoon Effluent	0.11 ng/L	4	n.a.		n.a.		Gall et al., 2014
		Lagoon Effluent	3000 ng/L		n.a.		n.a.		Fine et al., 2003
	Estriol	Lagoon Effluent	2.1 ng/L	4	n.a.		n.a.		Gall et al., 2014
		Lagoon Effluent	45,379 ng/L		n.a.		n.a.		Gall et al., 2014

[1] Farm size: L = large, M = Medium, S = Small, VS = Very small. See Table S1 for further details.
[2] Dose: R = Rough description in the reference study; D = Detailed description in the reference study.
n.a. = not available.

S3 (concentration in ng/g dm) and Figs. S6–S7 (concentration in ng/L) report and compare the values for raw and treated manure from cattle, Figs. S4–S5 (concentration in ng/g dm) and Figs. S8–S9 (concentration in ng/L) for raw and treated manure from swine.

It was found that operational conditions may greatly affect the removal of specific compounds. For instance, in composting, temperature has a key role: according to the investigations on pig and poultry solid manure composting by Zhang et al. (2019), swine manure by Liu et al.

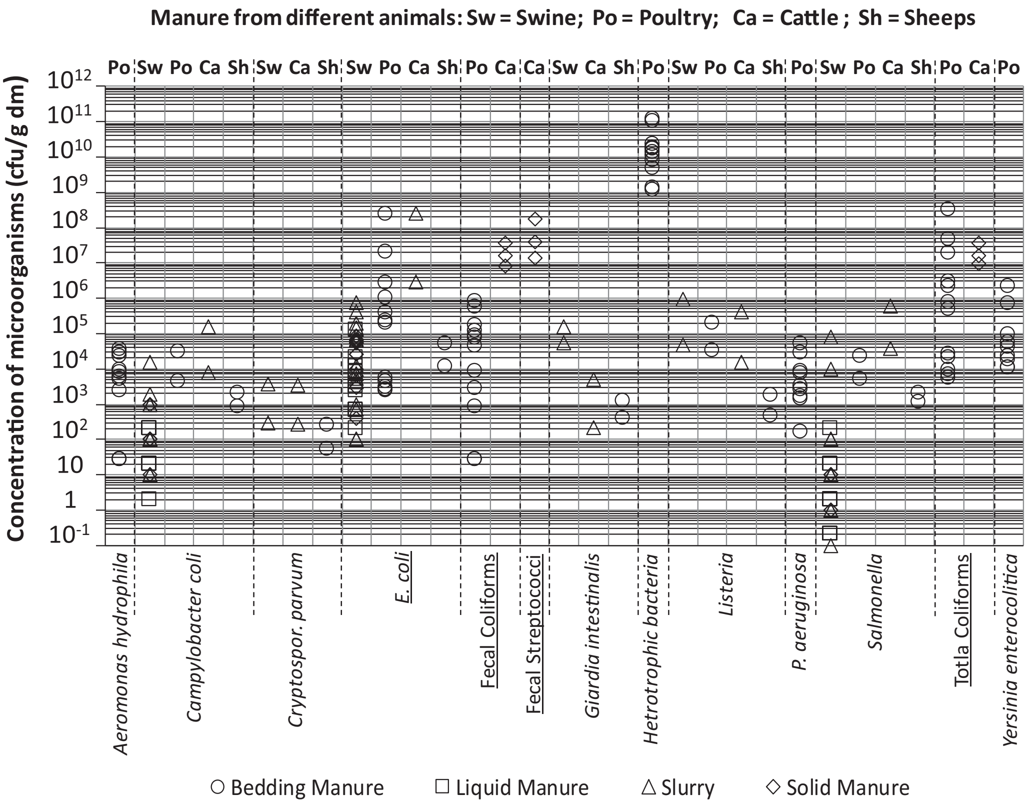

Fig. 17. Observed concentrations of microorganisms in different types of raw manure generated by different animals (swine, poultry, cattle and sheep). (On the X axis, the underlined names correspond to indicator organisms, those not underlined to pathogens). Data from: Hutchison et al. (2004), Kelley et al. (1994), Patten et al. (1980), Van den Meersche et al. (2019).

(2015), and cattle solid manure by Ray et al. (2017), thermophilic conditions allow higher removal efficiency for a wide spectrum of antibiotics. Arikan et al. (2016) found that in the composting of dairy and poultry bedding manure, temperatures in the range 45–65 °C lead to a high removal of salinomycin, whereas ambient temperatures may guarantee a high removal of monensin, lasalocid and amprolium. Bao et al. (2009) and Ho et al. (2013) reported that in the composting of poultry manure, antibiotic removal is strictly correlated not only to temperature, but also to total organic carbon, total nitrogen, C/N ratio and metal content, such as copper as found by Liu et al., 2015

Aerobic conditions seem to favour the degradation of antibiotics in lagoons (Hafner et al., 2017). Some antibiotics such as chlortetracycline,

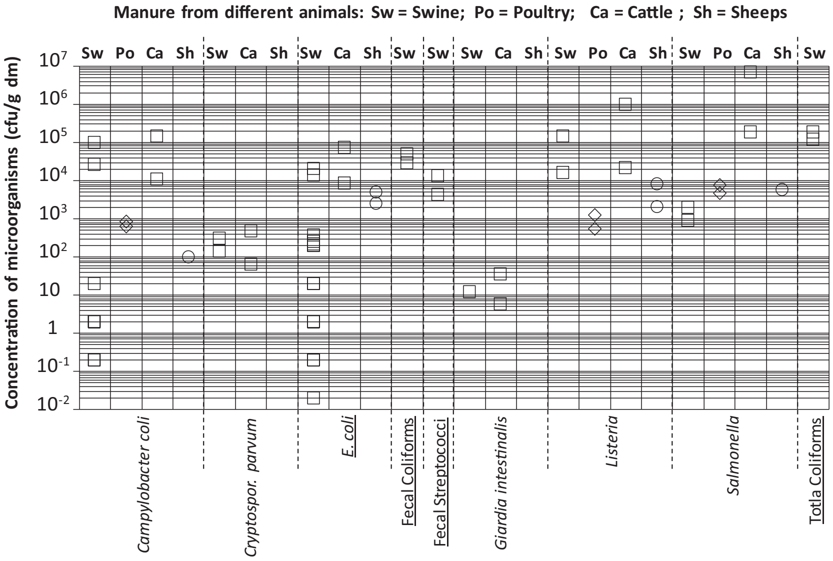

Fig. 18. Observed concentrations of microorganisms in treated manures from different animals. Data from: Frey et al. (2013), Hutchison et al. (2004), Van den Meersche et al. (2019).

Table 5
PEC soils by assuming the two application rates discussed in the manuscript (2200 kg dm/ha year; 9500 kg dm/ha year) and ranges of measured concentrations found in the literature.

Compound	Swine slurry c_i [ng/g dm]			PEC_{soil} [ng/g dm]–2200 kg dm/(ha year)			PEC_{soil} [ng/g dm]–9500 kg dm/(ha year)			MEC_{soil} [ng/g dm]	References for MEC
	Min	Max	Average	Min	Max	Average	Min	Max	Average	(Literature)	
Chlortetracycline	0.95	764,400	82,313	6.15E−04	495	53.26	2.65E−03	2136	230	ND–1430	Gros et al., 2019; Zhou et al., 2013a
Ciprofloxacin	880	3400	2140	5.69E−01	2.20	1.38	2.46E+00	9.50	5.98	ND–32.8	Gros et al., 2019; Zhou et al., 2013a
Doxycycline	5.4	106,000	8383	3.49E−03	68.6	5.42	1.51E−02	296	23.4	ND-499	Zhou et al., 2013a
Enrofloxacin	0.75	6010	2216	4.85E−04	3.89	1.43	2.10E−03	16.8	6.19	2.3–151	Gros et al., 2019
Oxytetracycline	0.425	100,000	7950	2.75E−04	64.7	5.14	1.19E−03	279	22.2	1–75	Gros et al., 2019
Sulfamethazine	0.11	20,000	1912	7.12E−05	12.9	1.24	3.07E−04	55.9	5.34	ND-15	Christian et al., 2003
Sulfamethoxazole	0.175	570	173	1.13E−04	0.37	0.11	4.89E−04	1.59	0.48	ND	Gros et al., 2019
Tetracycline	0.425	23,000	2424	2.75E−04	14.9	1.57	1.19E−03	64.3	6.77	0.22–10.25	Gros et al., 2019
Tiamulin	0.4	120	37.9	2.59E−04	7.76E−03	2.45E−02	1.12E−03	0.34	0.11	ND	Gros et al., 2019
Tylosin	5.2	32,500	2597	3.36E−03	21.0	1.68	1.45E−02	90.81	7.26	ND	Gros et al., 2019; Zhou et al., 2013b

sulfamethazine and tylosin seem to be degraded better under the anaerobic conditions occurring in stockpiling instead of in composting processes (see for instance: Sura et al., 2014 and Cessna et al., 2011).

5.2.1. Influence of the main parameters on the concentration of selected pharmaceuticals in manure

A rapid look at the graphs of the concentrations of selected micropollutants in the different manures points out that pharmaceuticals and hormones may occur with a wide range of variability. To better understand which could be the main reasons of this variability, Tables 3 and 4 report minimum and maximum values for the compounds exhibiting the highest variability ranges in raw and treated manure respectively, together with (when available) manure type, farm size, animal type, administered dose of pharmaceutical, manure age, and corresponding reference.

It emerges that it is not possible to correlate the maximum values with specific conditions and also to explain the widest ranges of variability. An in depth anaylisis showed that pharmaceutical administration pattern (in terms of pharmaceutical dose and administration time interval), manure sampling time, animals in different growing stages (namely piglets (14–20 kg and 20–35 kg), growing pigs (2–3 months, 35–55 kg), finising pigs (3–7 months, 55–125 kg) and sows (7–50 months)), different manure production area (for instance: milking area and stalls in case of dairy cattle farms), open or closed feedlot, farm management operations (mode and frequency), manure age may strongly influence the occurrence of microcontaminants. Unfortunately an in depth description of all these aspects is not always available. The main lessons learned from this analysis are herein reported.

Farm size. Chen et al. (2012) reported that there are not great differences in antibiotic concentrations in manure provided by animal farms of different size.

Animal growing stage (*swine manure*). Antibiotics are generally administrated to prevent disease (higher amounts to young pigs) and to improve feeding efficiency (mainly for to fattening pigs). The highest concentrations in manure were found for young pigs followed by fattening pigs. Very lower values were found in manure generated by sows (Chen et al., 2012; Hou et al., 2015; Zhou et al., 2013b). As to the hormones estrone, 17β-estradiol, 17α-estradiol and estriol, the highest values of concentrations in manure were found for sows with respect to piglets and finishing pigs (Gall et al., 2014).

Dairy and beef cattle farms (*cattle manure*). Figs. S10 and S11 report the same values of concentrations in raw cattle manure of Figs. 1 and 2, but grouped according to the two types of cattle farms (diary and beef cattle). A comparison of the distribution of values shows that in beef cattle raw manure the highest concentrations were found for oxytetracycline (225,000 ng/g dm, Arikan et al., 2007) and sulfamethazine (30,250 ng/g dm, Aust et al., 2008). In diary cattle raw manure, the highest values were for oxytetracycline (59,590 ng/g dm, Zhao et al.,

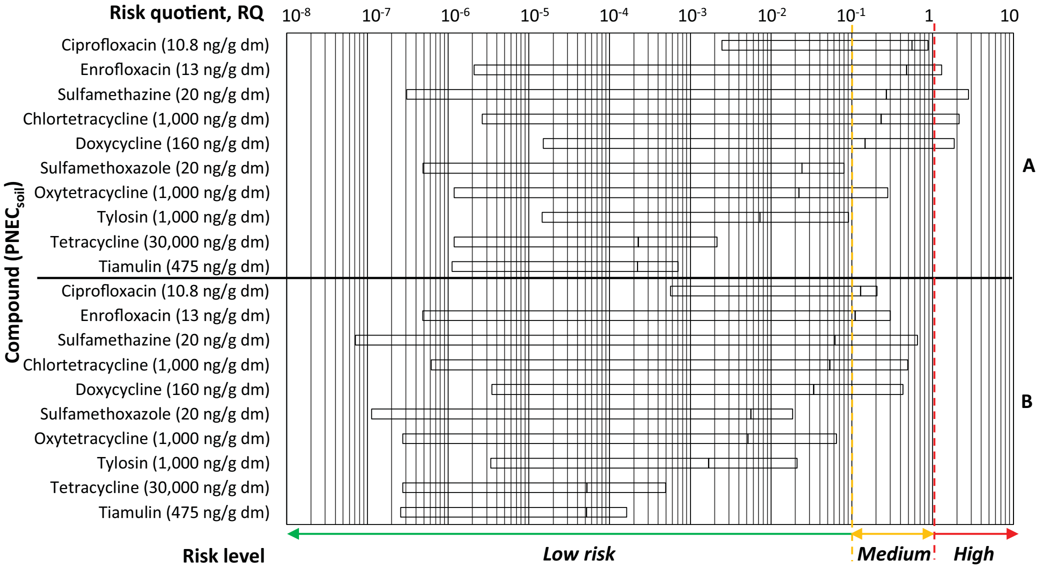

Fig. 19. RQ for a selection of antibiotics under review in the case of the different application rate of swine slurry. The number in brackets after the name of the antibiotic corresponds to the adopted $PNEC_{soil}$. Application rate of: A) 9500 kg dm/(ha year); B) 2200 kg dm/(ha year).

2010), enrofloxacin (46,700 ng/g dm Zhao et al., 2010), followed by ciprofloxacin (29,590 ng/g dm, Zhao et al., 2010) and chlortetracycline (27,590 ng/g dm, Zhao et al., 2010). The ranges of variability are still wide for some antibiotics in both graphs. This is the case of oxytetracycline (7 orders of magnitude in both dairy and milk cattle manure), enrofloxacin (6 orders of magnitude in dairy cattle manure), chlortetracycline (5 orders of magnitude in dairy cattle manure and 4 in beef cattle manure), sulfamethazine (5 orders in beef cattle manure and 4 in dairy cattle manure). Finally, dairy cattle raw manure contains hormones up to 1001,000 ng/g dm. In beef cattle manure hormone concentrations were always found in the range 0.1180 ng/g dm and they refer to a higher number of compounds.

Broiler and layer poultry farms.This two types of farms lead to the production of poultry litter and poultry manure. These kinds of manure are exactly what reported in the figures of this study.

Farm management. On the basis of the collected literature data it is not possible to correlate concentrations of antibiotics and hormones in manure with respect to the different farm management operations.

5.3. Occurrence of microorganisms in raw and treated manures

The investigations on microorganisms included in this review referred to different types of manure (bedding manure, liquid manure, slurry and solid manure, according to Table 1) produced by four different animals (cattle, swine, poultry and sheep).

The investigations dealt with the occurrence of indicator bacteria (Heterotrophic bacteria, Total coliforms, Faecal coliforms, *E. coli*, Faecal streptococci) and selected pathogens (mainly *Aeromonas hydrophila*, *Campylobacter coli*, *Cryptosporidium parvum*, *Giardia intestinalis*, *Listeria*, *Pseudomonas aeruginosa*, *Salmonella* and *Yersinia enterocolitica*). Limiting the attention to concentrations provided in cfu/g dm, the collected data are those reported in Fig. 17 (referring to 13 microorganisms in different types of raw manure) and Fig. 18 (9 microorganisms in three different treated manures). In addition, Tables S15 and S16 report a descriptive statistical analysis of the literature data under review for raw and treated manures, respectively.

The literature also provides concentrations of microorganisms in manure expressed in other units of measurement: for instance, cells/g dm, MPN/100 g dm and cfu/100 mL. For the sake of completeness, bacteria are listed in Table S2 with the corresponding references, but due to the limited number of values available we chose not to include all of them in this discussion.

As for raw manure, it emerges that poultry manure was the object of 10 studies, cattle manure of 9 and sheep and swine manure of 6 studies each. The highest number of concentrations were found for *E. coli* (54 concentrations), followed by *Campylobacter coli* (32 values) and *Salmonella* (30 values). All of them were observed in manure from the four different animals listed.

The group of heterotrophic bacteria exhibited the highest concentrations (in poultry they were found in the range 10^9–10^{11} cfu/g dm) followed by other indicator bacteria (Total coliform in poultry manure equal to 3.8 10^8 cfu/g dm; *E. coli* in poultry and cattle manure around 2.3–2.6 10^8 cfu/g dm; faecal streptococci in cattle manure 1.7 10^8 cfu/g dm, and faecal coliform equal to 3.7 10^7 cfu/g dm in cattle manure).

As for pathogens, the highest concentrations were found for *Yersinia enterocolitica* (2.1 10^6 in poultry manure), *Listeria* (9.7 10^5 cfu/g dm in swine manure), *Salmonella* (5.8 10^5 cfu/g dm in cattle manure) and also *Giardia intestinalis* (1.6 10^5 cfu/g dm in swine manure), and *Campylobacter coli* (1.5 10^5 cfu/g dm in cattle manure). The highest concentrations were more frequently detected in cattle manure followed by poultry manure. With regard to the type of manure sampled, it emerges that bedding manure more frequently presented the highest values (around 50% of cases).

A focus on the content of indicator bacteria and pathogens in the different animal manures shows that there are some orders of magnitude difference between the concentrations detected in the two groups. In particular, in cattle manure, average concentrations of the reviewed indicator bacteria range between 2.1 10^7 cfu/g dm and 1.3 10^8 cfu/g dm, whereas average concentrations of pathogens between 1.9 10^3 cfu/g dm and 3.1 10^5 cfu/g dm; in poultry manure, average concentrations of indicator bacteria range between 1.5 10^5 cfu/g dm and 2.7 10^{10} cfu/g dm, those of pathogens vary between 1.1 10^4 cfu/g dm and 3.4 10^5 cfu/g dm. In swine manure, concentrations of indicator bacteria (the only available is *E. coli*) vary between 10^2 cfu/g dm and 7.5 10^5 cfu/g dm, whereas average concentrations of pathogens vary between 9 10^2 cfu/g dm and 5.1 10^5 cfu/g dm. Finally, in sheep manure, the concentration of *E. coli* in raw manure varies from 1.1 10^4 cfu/g dm to 4.9 10^4 cfu/g dm and average concentrations of pathogens between 1.5 10^2 cfu/g dm and 1.5 10^3 cfu/g dm.

A lower number of values are available for the content of microorganisms in treated manure, as shown in Fig. 18. Most of them (50 out of 64 values) refer to concentrations of microorganisms found in the sediments of lagoons (lagooning sludge) receiving swine manure (Hutchison et al., 2004; Frey et al., 2013; Van den Meersche et al., 2019). 8 values refer to different microorganisms in composted bedding sheep manure (Hutchison et al., 2004) and composted solid poultry manure. Unfortunately, it is not possible to make a comparison of the different treatments for the same type of manure.

As with the raw manure, the most studied microorganisms are the indicator *E. coli* (20 values), followed by the pathogen *Campylobacter coli* (13 values). With regard to the maximum observed values, it emerges that *Salmonella* occurred up to 7.2 10^6 cfu/g dm (Hutchison et al., 2004) and *Listeria* up to 9.8 10^5 cfu/g dm, and all the other microorganisms generally presented <2 10^5 cfu/g dm.

The average concentrations of indicator bacteria in treated manure vary between 2.3 10^3 cfu/g dm (*E. coli* in swine lagooning sludge) and 1.6 10^5 cfu/g dm (total coliform in swine lagooning sludge) and for pathogens between 1.2 10 cfu/g dm (*Giardia intestinalis* in swine lagooning sludge) and 3.7 10^6 cfu/g dm (Salmonella in cattle lagooning sludge).

The highest average concentrations were 3.7 10^6 cfu/g dm (*Salmonella* in cattle lagooning sludge), 5.0 10^5 cfu/g dm for *Listeria* in cattle lagooning sludge), 1.6 10^5 cfu/g dm for total coliform in swine lagooning sludge. All the other values of average concentrations referred to the reviewed microorganisms, the different animals and treatment may be found in Table S16. A comparison with the above reported range of variability in the case of raw manure shows that the treatment is generally able to reduce the content of indicator bacteria.

6. Manure land application and predicted concentration of micropollutants in soil – comparison with measured concentrations

Predicted concentrations in manure-amended soil (PEC) for a selection of antibiotics under review was carried out under the following assumptions:

- swine slurry (with PhC concentrations in ng/g dm) was applied to soil;
- two application rates of this slurry 2200 kg dm/(ha year) (scenario 1) and 9500 kg dm/(ha year) (scenario 2), were applied to soil. These application rates would be able to respect the limit of 170 kg N/ha year discussed above (the first defined by Pappas et al. (2008) should be able to guarantee the respect of 168 kg N/ha year and the second rate, according to Joy et al. (2013) should respect 151 kg N/ha year);
- the estimation of the concentration in soil was made for compounds for which predicted no effect concentrations in soil ($PNEC_{soil}$) are known from the literature, namely the antibiotics: chlortetracycline, ciprofloxacin, doxycycline, enrofloxacin, oxytetracycline, sulfamethazine, sulfamethoxazole tetracycline, tiamulin and tylosin (see Table S17 for details).

PECs were evaluated according to the well-known Eq. (1), recommended by the European Technical Guidance Document on risk assessment EUR 20418 EN/2 (EC-TGD, 2003).

$$PEC_{i,soil} = c_{0,soil} + \frac{c_{i,slurry}\ x\ APP_{slurry}}{DEPTH_{soil}\ x\ RHO_{soil}} \quad (1)$$

where $c_{0,soil}$ corresponds to the background concentration in the soil (ng/g dm), before the manure has been spread on it (in this study it was assumed equal to zero); $c_{i,slurry}$ is the measured concentration (MEC) in swine slurry (ng/g dm); APP_{slurry} is the yearly application rate of dry slurry on soil; $DEPTH_{\mathrm{soil}}$ is the mixing depth (0.10–0.20 m is generally the depth of the mixing during application (Dutta et al., 2012; Ghirardini and Verlicchi, 2019)). Here the value of 0.20 m is used as well as discussed in Ghirardini and Verlicchi et al. (2019). RHO_{soil} is the bulk density of wet soil (1700 kg/m^3 for agricultural soils as discussed in Verlicchi and Zambello, 2015 and recommended by EC-TGD, 2003).

The evaluation of the PEC in soil was carried out assuming the measured minimum, maximum and average concentrations of the selected antibiotics in swine slurry for the two scenarios of the manure application rate (the lowest and the highest values of slurry application rates mentioned above).

Table 5 reports $c_{i,slurry}$, APP_{slurry}, the corresponding maximum, minimum and average PEC$_{\mathrm{soil}}$ in the two situations. It also compiles ranges of measured concentrations in soil (MEC$_{\mathrm{soil}}$) found in the literature for the same antibiotics in swine slurry-amended soil (Gros et al., 2019; Solliec et al., 2016; Christian et al., 2003) or in the lagoon effluent (receiving pig slurry) applied to arable land (Zhou et al., 2013a, 2013b).

A comparison between PECs and MECs in soil shows that average PECs$_{\mathrm{soil}}$ is always in the range of the reported MEC$_{\mathrm{soil}}$. Maximum PEC$_{\mathrm{soil}}$ exceed the MEC$_{\mathrm{soil}}$ for tetracycline, tiamulin and tylosin at both application rates and for chlortetracycline, oxytetracycline, sulfamethazine and sulfamethoxazole at the highest allowed application rate (see the underlined values in Table 5).

An in-depth description of the studies from which MECs$_{\mathrm{soil}}$ are taken is reported in Table S18, in particular, MECs$_{\mathrm{soil}}$ refer to soils amended with pig slurry or effluent from lagoons receiving pig wastewater or slurry.

7. Environmental risk assessment in the case of swine slurry-amended soil

The environmental risk assessment was based on the risk quotient evaluation, as discussed in Verlicchi and Zambello (2015), as the ratio between PEC$_{\mathrm{soil}}$ and PNEC$_{\mathrm{soil}}$.

With regard to PNEC$_{\mathrm{soil}}$, different proposals are available in the literature including Eriksen et al. (2009), Munoz et al. (2009), Chen et al. (2018), Thomaidi et al. (2016), Bourdat-Deschamps et al. (2017) and Gros et al. (2019). In this study, PNEC$_{\mathrm{soil}}$ were those used in the study by Bourdat-Deschamps et al. (2017) and Gros et al. (2019) obtained from a literature survey targeting relevant endpoints for (micro)-organisms in agrosystems.

The corresponding risk quotient values are reported in Fig. 19 grouped according to the slurry rate applied (2200 kg dm/(ha year) and 9500 kg dm/(ha year)) and ordered according to the average RQ value (descending order). On the y-axis, the number appearing in brackets after the name of the antibiotic is the adopted PNEC$_{\mathrm{soil}}$.

It emerges that average RQ are always <1. A medium risk was found for ciprofloxacin, sulfamethazine, chlortetracycline and doxycycline (at an application rate of 9500 kg dm/(ha year)) and only for ciprofloxacin in the case of the lowest application rate. A high risk was found in the case of maximum concentrations of sulfamethazine, chlortetracycline and doxycycline in swine slurry in the case of 9500 kg dm/(ha year) of the application rate.

It is worth noting that the environmental risk assessment carried out in this study refers to a vulnerable area where a maximum application rate is set according to the maximum quantity of nitrogen disposed on soil with the manure. In case of no vulnerable area, the application rate should be higher than the assumed value and also the subsequent soil concentrations of micropollutants resulting in higher RQ values.

8. Discussion and future perspectives – conclusions

The overview highlighted that concentrations of antibiotics are higher in swine manure rather than in cattle manure.This could be explained with the fact that antibiotics are administered at higher dosages and with higher frequencies in pig farms than in the other type of farm as comparing the living environments, pigs have much smaller space available with worse air and more pathogenic bacteria which make pigs prone to catch diseases.

Hou et al., 2015 compared concentrations of sulphonamides, tetracyclines, quinolones and macrolidesantibiotics in manure from different types of animal farms and they found them in this order: swin manure > poultry manure > cattle manure.

The antibiotic oxytetracycline was the compound with the highest concentrations in all types of raw manure (concentrations in ng/g dm) and chlortetracycline in the case of semiliquid and liquid raw manure (concentrations in ng/L). As for treated manure, chlortetracycline and its main metabolites (iso- and epi-chlortetracycline) presented the highest values (both ng/g dm and ng/L) in cattle and swine treated manure.

Looking at the hormones, they always occurred at lower concentrations: the highest were 17β-estradiol-3-glucuronide in raw swine manure (2.8 10^4 ng/L), and progesterone in raw cattle manure (2.1 10^4 ng/g dm), around 3 orders of magnitude lower than the highest concentrations of the antibiotic in the same type of manure. Only in cattle treated manure, estrone was found at a concentration only one order of magnitude lower than the top antibiotic (8.5 10^2 ng/g dm versus iso-chlortetracycline 4 10^3 ng/g dm).

A summary of the highest concentrations is reported in Table 6 with regard to the different manure (source and untreated/treated).

As remarked in the previous sections, great differences may be found in the occurrence of micropollutants in different tyes of manures. These

Table 6
Top pharmaceuticals in the three types of manures most investigated (raw and treated).

Source	Untreated [ng/g dm]	Untreated [ng/L]	Treated [ng/g dm]	Treated [ng/L]
Cattle	Oxytetracycline, 2.3 10^5 Enrofloxacin 4.7 10^4 Sulfamethazine 3.0 10^4	Chlortetracycline 5.9 10^6 epi-Chlortetracycline 4.1 10^6 iso-Chlortetracycline 2.4 10^6	iso-Chlortetracycline 4 10^3 Estrone, 8.5 10^2	Oxytetracycline, 6.8 10^6 iso-Chlortetracycline 4.6 10^6
Poultry	Enrofloxacin, 1.4 10^6 Oxytetracycline, 4.2 10^5 Norfloxacin, 2.3 10^5	Oxytetracycline 2.1 10^4		
Swine	Chlortetracycline, 8.8 10^5 Bacitracin A, 3.2 10^5 Oxytetracycline, 3.5 10^5	Chlortetracycline, 1.1 10^8 Sulfamethazine 1.1 10^7 Lincomycin, 2.0 10^5	Chlortetracycline, 8.8 10^5 iso-Chlortetracycline 3.3 10^5 epi-Chlortetracycline, 2.5 10^5	Tylosin, 4.9 10^6 Chlortetracycline, 1 10^6

differences may be attributed to variations in the dosage levels, different metabolic characteristics of the animals, geographical variations due to different prescribing habits in different regions. These were confirmed by Zhao et al. (2010) who statistically analysed samples taken from different farms in different regions in China.

With regard to the potential environmental risk posed by residues in manure-amended soil, the analysis referred to the application of swine manure (Fig. 19) noted that sulfamethazine, chlortetracycline and doxycycline presented the maximum values in the case of an application rate of 9500 kg dm/(ha year).

Interesting results are provided by Zhang et al. (2015), who compared the environmental risk posed by residues of veterinary antibiotics in raw and treated manures from different animal farms (chickens, ducks, pigs and cattle) if applied to soil as a fertilizer. It emerged that the risk is higher (in terms of risk quotient) for raw manures than in the composted mixture of the different manures, for all the investigated antibiotics. In more detail: in raw manure RQ was found >1 for tetracycline (pigs, chickens and cattle), oxytetracycline (pigs, chickens and cattle), chlortetracycline (pigs), sulfadiazine (chickens and pigs), ciprofloxacin (chickens and cattle), and after composting the mixture of the different types of manure RQ > 1 for tetracycline, oxytetracycline, sulfadiazine and ciprofloxacin. The study by Gros et al. (2019) showed that RQ > 1 in the case of swine slurry application on soil for enrofloxacin.

If a liquid or semiliquid manure is spread on the land it may percolate through the soil more easily than manures with a higher content of suspended solid and, in particular, if there is rain soon after it has been applied to the land, it is more likely to reach the groundwater.

Zheng et al. (2008) found that the use of sequencing lagoons (that is a multi-stage lagooning system) and increasing manure-piling time promotes degradation processes of pharmaceuticals and hormones (in particular) and thus represents feasible, efficient and promising practices to reduce the risk of environmental contamination due to pharmaceuticals and hormones commonly administered to animals.

Once disposed on soil, the behaviour of the residues of micropollutants depends on their nature. For instance, oxytetracycline demonstrates a high persistence in pig slurry-amended soil: Aga et al. (2005) reported that it has a strong potential to absorb on solid matter which makes it unavailable for microbial attack, but at the same time it remains in the soil without being mobilized in the aqueous phase (this means that it is not present in the runoff or tile drainage induced by rain). However, the subsequent application of the same type of manure on the same soil over the years will cause it to accumulated (Blackwell et al., 2007). Other investigations pointed out that the persistence of antibiotics in soil is higher in the case of composted-amended soil instead of raw manure-amended soil. The advantage to spread composted manure is due to the (expected and observed) lower concentrations of antibiotics, but sometimes these compounds could resist biodegradation in the soil.

To sum up, based on the collected results, it emerges that future researches should focus on:

- the detection in raw and treated manure not only of the parent compounds, but also of their main metabolites, as they may retransform into their parent compounds during piling or treatments as noted by Lamshöft et al. (2010);
- the dispersion in the environment not only of antibiotics, but also of antibiotic-resistant genes and different types of microorganisms, especially if a high rate of manure is applied on arable land and on the subsequent risks for the environment as noted by Van den Meersche et al. (2019) and Gros et al. (2019). In fact, exceptional quantities of rain may lead to the unexpected release (through drainage and surface runoff) of very higher amounts of residues (and microorganisms) that have accumulated over the years on and in the soil, due to recurrent manure application and (Gall et al., 2014);
- the mobility mechanisms which may take place within the soil over time, after manure application;
- an investigation on the relationship between occurrence of micropollutants in manure-amended soil and soils and manure characteristics;
- a prediction of the fate and dissipation of selected micropollutants, based on their chemical and physical properties.

Lessons learned from municipal sludge application on rural land could be useful, keeping in mind the differences between their compositions in terms of macro- and microcontaminants which may affect their behaviour once they have entered the environment.

As for microorganisms, it was found that in the top 10 cm of soil (where manure was not added), the concentration of *E. coli* was in the range 10^2–10^5 cfu/g dm (Stocker et al., 2015) and faecal coliform around 2.58 10^4 CFU/g dm (Gondim-Porto et al., 2016). If manure is spread, the concentration increases up to 2 log units and the environmental conditions define its survival/decay: a removal of 1 log unit may be reached from a few days (Hutchison et al., 2005) to 19 weeks (Lau and Ingham, 2001). Rainfall intensity occurring soon after manure amendment results in an immediate reduction of deposited bacteria within the first cm of soil (Stocker et al., 2015).

It is worth noting that the contribution to microorganism release in surface water in the case of heavy rainfall on manure-amended soil may be critical especially in the case of a catchment area discharging in a bathing area or close to water that is used for drinking.

Declaration of competing interest

There is no conflict of interest in publishing this paper.

Supplementary data

The Supplementary Data includes tables referring to (i) the main issues addressed in the papers included in the review; (ii) the main characteristics of the selected contaminants: chemical and physical properties, excretion factors with regard to the different types of animals, half-life time in manure and in manure-amended soil; (iii) the descriptive statistical analysis of the selected compounds and microorganisms in the different types of raw and treated manure; (iv) predicted no effect concentrations for a selection of compounds with regard to terrestrial organisms; (v) measured environmental concentrations in soil for a selection of antibiotics. The Supplementary data also includes figures providing the worldwide distribution of the investigations included in the review, the comparisons between concentrations of selected contaminants in raw and treated manures for the different types of animal farms and the comparison among concentrations of micropollutants in manure produced in dairy and beef cattle farms. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.136118.

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Most relevant sources and emission pathways of pollution for selected pharmaceuticals in a catchment area based on substance flow analysis

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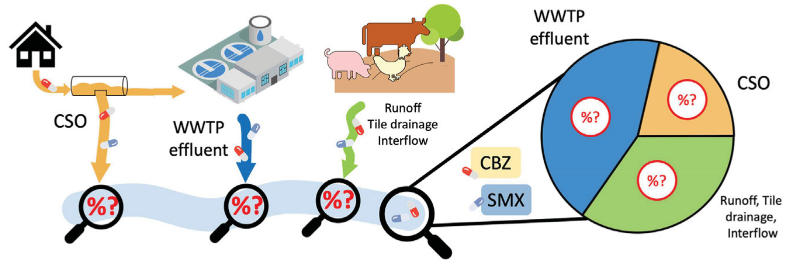
HIGHLIGHTS

- Prediction of pharmaceutical release in river by different emission pathways
- Carbamazepine and sulphamethoxazole selected as key compounds.
- Carbamazepine mainly released by wastewater treatment plant effluent.
- Sulfamethoxazole mainly released by land runoff.
- Uncertainties mainly due to the fate of pharmaceuticals in soil and surface water.

GRAPHICAL ABSTRACT



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ABSTRACT

The release of micropollutants in surface water depends on different sources and on different pathways. Through substance flow analysis, this study estimates the annual load of two pharmaceuticals (carbamazepine and sulfamethoxazole) in a catchment area, due to different emission pathways: wastewater treatment plant effluent, combined sewer overflows, and runoff from sludge and manure amended soil. It emerged that wastewater treatment plant effluent is the main emission pathway for carbamazepine (98.5% referring to the total released annual load) and land runoff (98%) for sulfamethoxazole.

The study also investigates the parameters (including manure disposed on the land, removal efficiency and combined sewer overflow flowrate) which mostly influence the results, and those which are affected by higher uncertainty. The most uncertain parameters are those determining the fate of pharmaceuticals once in soil and surface water.

The study concludes with a comparison between the predicted concentrations in different points of the receiving water body of the two key compounds, modeled with substance flow analysis, and those directly measured in a dedicated sampling campaign. The main discrepancies were found for sulfamethoxazole. Future research focusing on monitoring campaigns under different weather conditions and in different environmental compartments (soil and water) will certainly provide new insights in this kind of study.

1. Introduction

In recent years, many investigations have dealt with the occurrence of selected pharmaceuticals (PhACs) in different water compartments (among them Luo et al., 2014, Meffe and de Bustamante, 2014 and Yang et al., 2017) and less frequently in raw and treated sludge and manure (among them Bloem et al., 2017, Gros et al., 2019 and Martín et al.,

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2012). Great attention has mainly been paid to the release of wastewater treatment plants (WWTPs) in the receiving water (Al Aukidy et al., 2012; Collado et al., 2014) and to the assessment of the environmental risk posed by their residues once in the water body (Su et al., 2020). At the same time, studies have also tackled removal efficiencies for a wide spectrum of PhACs at the different treatment levels as well as their variability depending on the feeding, biological reactor configuration, and operational and environmental conditions (Luo et al., 2014; Radjenović et al., 2009; Verlicchi et al., 2015). More recent research has also referred to the environmental risks related to soil amendment with treated sludge and manure (Bourdat-Deschamps et al., 2017; Ghirardini et al., 2020; Gros et al., 2019) and has remarked that land runoff could be a relevant emission pathway in surface water for some persistent compounds. Other studies have evaluated the impact of combined sewage overflows (CSOs) during heavy rain events on the receiving body mainly in terms of concentrations of microorganisms and some key PhACs (Al Aukidy and Verlicchi, 2017; Galfi et al., 2016; Launay et al., 2016; Phillips et al., 2012).

Other efforts have been made to evaluate the contributions of the different emission pathways to the release of selected PhACs in surface water. A comparison between the annual load of PhACs released in the surface water body by WWTP and CSO outlets was carried out by different research groups. Among them: an investigation was carried out by Chèvre et al. (2013) in a Swiss catchment area referring to the antibiotic ciprofloxacin, the analgesic diclofenac and the anti-epileptics carbamazepine and gabapentin; by Baranauskaite-Fedorova et al. (2016) in a Lithuanian river basin referring to diclofenac; and by Verlicchi et al. (2017) in an Italian coastal, tourist area with regard to caffeine, paracetamol and carbamazepine.

By following other approaches, Hanamoto et al. (2018) first carried out a comprehensive monitoring campaign in the Yodo watershed in Japan referring to 55 PhACs and they then tried to correlate their concentrations to the potential sources (animals and humans) and emission pathways (WWTP effluent, scattered septic tank release and rural soil emission). Castiglioni et al. (2018) compared the contributions to the release in surface water bodies of the effluent of three WWTPs in the large urbanized and industrialized area of Milan (Italy).

Polesel et al. (2015) and Delli Compagni et al. (2020) performed a mass balance from consumption to the crop for different PhACs, including ciprofloxacin, furosemide and triclosan, carbamazepine and sulfamethoxazole, and some hormones. They focused on specific catchment areas where treated sewage sludge is applied on the soil as a crop fertilizer and where WWTP effluent is released into the surface water body, which in turn is used for irrigation.

All these studies consist of a mass balance applied to differently-sized catchment areas. Some (Chèvre et al., 2013; Baranauskaite-Fedorova et al., 2016) followed the systematic approach of substance flow analysis (SFA). According to the well-known manual by Brunner and Rechberger (2016), SFA is a comprehensive evaluation of material flows and stocks limited to a specific system defined in space and time. In all the cited studies, the system is the selected catchment area and the time interval is the year.

This kind of analysis has successfully been adopted in many previous investigations referring to other pollutants, including heavy metals: copper in Chèvre et al. (2011), mercury in Wongsoonthornchai et al. (2016), and nutrients: phosphorus in Zoboli et al. (2016). The results clearly show that it is useful to include all the sources and emission pathways in the study as they may have a different contribution to the release of pollutants.

Limiting the attention to PhACs, the contribution of the different emission pathways has scarcely been investigated and, to the best of the authors knowledge, SFA which includes SFA of CSOs, WWTP effluent and arable land runoff, was not performed.

In this context, this study estimates the load of two PhACs, carbamazepine (CBZ) and sulfamethoxazole (SMZ) (the first consumed by humans, the second by both humans and animals), due to WWTP effluent, CSOs, sludge and manure amended-soil release in a flat and hilly catchment area in Austria on an annual basis. It also investigates the parameters (including the amount of manure disposed on the land, WWTP removal efficiency and CSO flowrate) that mostly influence the results, and those which are affected by higher uncertainty. To do this, an SFA was implemented to quantify and describe the different pathways the PhACs may take. The study concludes with a comparison between predicted (modeled with the SFA) concentrations of the two key compounds and those directly measured in the receiving river in a dedicated sampling campaign.

2. Materials and methods

2.1. Definition of the terms used in the SFA

The terms and definitions adopted in this study are those of the SFA terminology as reported in Brunner and Rechberger (2016) with a few exceptions. In particular, and as mentioned above, a substance flow analysis is a comprehensive evaluation of flows and stocks of materials limited to a specific *system* defined in terms of space and time. Within a system, a *compartment* is defined as a point where the substance flow splits (*node*), or where the substance is stored (*stock*). The term compartment has been preferred to the term *process*, more commonly used in SFA, in order to avoid confusion with the treatment processes to which wastewater and sludge are subjected. A compartment can be composed of multiple nodes or stocks, which are termed *sub-compartments*.

Compartments are linked by *flows*, expressed as mass per time, which represent the potential direction a substance may take in the system. A flow of a substance entering the system is called *input*, while a flow exiting the system is called *output*.

In addition to the basic terms necessary to analyze substance flows and stocks, the term *emission pathway* has been used herein to identify the specific flows that directly contribute to the final output flow.

Finally, from an overall point of view, a *parameter* is defined as a value which will be assumed in the SFA, such as the fraction of the flow that splits in a node as well as the substance load taken as the input.

In this context, considering a one-year time interval, the study aims to evaluate the *flows* (g year^{-1}) of CBZ and SMX within an East Austrian catchment (the studied *system*), taking as *inputs* the human PhAC consumption and PhAC content in manure applied on arable land. The *output* is herein represented by the load of CBZ and SMX that reaches the river endpoint of the catchment under study through 3 different *emission pathways*: WWTP effluent, CSOs and arable land vadose zone flow (surface runoff, tile drainage and interflow). The pathways the PhACs may take from their inputs to the final output are described by the flow connection between the different *compartments* and *sub-compartments*: sewer system, WWTP, CSO outlets, arable land and receiving river. A brief description of the terms adopted in the current study and corresponding examples are reported in Table S1.

SFA was performed with the software STAN (Cencic and Rechberger, 2008), which allows the system to be balanced with nonlinear data reconciliation based on the conventional weighted least-squares minimization approach and error propagation (Cencic, 2016).

The two PhACs considered in this study were selected for different reasons. In particular, CBZ was chosen for its high persistence during its passage in conventional WWTPs (Verlicchi et al., 2012) and in the agricultural environment (Kodešová et al., 2016), which makes it an ideal anthropogenic marker (Clara et al., 2004). SMX was selected because it is used as an antibiotic both for humans and animals, thus it could be found in treated sewage sludge (Verlicchi and Zambello, 2015) as well as in different manure types (Ghirardini et al., 2020). No metabolite or transformation product of CBZ and SMX is considered in this study.

The following sections describe the methodology used to evaluate the fractions of PhAC load inputs that have split into the different flows through the selected compartments, until their release into the surface water body of the study area.

2.2. Site description

The watershed under study is in the eastern part of Austria and it extends for 404.1 km^2. Here, more than half of the total surface is covered by arable land (50.4%), followed by forests (40.5%), small urban areas (5.5%) and grasslands (2.5%). The annual average rainfall is about 573 mm, the annual average temperature is about 10.2 °C (with a minimum monthly average temperature of 5.8 °C and a maximum average temperature of 14.3 °C) and the mean solar radiation is 11,797 kJ m^{-2} day^{-1} (Fick and Hijmans, 2017). As represented in Fig. 1, the area was schematically divided into 4 sub-catchments (called SC1, SC2, SC3 and SC4, respectively). Each of them is characterized by a similar urban area coverage (5.3% of the total surface area).

The population was approximately 108,879 in 2017 (of which 21,579 in SC1; 42,717 in SC2; 20,570 in SC3 and 24,013 in SC4). The local sewer system is combined and includes some sewer overflow outfalls.

As to the receiving surface water body, the river starts its flow in SC1 (point A in Fig. 1) and, after 11 km, reaches SC2 (point B), in which the flow continues for 23 km towards SC4 (C). Here, the river flows for 56 km until reaching the catchment outlet (D). In addition, another small tributary starts in SC3 (F) and reaches the main river in SC4 after 16 km (E). The travel time from the starting point of the river in SC1 (A) to the end in SC4 (D) is approximately 24–25 h (surface water velocity could be assumed around 1 m s^{-1}). The annual average river discharge at the outlet of the sub-catchments is about 0.23 m^3 s^{-1} at SC1 (point B), 0.53 m^3 s^{-1} at SC2 (point C), 0.09 m^3 s^{-1} at SC3 (point E) and 1.15 m^3 s^{-1} at SC4 (point D).

In SC4 there are three WWTPs (WWTP 1, 2 and 3) which treat the wastewater of the population resident in the whole catchment, serving respectively 22%, 58% and 20% of the population. There is no significant industrial activity in the studied area, thus the sewer network dominantly conveys domestic wastewater and rainwater. According to Council Directive 81/271/EEC, the mixing of domestic and drained rainwater is called urban wastewater and this term will be generally used in the following also when referring to dry periods.

The three WWTPs have a capacity of 42,000 person equivalent (PE), 110,000 PE and 66,500 PE, respectively, and consist of pre-treatments, primary sedimentation and secondary treatment based on conventional activated sludge (nitrification and denitrification stages working at 0.06 kg BOD kgSS^{-1} d^{-1} with bubble aeration, a hydraulic retention time (HRT) of 24 h and a sludge retention time (SRT) of more than 15 days. Phosphorus is removed by precipitation with aluminum and iron coagulants).

Between 2009 and 2016, the 3 WWTPs had the following average flow rates: 4 10^6 m^{3}year^{-1} (WWTP1), 10^7 m^{3}year^{-1} (WWTP2) and 3 10^6 m^{3}year^{-1} (WWTP3) (see table S7). The WWTP1 and WWTP2 effluents are directly released into the surface water body inside SC4, whereas the WWTP3 discharges into a watercourse outside the catchment area and for this reason its effluent is not considered in this SFA.

WWTP2 treats the sewage sludge produced during its treatment as well as that produced in WWTP1, whereas sludges from WWTP3 are treated locally. In both WWTP2 and WWTP3, sludge treatment consists of dewatering followed by an aerobic digestion carried out at mesophilic conditions (35 °C) with a minimum SRT of 20 days.

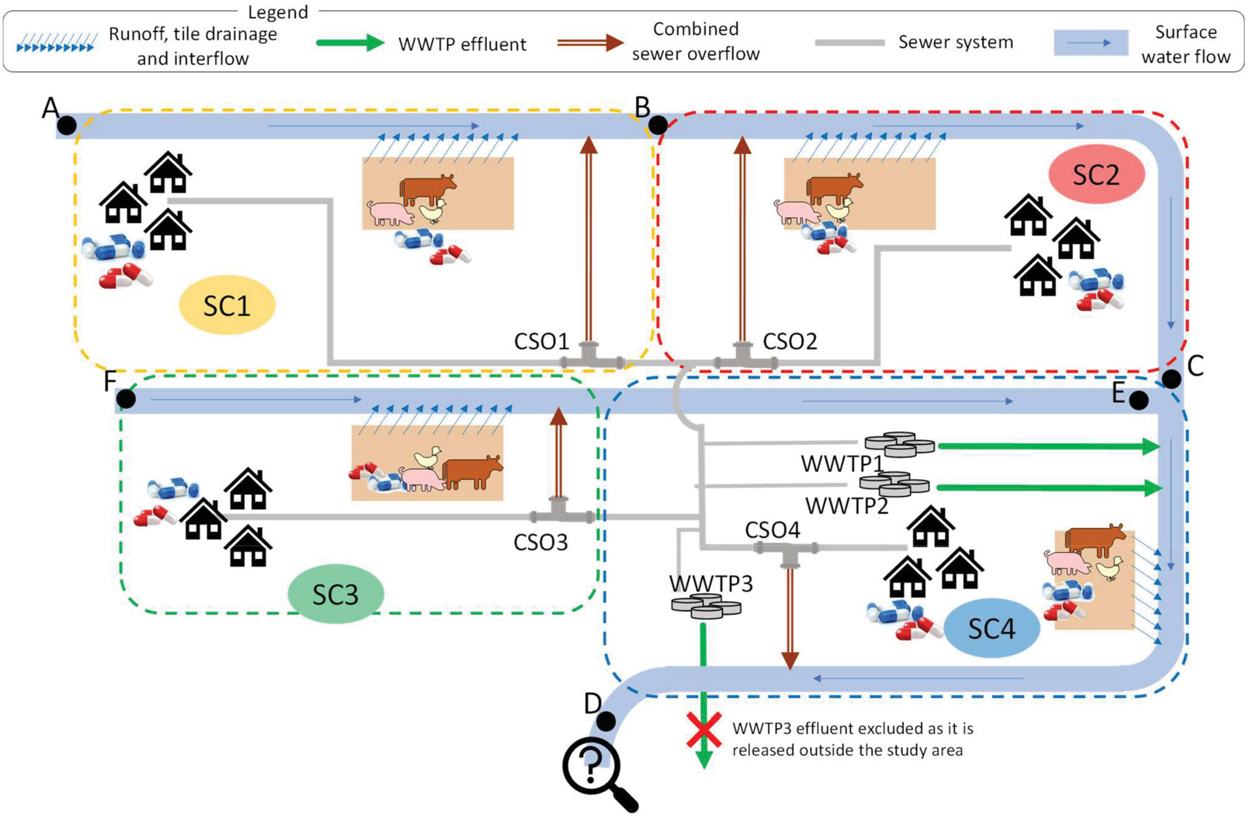

Fig. 1. Schematic representation of the different water flows (raw wastewater, treated effluent, CSOs and surface water) in the catchment under study. A, B, C, D, E and F are reference points for the study. Surface water samples were collected at points B, C, D and E.

2.3. Household pharmaceutical input

Data on the SMX and CBZ *per capita* released loads in the sewer system were found in Clara et al. (2013), who measured influent concentration of two WWTPs in Styria (Austria). The region has characteristics similar to those in the current study area and the estimated *per capita* consumption is based on the population served by the two WWTPs, assuming that there was no degradation of the compounds in the sewer. The *per capita* load released into the sewer system was equal to 4.7 mg inhabitant^{-1} year^{-1} for SMX and 118.6 mg inhabitant^{-1} year^{-1} for CBZ.

The total PhAC loads released into the 4 SCs sewer system on a yearly basis were evaluated by multiplying the population of each SC by the corresponding *per capita* release. The details of these evaluations are reported in Table S2, whereas, for the sake of clarity, the resulting loads are summarized in Appendix 1.

2.4. Livestock pharmaceutical input

Among the two PhACs, only SMX has been considered in this evaluation as CBZ is not commonly used for veterinary purposes and thus does not occur in animal manure (Ghirardini et al., 2020).

As it was not possible to obtain the veterinary consumptions, and consecutively the excreted amount due to animal metabolism, the SMX input due to livestock was evaluated by considering the annual load of SMX applied on arable land *via* manure amendment.

In the studied catchment, swine, cattle and poultry are the main animal farms both in terms of the amount of manure produced (BMLFUW, 2017a) and number of heads (Gilbert et al., 2018).

The SMX load annually applied onto arable land by means of manure spreading was evaluated by multiplying the tons of different types of manure produced every year in the catchment by the corresponding SMX concentrations.

In particular, as to manure amount evaluation, data about the number of swine, cattle and poultry heads in the studied catchment (referring to 2017) were taken from the dataset of the Austrian Federal Ministry of Sustainability and Tourism (Integriertes Verwaltung – und Kontrollsystem, available at https://www.bmnt.gv.at/land/direktzahlungen/Invekos.html) and are provided in aggregated form in Table S3.

The total number of animals was then multiplied for the average annual tons of manure produced by each type of animal, taking into account both solid manure and slurry, depending on the stall type. Here, data about the average annual volumes of different types of manure produced by each animal were found in (BMLFUW, 2017a) and the typical manure values of bulk density and dry matter were taken from (Lfl, 2018). On the basis of the values reported in Table S3, it is possible to evaluate the quantity of manure produced by each type of animal in each SC.

The obtained tons of animal manure produced in the whole catchment were 501 t dry matter (DM) year^{-1} of cattle slurry, 21,842 t DM year^{-1} of cattle solid manure, 7927 t DM year^{-1} of poultry solid manure, 181,672 t DM year^{-1} of swine slurry and 1088 t DM year^{-1} of swine solid manure (Table S4).

In order to evaluate the SMX annual load applied onto soil with manure, the average concentration in different types of raw manure found in Ghirardini et al. (2020) were used (detailed calculations are given in Tables S5 and S6). In the studied catchment, manure treatment is an uncommon practice, thus, following a conservative approach, no further degradation of SMX was assumed to occur before land spreading.

Finally, the manure is considered to be uniformly applied over all the arable land in the catchment, thus the total SMX mass load was divided into four SCs proportionally to the arable land surface of each one. The average SMX load input was equal to 3762 g year^{-1} for SC1, 15,884 g year^{-1} for SC2, 7752 g year^{-1} for SC3 and 11,305 g year^{-1} for SC4 (see Table S6 and also Appendix 1 for details).

2.5. Sewer network and wastewater treatment plant

2.5.1. Sewer system flow rates

Urban wastewater may be conveyed to the treatment plant or, during intense rainfall events, may be directly released into the surface water body through CSO outfalls. As schematically represented in Fig. 1, CSO outfalls are located before the WWTPs acting as a barrier to hydraulically protect the WWTP receiving the conveyed wastewater. When in operation, CSOs discharge the excess wastewater flow at different points along the same river within the study area.

Data about the annual flow rate of the effluent (assumed equal to the influent) of the three WWTPs and of the CSOs in the four SCs come from Clara et al., 2020. Specifically, annual effluents of the WWTPs ($V_{WWTP1,2,3}$) stem from the Austrian official register of emissions into surface waters (BMLFUW, 2017c), whereas the annual flow rates of the CSOs ($V_{CSO\text{-}SC1,2,3}$) were estimated for the whole of the Austrian territory based on (Clara et al., 2014). These data are summarized in Table S7 with regard to different years. As to the released treated effluents, it emerges that WWTP1 contributes on average to 23.4%, WWTP2 to 59.0% and WWTP3 to 17.6%.

2.5.2. PhAC load discharged via CSOs and conveyed to the WWTP

By focusing on each SC, in order to evaluate the PhAC load exiting households that is directly discharged with CSO outfalls and, respectively, the PhAC load conveyed to the treatment plant on annual basis, it was assumed that each PhAC follows the water flow passively and does not degrade in the sewer system. With this assumption, and focusing on a generic high intensity rainfall event which led to the CSOs, the PhAC concentration in the CSOs may be calculated with the following equation:

$$c_{PhAC,CSO} = \frac{\dot{m}_{PhAC,in}\big|_{yearly} \times \dfrac{t_{CSO}}{1\ year}}{\left(V_{WW,dry} + V_{RAIN,overflow}\right) \times \dfrac{t_{CSO}}{1\ year}} \tag{1}$$

where, $\dot{m}_{PhAC,in}$ is the PhAC load annually released into the sewer system from the household (kg year^{-1}) evaluated above in Section 2.3, t_{CSO} is the time of the overflow duration (h), $V_{WW,dry}$ the wastewater volume discharged into the sewer system by the household in dry periods (m^3 year^{-1}) and $V_{RAIN,overflow}$ the rainfall volume due to urban runoff which led to the CSOs (m^3 year^{-1}). Here, $V_{WW,dry}$ can be calculated assuming a *per capita* wastewater discharge of 150 L inhabitant^{-1} d^{-1} as a standard value for peri-urban areas (Metcalfe and Eddy, 2014) and $V_{RAIN,overflow}$ may be assumed equal to:

$$V_{RAIN,overflow} = d \times V_{WW,dry} \tag{2}$$

In which d is the factor of dilution of $V_{WW,dry}$ which, according to technical principles and norms applied in Austria, was estimated to be equal to 50 on average annually, with a minimum of 30 and a maximum of 100 in this area (ÖWAV, 2007; Fenz, 2002).

Thus, the percentage of PhAC load entered into the sewer system and then discharged *via* the CSOs can be evaluated with the equation:

$$\%_{PhAC\ discharged\ via\ CSO} = \frac{c_{PhAC,CSO} \times V_{CSO}}{\dot{m}_{PhAC,in}}\bigg|_{yearly} \tag{3}$$

as the numerator corresponds to the PhAC load discharged *via* the CSOs ($\dot{m}_{PhAC,CSO}$) as shown in Eq. (4):

$$\dot{m}_{PhAC,CSO} = c_{PhAC,CSO} \times V_{CSO} \tag{4}$$

In this context, removing t_{CSO} from Eq. (1), as it appears both in the numerator and denominator, and including Eq. (1) in Eq. (4), it is possible to write Eq. (3) as follows:

$$\%_{PhAC\ discharged\ via\ CSO} = \frac{V_{CSO}}{\left(V_{WW,dry} + V_{RAIN,overflow}\right)} \tag{5}$$

In which $\dot{m}_{PhAC,in}$ was removed, for the same reason as t_{CSO}.

The last simplified equation was used to evaluate the average fraction of the CBZ and SMX load discharged *via* the CSOs in each SC on an annual basis, and, respectively, the average fraction which remains in the sewer system and which is conveyed to the WWTP.

As reported in Table S8, with the details about the calculations, the resulting percentage of the PhAC load that moved into the surface water through the CSOs ranges from 0.9% to 1.2% in SC2 and SC4, respectively, and thus about 99% of the PhAC annual load is conveyed to the treatment plant.

2.5.3. PhAC fate in the WWTPs

The fate of the two selected PhACs in the three WWTPs was assessed with the SimpleTreat 4.0 model (Struijs, 2014), an improved version of the SimpleTreat 3.1 applied in the European REACH (Registration, Evaluation, Authorization and restriction of Chemicals) legislation (EC, 2004). Briefly, the model can evaluate the fraction of PhAC load influent released with the WWTP effluent, removed *via* biotransformation and adsorbed into primary and secondary sludge. To do this, the physical and chemical characteristics (molecular weight, octanol-water partition coefficient (K_{ow}), dissociation constant (pK_a), vapor pressure, solubility in water, chemical classification based on the ionized form of a chemical (base, acid or neutral) and biodegradation rate constant) of the PhACs are required as well as the WWTP design and operational conditions (*i.e.* served inhabitants, hydraulic retention time (HRT), sludge retention time (SRT), sludge loading rate and aeration type).

As the three WWTPs present in the area are similar in terms of configuration and operational conditions, in this study, the three WWTPs have been processed as a single plant with the same configuration, operating at the same conditions and with an influent equal to the sum of the three influent flow rates (Fig. S1).

Table S9 includes the parameters used as the input of the model. Among them, the WWTP operational conditions are those presented in Section 2.2 and the physical and chemical characteristics of SMX and CBZ were found in PubChem (Kim et al., 2016) and in (Lautz et al., 2017).

Finally, as WWTP3 discharges outside the study area (Fig. 1), the total PhAC load exiting the modeled WWTP obtained by applying SimpleTreat 4.0 was reduced of the WWTP3 corresponding contribution, equal to 17.6% as reported in Table S7.

There is no reuse of reclaimed wastewater in the area, thus the fraction of WWTP effluent destined to direct reuse was not considered in this analysis.

2.5.4. PhAC fate in sewage sludge treatment and disposal

PhACs adsorbed onto primary and secondary sludge may be removed during sludge treatment. Here, the literature data about removal of CBZ and SMX during anaerobic digestion at 35 °C (mesophilic conditions) for 20 days were averaged to evaluate the fraction of the PhACs which may be degraded during the treatment. A removal of 13.3% for CBZ and 88.9% for SMX was assumed in this study as the average of the experimental data reported in literature (Carballa et al., 2007, Gonzalez-Gil et al., 2016 and Yang et al., 2016, see Table S10 for more details).

Treated sludge may be disposed on arable land as an amendment or a fertilizer, it may also be landfilled or incinerated (Fig. S2). It is necessary thus to estimate the fraction which can be disposed on the soil, according to local regulations and current agricultural practices, and the consequent PhAC mass sequestered in this fraction.

In order to estimate these fractions, local data were used (from 2015, 2016 and 2017) of tons of treated sludge produced every year by WWTP2 and WWTP3 and tons *per* year disposed in agriculture (provided by local wastewater management companies). The collected data are reported in Table S11. They show that the amount treated in WWTP2 spread as an amendment is higher (on average 82%) than the percentage referring to WWTP3 (21% as a mean value).

The fraction of treated sludge spread onto soil used in this study was evaluated as the weighted average of the two percentages (respectively 82% and 21%), on the basis of the treated sludge associated with WWTP1 and WWTP2 from one side and WWTP3 from the other (the details and data are given in Table S11). This is equal to 60% and is also the fraction of mass load of each of the two PhACs spread onto the soil.

Treated sludge has been considered as being spread onto the four SCs proportionally to the surface of the arable lands in each of them, assuming a uniform disposal in the whole catchment and considering that the sludge characteristics (in terms of concentrations of metals and nutrients) are below the limits set by the Austrian legislation (BMLFUW, 2017b).

Data about the arable land surface in the catchment are reported in Table S12. With these considerations and assuming that CBZ and SMX do not further degrade during the time between sludge treatment and agricultural application, the fractions of the PhAC load received by the four catchments are equal to 9.7%, 41.0%, 20.0% and 29.3%, respectively, with regard to the total load associated with the sludge destined to arable land (Appendix 1).

2.6. PhAC in arable land in the vadose zone

With regard to the soil compartment, the maximum load of PhACs that may be potentially released into the surface water body occurs when rainfall immediately follows the soil application of sewage sludge or manure (Ghirardini and Verlicchi, 2019). Soil chemical and physical characteristics, type of manure and treated sludge affect the quality of the water released from the soil.

Thus, due to the uncertainty about the days of the year in which these organic wastes are spread onto soil in the studied catchment, and in order to estimate the worst case scenario of PhAC occurrence in a surface water body due to this specific agricultural practice, the whole PhAC mass sequestered within the manure and treated sludge was assumed to be available for remobilization in soil vadose zone water flows. These correspond to three types of contributions: surface runoff, tile drainage and interflow, as schematically shown in Fig. S3. For the sake of brevity, these flows will be identified as “vadose zone flow” below.

In this context, the maximum load of SMX and CBZ that may reach the surface water body after manure and sludge amendment was estimated following the approach by Langdon et al. (2010).

Briefly, the method splits the mass of a PhAC applied onto soil through manure and sludge over one year between the mass that could be sequestered in the solid phase (M_S) and the mass that could be moved in the aqueous phase (M_A). The ratio between M_S and M_A can then be expressed with Eq. (6):

$$\frac{M_S}{M_A} = \frac{K_d \times \rho_b}{v_0} \tag{6}$$

where ρ_b is the bulk density of the soil (g cm^{-3}), v_0 the porosity of the soil and K_d the partition coefficient that takes into account the carbon-water partition coefficient of the PhACs (K_{OC}) and the fraction of organic carbon of the soil (f_{OC}) as defined by Eq. (7):

$$K_d = K_{OC} \times f_{OC} \tag{7}$$

In this study, f_{OC} was assumed to be 1.51% as suggested by Gerzabek et al. (2005) for east Austrian agricultural loamy soils, and, following Langdon et al. (2010), K_{OC} was evaluated with Eq. (8) (Kenaga and Goring, 1980):

$$\log K_{OC} = 0.544\ \log K_{OW} + 1.377 \tag{8}$$

where K_{OW} is the octanol-water partition coefficient that is equal to 0.89 for SMX and 2.45 for CBZ (Pubchem, Kim et al., 2016).

With these considerations, K_d results equal to 1.10 $cm^3\ g^{-1}$ for SMX and 7.74 $cm^3\ g^{-1}$ for CBZ.

Thus, assuming a soil bulk density of 1.28 $g\ cm^{-3}$ and a soil porosity of 0.52 (as suggested by Marshall et al. (1996) as typical values for agricultural loamy soils), the ratio between M_S and M_A resulted 2.7 and 19.0 for SMX and CBZ, respectively.

This means that 27% and 5% (for SMX and CBZ, respectively) of the PhAC mass applied onto soil are moved in the aqueous phase, while the remaining 73% and the 95% (for SMX and CBZ, respectively) are bonded in the solid phase.

This method represents a worst-case scenario for PhAC occurrence in surface water and does not consider many different processes that could take place in the soil matrix, specifically if the time between manure or sludge application and rainfall events becomes considerably higher than 1 or 2 days. These processes may include: PhAC degradation in soil/manure-sludge matrix (Albero et al., 2018), alterations of soil carbon fraction (f_{OC}) due to organic waste amendment (Monteiro and Boxall, 2009), direct flowing of PhAC to groundwater due to soil macropores (especially when the solid content of the organic waste is less than 18%) (Lapen et al., 2008) and many others. On the other hand, at catchment scale, it is difficult to accurately define the behavior of PhACs in the soil (the agricultural land may be amended with sewage sludge and/or different types of manure from different animals. In addition, the amendment may occur in different times and amounts. Thus, the assumptions by Langdon et al. (2010), were taken as an unavoidable choice, and the uncertainty of the resulted values was assessed in the uncertainty analysis (Section 2.8).

2.7. PhAC in the receiving surface water body

Once in the surface water, a PhAC may be degraded, released in agricultural fields through land irrigation or may flow into the river as it is.

PhAC degradation mechanisms include photolysis, biodegradation and adsorption in soil particles (Maldonado-Torres et al., 2018), and due to the various environmental factors affecting these phenomena, it is difficult to estimate an overall half-life that well describes its fate while remaining in the surface water body. Thus, in this study, a conservative approach was adopted in the choice of the half-life values of the two selected PhACs.

In particular, CBZ reduction was assumed to be negligible and a first order kinetic decay model with a half-life of 15.5 h was assumed for SMX, according to Aymerich et al. (2016), who estimated the total mass reduction of the two compounds by carrying out a sampling campaign in the Ebro River in similar environmental conditions. They carried out their investigation under natural sunlight exposure and according to (Charuaud et al., 2019) photolysis seems to be the most important mechanism responsible for the reduction of sulfonamides once in the aquatic environment. For this reason the results by Aymerich et al. (2016) were preferred to those presented by others, which measured the three attenuation processes (photolysis, biodegradation and adsorption into sediments) separately at laboratory scale (among them Baena-Nogueras et al., 2017 and Liu et al., 2019).

In order to calculate the SMX mass that persists in the river from its release (through CSOs, arable land vadose zone flow and, only for SC4, WWTP effluent) to the outlet in SC4 (point D in Fig. 1), the river was schematically divided into 4 branches (one for each SC), as represented in Fig. 1. Thus, assuming the SMX load is released in the outlet of the river in each SC, the percentage of degraded mass was calculated by considering the remaining travel time until the outlet of the subsequent SC (keeping in mind that SC1 discharges in SC2, SC2 in SC4 and SC3 in SC4).

As already described in Section 2.2, the river travel time from the starting point SC1 (point A) to the outlet in SC4 (point D) is about 24 h; thus, since the total length is about 90 km, the water course velocity is assumed equal to 1 $m\ s^{-1}$. With these considerations, the resulting degraded mass in the surface water of the catchment resulted to be 25% from the outlet of SC1 (point B) to the outlet of SC2 (point C) and 50% from the inlet to the outlet of SC4 (points C and D, respectively) (Table S13).

Agriculture water needs in the catchment are met by irrigation *via* groundwater abstraction. Thus, in the study, no PhAC mass was conveyed to the arable land through surface water irrigation.

2.8. Uncertainty analysis

Due to SFA peculiarities, in terms of geographical frame and time period, it is often difficult to obtain an appropriate data set that accurately describes the actual system under investigation (Do et al., 2014). Therefore a rigorous, transparent and reproducible method to assess SFA parameters uncertainty is required (Laner et al., 2014). This was evaluated herein following the approach by (Laner et al., 2016). Briefly, the method firstly evaluates the quality of each parameter defining a *score* (between 1 and 4, being 1 the best and 4 the worst evaluation) with respect to defined *indicators* (namely: reliability, completeness, temporal and geographical correlation, and others, such as technical correlation). These indicator scores are established according to the scheme reported in Table S14. Then, the model translates each score into a *coefficient of variation* (*CV*) by the use of an exponential-type equation (Eq. (9)):

$$CV = a \times e^{b \times score} \tag{9}$$

where *a* and *b* are user defined coefficients which provide the quantification of the significance of the indicator under study (low L, medium M and high sensitivity H), with respect of each input parameter.

According to Laner et al. (2016), *a* was set equal to 0.375 for low sensitive indicators, 0.75 for medium sensitivity and 1.5 for high sensitivity and *b* was set equal to 1.105 in all the cases. The *CV* may be evaluated by choosing one of the three equations for each indicator (according to the sensitivity level defined by the user), with the exception of reliability which is always translated as a medium sensitive indicator (M).

Eq. (9) is continuous, allowing the translation of both integer and irrational scores.

Finally, the overall uncertainty of the specific parameter is determined by the use of Eq. (10):

$$CV_{tot} = \sqrt{CV^2_{reliability} + CV^2_{completeness} + CV^2_{geogr.corr} + CV^2_{temp.corr} + CV^2_{other\ corr.}} \tag{10}$$

The graphical result of the different function (for low, medium and high sensitivity, and for the reliability indicator) is represented in Fig. S4.

In this study, when more than 1 score may be chosen in a single parameter for the same indicator (for example, when a parameter is evaluated as the average of different data found in the literature as in the case of PhAC degradation during sewage sludge treatment), the highest score (corresponding to the worst evaluation) has been set in order to maintain a conservative approach.

All the SFA parameters were entered in the STAN software with their corresponding *CV* as their standard deviation. The overall uncertainty of the model output was then estimated by STAN using Gaussian error propagation and data reconciliation (Laner et al., 2014).

2.9. Sensitivity analysis

In order to identify the most significative parameters and to quantify the influence of their variation on PhAC load in SC4 output, a one-at-time (OAT) sensitivity analysis (Pianosi et al., 2016) was carried out.

Briefly, the SFA model implemented in STAN was run changing one parameter at a time and evaluating the corresponding variation of PhAC load in SC4 output. The influence of the analyzed parameter x_i

on the output was then evaluated by calculating the Sensitivity Index (S_i) with Eq. (11):

$$S_i = \frac{100}{p} \times \frac{OUT_i - \beta_0}{\beta_0} \tag{11}$$

where p is the percentage of parameter variation, OUT_i the new output value calculated by varying the parameter x_i, and β_0 the base solution (output value before any parameter variation). The range of variation of p may be fixed assuming a default value (for instance ±10% of x_i) (Delli Compagni et al., 2020; Verlicchi et al., 2011) or defined on the basis of the literature findings (Verlicchi and Zambello, 2016). In this study, this second option was preferred as it provides a better variability range of potential *real* scenarios.

In particular:

- the maximum CBZ and SMX load entering the system with human consumption was evaluated considering the consumption data reported in Clara et al. (2005), corresponding to 795 mg inhabitant^{-1} year^{-1} and 121 mg inhabitant^{-1} year^{-1} for CBZ and SMX, respectively. In this context, only 31% and 28% of the total load, for CBZ and SMX, respectively, was considered to reach the sewer system, according to the values of excretion factor derived from the analysis in Verlicchi and Zambello (2016) (average of the literature values). The maximum load of CBZ and SMX resulting from these calculations is reported in Tables S5 and S6. Due to the lack of data about local consumption it was not possible to evaluate the minimum value to set for CBZ and SMX. Thus, the minimum PhAC consumption was assumed equal to 0 in both cases;
- the minimum and maximum SMX load in manure was evaluated by multiplying the amount assessed above in Section 2.4 by the corresponding minimum and maximum concentrations reported in the review by (Ghirardini et al., 2020) (Tables S5 and S6);
- the minimum and maximum percentage value was assessed as described in Section 2.5.2 assuming different values of dilution (d). In particular, as reported in Table S8, the maximum percentage of wastewater discharged *via* the CSOs (1.8% in SC1, 1.4% in SC2, 1.6% in SC3 and 2.1% in SC4) corresponds to the lowest value of dilution ($d = 30$), and the minimum percentage of wastewater discharged *via* the CSOs (0.6% in SC1, 0.4% in SC2, 0.5% in SC3 and 0.6% in SC4) corresponds to the highest value of dilution ($d = 100$).
- the minimum and maximum percentages of load released with WWTP effluent were set to 10% (Göbel et al., 2007) and 80% (Suárez et al., 2005) for SMX and 60% (Joss et al., 2005) and 95% (Jelic et al., 2011) for CBZ, according to the literature data reviewed in Verlicchi et al., 2012;
- the variability range of PhAC load released outside the catchment through WWTP3 effluent was assessed as reported above in Section 2.5.3, considering the minimum and maximum resulting percentages from the local data reported in Table S7, instead of their mean value (further details are provided in Tables S15 and S16).
- the minimum and maximum percentages of PhAC that degraded during sewage sludge treatment was set equal to 0% (S. Yang et al., 2016) and 40% (Gonzalez-Gil et al., 2016) for CBZ and to 79% (Gonzalez-Gil et al., 2016) and 99% (Carballa et al., 2007) for SMX;
- the variability range of PhAC load released in arable soil through treated sludge amendment was assessed as reported above in Section 2.5.4 but by considering the minimum and maximum resulting percentages from the local data reported in Table S11, instead of their mean value (further details are provided in Tables S15 and S16).
- the variability range of PhAC mass moved in the soil aqueous phase after sludge and/or manure amendment was evaluated as reported above in Section 2.6, but considering different values of soil characteristics (namely organic carbon fraction (f_{OC}), bulk density (ρ_b) and porosity (v_0)). In particular, the K_d minimum value was calculated assuming wet clay soil (ρ_b equal to 1.1 g cm^{-3} and v_0 equal to 0.58 according to Marshall et al. (1996)) and a f_{OC} of 0.92 as the minimum value found in Austrian arable lands (Gerzabek et al., 2005). The K_d maximum value was calculated assuming sandy loam soil compacted by heavy traffic of agricultural vehicles (ρ_b equal to 1.9 g cm^{-3} and v_0 equal to 0.28 according to Marshall et al. (1996)) and a f_{OC} of 3.62 as the maximum value found in Austrian arable lands (Gerzabek et al., 2005);
- the minimum and maximum percentages of PhAC that degraded once in the surface water body were assessed as reported above in Section 2.7, considering their maximum and minimum half-life found in the literature. As to SMZ, they were assumed equal to 17.8 days (Liu et al., 2019) and 3.7 h (Baena-Nogueras et al., 2017). Regarding CBZ, it was already discussed that a negligible degradation may occur in the water compartment (*i.e.* half-time undefined) and thus only the scenario corresponding to a potential minimum half-life of 100 days (Andreozzi et al., 2003) was considered.

Eight parameters were varied for CBZ and 9 for SMX (the amount of PhAC in manure may only be varied in SMX).

Tables S15 and S16 compile the evaluated p values for the two key compounds.

2.10. Model testing

In order to compare the SFA results with the measured CBZ and SMX concentrations in the catchment surface water, a sampling campaign of the river water was carried out. Analysis referred to the liquid phase and not to the sediment particles which may be subjected to transport in the river.

The endpoint of each of the four SCs (points B of Fig. 1 for SC1, C for SC2, E for SC3 and D for SC4) was sampled 10 times, namely in November 2018 and with approximately each month between March and October 2019, in order to cover the whole period in which sewage sludge and manure might be applied onto the agricultural soils. Further, the sampling took place at different river discharge levels with the goal to include both baseflow conditions and situations with significant surface runoff and soil erosion. A total of 40 grab samples were taken between November 2018 and October 2019. At each sampling day, the weather conditions as well as the river conditions (turbidity, flow rate and concentrations of main pollutants) were monitored. Sampling sites were chosen based on available access to the stream banks. Surface water was collected from the central part of the streams. At each outlet, one liter of water was collected using a pre-cleaned glass bottle. The samples were kept refrigerated in the dark and were promptly processed in the laboratory. They were filtered *via* VWR® glass fiber filters with a diameter of 45 mm and a pore size of 1 μm and were analyzed through fully automated online solid phase extraction (SPE) coupled with liquid chromatography – tandem mass spectrometry (LC-MS/MS). For online SPE, a Phenomenex Strata X On-Line extraction cartridge (20 × 2.0 mm; 25 μm) was used. For HPLC, an analytical column Phenomenex Luna C-18 (150 × 3.0 mm; 5 μm) and Phenomenex C18-Security guard cartridges (40 × 3.0 mm) were utilized. High purity standards of Carbamazepine (CAS 298–46-4) and Sulfamethoxazole (CAS 723–46-6), HPLC grade organic solvents, namely Ethanol (CAS 64–17-5), Acetonitrile (CAS 75–05-8) and Acetic acid (CAS 64–19-17) as well as the mobile phase for online solid phase extraction and column chromatography were purchased from Sigma Aldrich. For CBZ, a limit of detection (LOD) of 0.05 ng L^{-1} and a limit of quantification (LOQ) of 0.2 ng L^{-1} were achieved, whereas the LOD and LOQ for SMZ were equal to 0.11 ng L^{-1} and 0.4 ng L^{-1}, respectively. Further information about the determination of PhAC concentrations in water by fully automated online solid phase extraction coupled with LCMSMS analysis are reported in the supplementary material from Tables S17 to S21, including Figs. S5 and S6.

The loads of CBZ and SMX in the river obtained with the SFA were divided by the average river flow rate related to each sampling point in order to obtain the average predicted CBZ and SMX concentrations on an annual basis ($CBZ_{c,p}$ and $SMX_{c,p}$) and to compare them to the measured concentrations that resulted from the sampling campaign ($CBZ_{c,m}$ and $SMX_{c,m}$). Data about river flow rate at each sampling point on the specific sampling days were obtained from the online portal of the hydrological service of the regional government of Burgenland (https://wasser.bgld.gv.at/hydrographie/die-fluesse).

3. Results and discussion

Fig. 2 schematically shows what is reported in a simplified way in Fig. 1: it represents the final STAN map with all the flows from the input to the output in the surface water body in each SC. Each flow is marked with a name like SC#_F$ where SC# corresponds to the sub-catchment area to which it belongs and F$ corresponds to the number assigned following the same order in which they are described in Sections 2.3 & 2.7. In addition, only for manure, the systems make a distinction between four different types of animal producing it and this corresponds to a further number added after the flow number SC#_F2.§ (with § = 1, 2, 3, 4). Simple compartments are represented as white rectangles while the compartments consisting of multiple sub-compartments are represented as grey rectangles (arable land, WWTP and sludge treatment, and Sludge stock).

An evaluation of the different flows will be performed and discussed in the following sections.

3.1. Estimation of PhAC loads in receiving water bodies and emission pathway contribution

The total annual CBZ and SMX loads released in the surface water body through the different emission pathways amounts to 9.5 kg year^{-1} and 10.6 kg year^{-1}, respectively (Figs. 3 and 4, Table S22).

Here, it is important to remark that the entire amount of CBZ reaches the catchment endpoint (point D in Fig. 3), while regarding SMX, a significant part is degraded between the release point and the final point (point D), resulting in a residual load of 4.6 kg year^{-1} in SC4 river endpoint (with the degraded loads being 1.3 kg year^{-1} and 4.7 kg year^{-1} in SC2 and SC4, respectively).

It was also found that emission pathway contributions, in terms of PhAC load, consistently differed for the 2 compounds. In particular, out of 9.5 kg year^{-1} of CBZ released into the catchment surface water, around 98.5% was due to WWTP effluent (9.4 kg year^{-1}), 1.5% to the CSOs (132 g year^{-1}, of which 28 g year^{-1} from SC1, 46 g year^{-1} from SC2, 24 g year^{-1} from SC3 and 34 g year^{-1} from SC4) and only a negligible percentage was from the vadose zone flow (3.7 g year^{-1}, of which 0.4 g year^{-1} from SC1, 1.5 g year^{-1} from SC2, 0.7 g year^{-1} from SC3 and 1.1 g year^{-1} from SC4).

Different results were found for SMX, for which out of 10.6 kg year^{-1} released in the river, 10.4 kg year^{-1} were related to the vadose zone flow, representing around 98% on the total released amount. WWTP effluent contributed to 229 g year^{-1} representing the remaining 2% of the total load released and the CSO contribution was only negligible (5.3 g year^{-1} of which 1.1 g year^{-1} from SC1, 1.8 g year^{-1} from SC2, 1.0 g year^{-1} from SC3 and 1.4 g year^{-1} from SC4).

These differences are due to the high amount of SMX entering into the catchment area with manure application, and with the assumption of negligible SMX degradation both in the manure and soil matrix.

In this context, considering the scenario with the lowest amount of SMX entering into the catchment area with manure amendment (as will be discussed in the sensitivity analysis), it emerges that wastewater-tied sources become relevant, resulting in a contribution of WWTP effluent, CSOs and vadose zone flow of 92%, 2% and 6%, respectively (Table S29).

For both CBZ and SMX, the results show that the CSO contribution to the release into surface water body is very low compared to that related to WWTP effluent, in accordance with the findings by Chèvre et al.

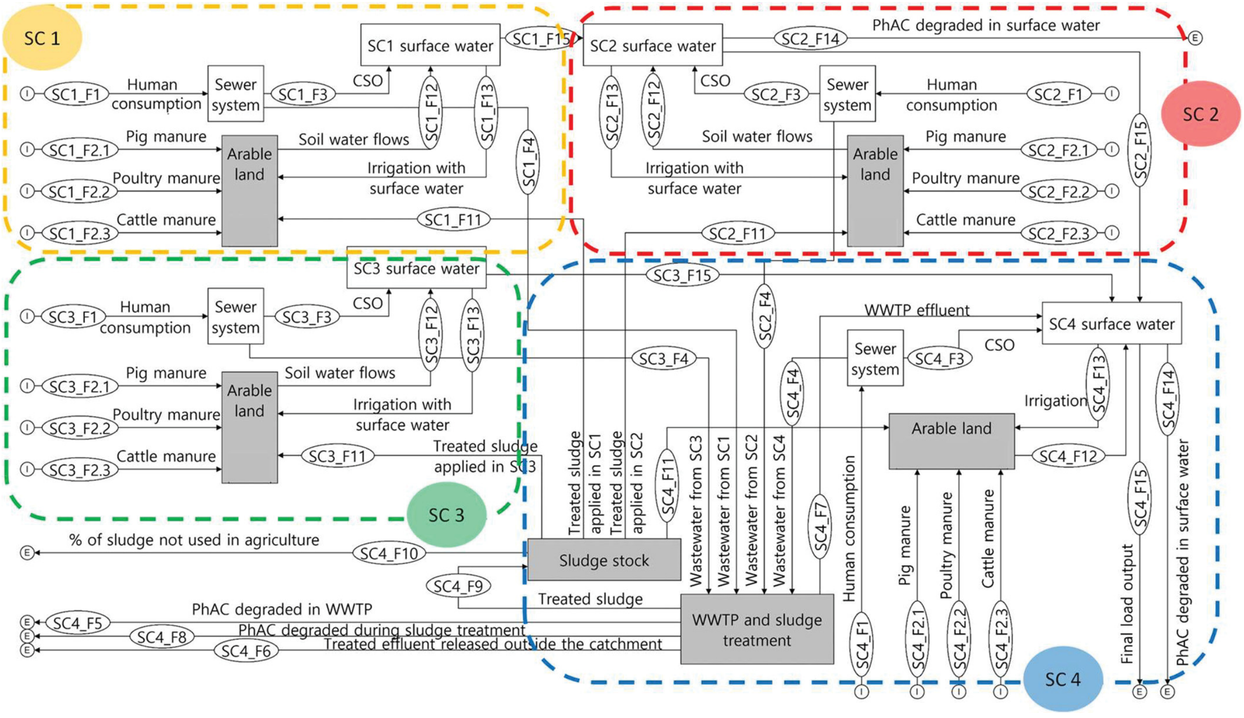

Fig. 2. STAN representation of the flows which may contain SMX and CBZ in the studied catchment.

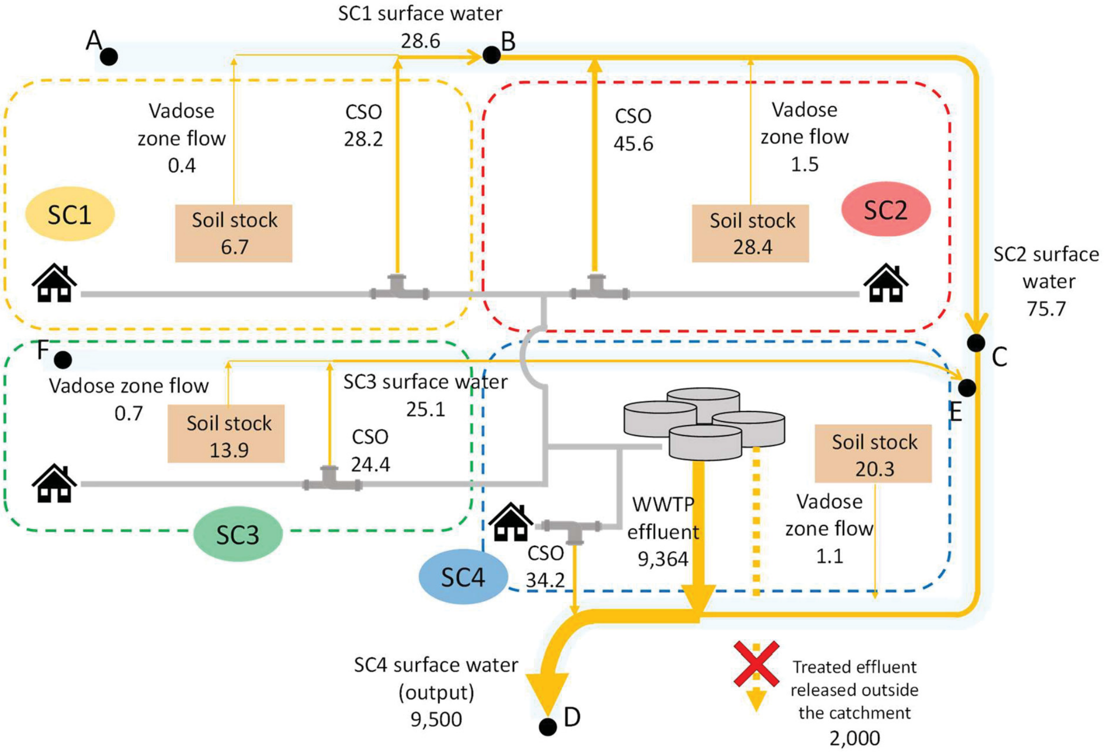

Fig. 3. Load of CBZ in surface water of each SC and in the main emission pathways: WWTP effluent, CSOs and vadose zone flows. All the loads are expressed in g year^{-1}.

(2013) and Baranauskaite-Fedorova et al. (2016) who analyzed the potential pathways that some selected human PhACs may take, with the implementation of an SFA and considering only CSOs and WWTP effluents in their catchment area. The compounds they considered were CBZ, ciprofloxacin, diclofenac and gabapentin (Chèvre et al., 2013) and diclofenac (Baranauskaite-Fedorova et al., 2016).

Hanamoto et al. (2018) investigated the contribution to the release of a wide spectrum of human and veterinary PhACs due to WWTP effluent, septic tank effluent for the scattered houses in the catchment area and pig manure-amended soil runoff. They based their study on a comprehensive sampling campaign in the Yodo river watershed (Japan) and, by means of a mass balance, they tried to correlate these measured concentrations to the different sources. As to SMX, they found that the main source was the WWTP effluent, unlike the results found in the current study. This could be explained by considering that, as noted by the same authors, in Japan, SMX is substantially consumed by humans (Ministry of Health, Labour and Welfare, 2010) and only at a lesser extent by animals which are considered the main source of SMX in the current study. In this respect, Hanamoto et al. (2018) confirmed that agricultural soil water may be the relevant source of pollution for drugs mainly administered to animals, such as sulfamonomethoxine and lincomycin.

From a comprehensive point of view, as reported in Table S22, out of 13 kg year^{-1} of CBZ entered as an input of the SFA, around 73.5% (9.5 kg year^{-1}) reached the output in SC4, while, regarding SMX, of 39 kg year^{-1} introduced in the system from human consumption and animal manure, only 12% (4.7 kg year^{-1}) reached the output. This difference could be explained when bearing in mind that, as SMX mainly entered into the system through manure application on soil, the soil matrix sequestration of this compound plays an important role in the analysis, accounting for around 72% (28 kg year^{-1}) of its total input amount.

Finally, due to the high persistence of CBZ, only 10% of the input annual amount (1.3 kg year^{-1} out of 13 kg year^{-1}) was degraded in the system (specifically, in WWTP and during sludge treatment), compared with 16% of SMX (6.2 kg year^{-1} out of 39 kg year^{-1}) which was partially degraded also in the surface water of SC2 and SC4.

Further details about all PhAC flows considered in the current SFA are reported in Table S22, Figs. S7 and S8 and are summarized in Table 1.

3.2. Uncertainty analysis

The uncertainty related to each SFA parameter is reported in Table S23 (CBZ), Table S24 (SMX) and Fig. S9 and expressed in terms of its coefficient of variation (*CV*).

It emerges that the parameter with the lowest *CV* ($CV = 7.6$), and therefore the lowest uncertainty, is the percentage of treated sludge that is applied in each SC arable land (SC#_F11). This could be explained by noting that its worst indicator score (equal to 2) refers to the indicator that least influences the *CV* calculation (temporal correlation) due to its low sensitivity. In this case, the low sensitivity of the temporal correlation indicator was set by considering that this parameter was evaluated proportionally to the arable land surface of each SC, which is expected to be the same over the years.

The highest *CV*, and therefore the highest uncertainty, was found for the percentage of PhAC load that was moved into the aqueous phase of the soil SC#_F12 ($CV = 65.8$) (Table 1). This result is not surprising, as the evaluation of this parameter did not consider many important processes occurring in the soil matrix (as already mentioned in Section 2.6). In this specific case, the completeness indicator score was set equal to 4, representing its worst evaluation among all the SFA parameters.

High values of *CV* were also found for the PhAC consumption data SC#_F1 ($CV = 41.8$), the percentage of wastewater conveyed to the treatment and, respectively, to CSO SC#_F3 and SC#_F4 ($CV = 43.8$),

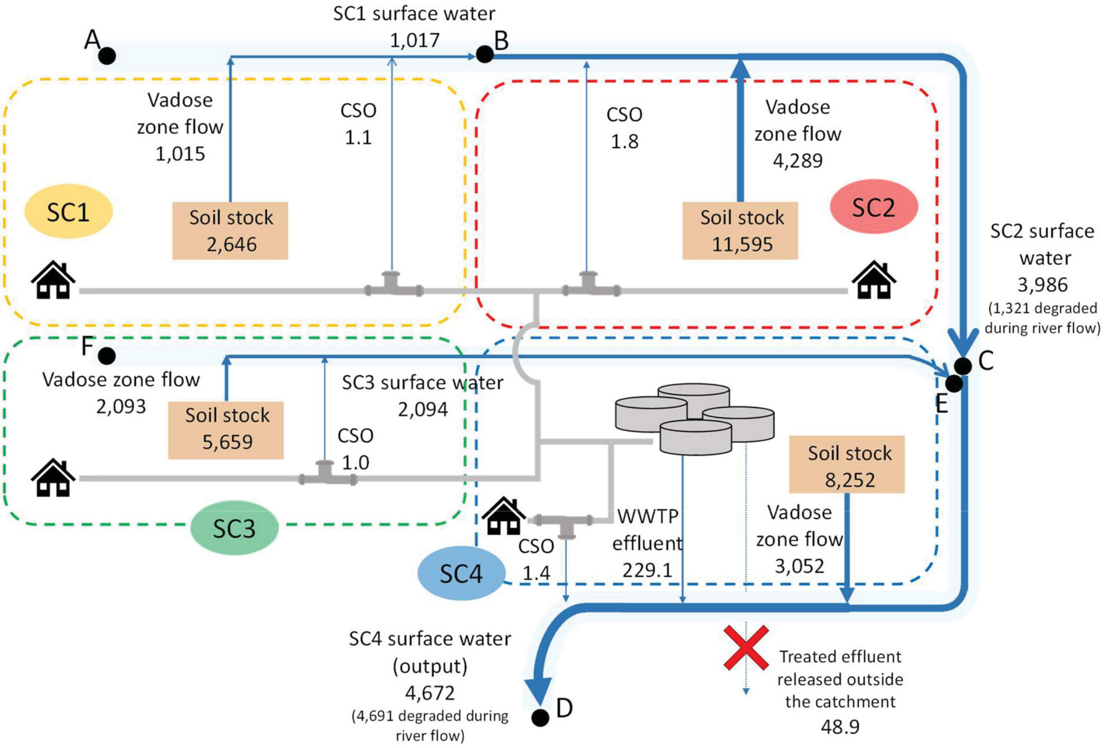

Fig. 4. Load of SMX in the surface water of each SC and in the main emission pathways: WWTP effluent, CSOs and vadose zone flows. All the loads are expressed in g year^{-1}.

the percentage of treated effluent released outside the catchment SC4_F6 ($CV = 43.8$) and the percentage of PhAC that degraded in surface water SC#_F14 ($CV = 43.8$) (Table 1). The high uncertainty related to the first three parameters was mainly due to the strong relevance of the time correlation indicator, while in the case of the percentage of PhAC that degraded in surface water SC#_F14, the uncertainty was strongly influenced by the difficulty in estimating the half-life that considers all the degradation processes which may occur in the surface water (as already described in Section 2.7).

For the other parameters, the *CV* ranges from 14.7 (for the percentage of PhAC that degraded in WWTP SC4_F5, sequestered into sewage sludge SC4_S·F2 or released with treated effluent SC4_S·F1) to 32.1 (for the percentage of PhAC that degraded during sewage sludge treatment SC4_F8). Further details about the chosen indicator sensitivity and indicator score are reported in Tables S23 and S24.

Finally, entering the uncertainty values in STAN as the parameter variance, the overall uncertainty related to the CBZ and SMX load in the SC4 output resulted equal to 45% and 113%, respectively.

It is important here to highlight that the uncertainty related to some intermediate value may be considerably higher than that related to the final output. In this regard, the uncertainty related to the CBZ load in the SC1, SC2 and SC3 river outlet was equal to 2779%, 2324% and 3006%, respectively, while for SMX, it was equal to 173%, 191% and 174% in the SC1, SC2 and SC3 outlet, respectively (Table S25). Here, although the values are high, they seem reasonable as STAN evaluates them using Gaussian error propagation (Laner et al., 2014).

In this study context, it is important to underline that an uncertainty higher than ±100% has a physical meaning only in its positive part, as a PhAC load or concentration cannot be negative. Thus, uncertainties >100% will be considered hereinafter as their actual value for the positive part, and as −100% for the negative part.

3.3. Sensitivity analysis

Regarding CBZ, 5 out of the 8 varied parameters had an impact on the final SFA output (PhAC load in the SC4 river endpoint) (Table 1). In particular, CBZ removal in WWTP (evaluated by varying the percentage of CBZ released with the treated effluent) showed the highest S_i range ($S_{i,\min} = -110\%$, $S_{i,\max} = 54.7\%$) and thus the highest impact on the final output, as reported in Fig. S10 and summarized in Table S26. It is important to note that the parameter with a highest S_i range, and therefore the highest significance, should be CBZ human consumption, but it has to be excluded in this discussion because it corresponds to a trivial solution as it is the only CBZ input in the system.

Since CBZ degradation in the surface water body was assumed to be negligible in SFA, it was impossible to assess both minimum and maximum variations in the sensitivity analysis. Therefore, only the maximum CBZ degradation of 0.4% (on average) was evaluated, resulting in a reduction of CBZ residual load in the SC4 output equal to 0.42%, which led to an S_i value equal to 109%, suggesting that degradation in surface water might have been the most significant parameter if it had been evaluated both positively and negatively.

Among the other parameters, it was found that the percentage of treated effluent released outside the catchment considerably affects the final output (S_i ranges from −21.1 to 21.1%), the percentage of wastewater discharge *via* the CSOs was only slightly significant (S_i ranges from −0.4% to 0.4%), and the percentage of CBZ degraded during sludge treatment, the percentage of treated sludge applied on agricultural soil and soil characteristics did not affect the CBZ load in the SC4 surface water ($S_i << 1\%$).

Regarding SMX, among the 9 analyzed parameters, the degradation in surface water proved to be the most relevant (S_i ranges from −72.7% to 131%), confirming the considerations reported above for CBZ. However, unlike CBZ, the soil characteristics (represented by the percentage

Table 1
Results summary of the SFA for CBZ and SMX: output load, emission pathway contribution, most uncertain parameters and most significant parameters.

	CBZ			SMX		
Load in SC4 river endpoint ± overall uncertainty (Section 3.1)	9.5 ± 4.2 kg year^{-1}			4.6 ± 5.1 kg year^{-1}		
Emission pathway contribution (Section 3.1)	WWTP effluent	CSOs	Runoff, tile drainage, interflow	WWTP effluent	CSOs	Runoff, tile drainage, interflow
	98.6%	1.4%	<< 1%	2.1%	<< 1%	97.8%
Most uncertain parameters (Section 3.2)	Flow splitting in soil system ($CV = 65.8$) PhAC degradation in surface water ($CV = 43.8$) Flow splitting in sewer system ($CV = 43.8$) Treated effluent released outside the catchment ($CV = 43.8$) PhAC human consumption ($CV = 41.8$)					
Most significant parameters (Section 3.3)	Human consumption CBZ removal in WWTP CBZ degradation in surface water Treated wastewater released outside the catchment			SMX degradation in surface water Soil characteristics SMX amount added with manure amendment		

of SMX load moved into the soil aqueous phase) played an important role in the analysis (S_i ranges from −97.5% to 97.5%). This is mainly due to the addition of SMX into soil by manure application that significantly increased the SMX load in runoff, tile drainage and interflow. A considerable influence on the SMX load output was observed from this additional parameter (which was not considered for CBZ), resulting in an S_i which ranges from −97.2% to 96.8% (Table 1).

As shown in Fig. S11 and summarized in Table S27, wastewater correlated parameters (namely, percentage of wastewater discharge *via* the CSOs, percentage of SMX released into the WWTP effluent and percentage of treated effluent released outside the catchment) only slightly affected the final SMX output or were even insignificant (S_i ranges from −2.4% and 2.5% for the percentage of SMX released with the WWTP effluent, from −0.5% to 0.5% for the percentage of treated effluent released outside the catchment and $< <$ 1% for the percentage of wastewater discharged *via* the CSOs). In this context, the percentage of SMX that degraded during sludge treatment and the percentage of treated sludge applied onto agricultural soil showed negligible sensitivity ($S_i < < 1$) due to manure amendment predominance, in terms of SMX load applied onto the soil. Here, also human consumption showed not to consistently influence the results, as the SMX amount reaching the sewer system after human consumption and metabolism is low anyway compared to the SMX amount added onto soil with manure amendment (S_i ranges from −2.5% to 2.5%).

The different results, in terms of PhAC residual load in the SC4 river endpoint and the source contribution of the WWTP effluent, CSOs and agricultural soil vadose zone flows, obtained with the different runs of the sensitivity analysis, are reported in Tables S28 and S29 for CBZ and SMX, respectively.

Focusing on CBZ, it emerges that the variation of the considered parameters did not consistently change the emission pathway contributions. In particular, the variations of the percentage of wastewater discharged *via* the CSOs led to the highest variation range of the WWTP effluent contribution (97.7% to 99.3% of the total CBZ load in surface water) as well as the highest variation range of the CSO contribution (ranging between 0.7% and 2.3%). On the other hand, the agricultural soil vadose zone flow did not play a relevant role in any case, resulting in a contribution ranging between <0.1% and 0.1%.

Different results have been found for SMX, for which the variation of the input load added with manure amendment onto the soil led to a substantial change in the emission pathway contributions, resulting in a WWTP effluent contribution ranging between 0.6% and 92.2%, a CSO contribution ranging between 0% and 2.1%, and an agricultural soil vadose zone flow ranging between 5.7% and 99.4% (Table S29). Variations of the other parameters did not consistently change the resulting emission pathway contributions, suggesting that the SMX load added with the amended manure is the most important source of pollution in the receiving water body, also considering the unclear level of treatment the manure is subjected to.

3.4. Measured PhAC concentrations

3.4.1. Sampling campaign results

The concentrations of CBZ and SMX measured in the surface water samples collected at the endpoint of each SC are reported in Table S30. It emerges that CBZ was quantified in 29 out of 40 samples with a concentration ranging from 1 ng L^{-1} (in SC1 and in SC3) to 221 ng L^{-1} (in SC4), while it was detected but not quantifiable in the rest of the samples (< 0.2 ng L^{-1}). SMX was only quantified in 8 out of 40 samples, and only in the SC3 and SC4 surface water, with concentrations between 1 ng L^{-1} (in SC3) and 42 ng L^{-1} (in SC4), whereas it was detected but not quantifiable in the remaining samples (< 0.4 ng L^{-1}). The average measured concentration in surface water of CBZ was equal to 1.7 ng L^{-1}, 5.1 ng L^{-1}, 3.4 ng L^{-1} and 120 ng L^{-1} for SC1, SC2, SC3 and SC4, respectively, while the average concentration of SMX can be estimated only in SC3 and in SC4, resulting equal to 0.8 ng L^{-1} and 6.8 ng L^{-1}, respectively. It is important to underline that the average concentrations reported above were estimated by only considering the concentrations equal or above the LOQ, instead of evaluating the average on the basis ofall the samples and assuming equal to LOQ for those values less than LOQ. In this way, the average concentrations obtained correspond to the highest or "worst-case" results based on the available dataset.

The river discharge at the endpoint of each SC is reported in Table S30 for each sampling day. On average, surface water flow rate was 0.2 m^3 s^{-1}, 0.4 m^3 s^{-1}, 0.05 m^3 s^{-1} and 1.1 m^3 s^{-1} in SC1, SC2, SC3 and SC4, respectively, with minimum discharge of 0.01 m^3 s^{-1} in SC3 and maximum discharge of 3.1 m^3 s^{-1} in SC4. These measurements are therefore in line with the expected average flow rates reported above in the site description (Section 2.2).

3.4.2. Comparison between modeled and measured concentrations

Table S31 reports the predicted PhAC concentrations ($CBZ_{c,p}$ and $SMX_{c,p}$) obtained by dividing the modeled loads at the endpoint of each SC by the annual average river flow rate in the corresponding locations (0.23 m^3 s^{-1}, 0.53 m^3 s^{-1}, 0.09 m^3 s^{-1} and 1.15 m^3 s^{-1} in SC1, SC2, SC3 and SC4), and their related uncertainty (evaluated as described above).

It emerges that, with the exception of CBZ in SC2, the average predicted concentration of both PhACs at the river endpoint of each SC is always higher than the measured concentrations found in the experimental campaign, showing that the SFA overestimates PhAC occurrence in surface water. This is not surprising bearing in mind the conservative approach maintained during the evaluation of the SFA parameters in which the worst-case scenario was commonly adopted, such as for load splitting in soil matrix, or PhAC degradation in surface water. In particular, for CBZ, the highest difference between predicted and measured concentrations was observed in SC4 (point E in Fig. 1) in which the average $CBZ_{c,p}$ was estimated equal to 262 ng L^{-1} while the maximum observed $CBZ_{c,m}$ was found equal to 120 ng L^{-1}.

The overestimation of the SMX concentration at the endpoint of SC3 was even higher, at which $SMX_{c,p}$ resulted equal to 738 ng L^{-1} while the maximum measured $SMX_{c,m}$ was found equal to 4 ng L^{-1} (Table S30 and S31). This discrepancy may be justified by the assumption of no SMX degradation during manure storage, and by the fact that the PhAC load moved into the aqueous phase of the soil was evaluated as the worst case scenario. However, due to the high uncertainty related to the modeled results, it emerges that $CBZ_{c,m}$ and $SMX_{c,m}$ are always (with the exception of $CBZ_{c,p}$ in SC4) within the minimum and maximum range of $CBZ_{c,p}$ and $SMX_{c,p}$ (Fig. 5a–b).

Fig. 5a shows that the measured CBZ concentrations in SC4 are at least one order of magnitude higher than those found in the other SCs, confirming the modeled findings for which the WWTP effluent was the main CBZ emission pathway. On the other hand, SMX was detected at low concentrations in all the SCs, with the exception of a single peak measure equal to 42 ng L^{-1} in the SC4 river outlet on August 14, 2019, which suggested that the WWTP may contribute more to the SMX load in surface water than what the model estimated, especially noting that the peak concentration was measured on a sunny day, during which no CSOs and no surface runoff are expected to occur.

Furthermore, the high number of no SMX detection in surface water samples suggests that SMX degradation may be significant both in case of its release from point (such as WWTP effluent and CSOs) and non-point (arable land surface runoff, tile drainage and interflow) emission pathways, and thus no other better considerations may be outlined concerning the comparison between the predicted and measured SMX concentrations.

4. Conclusions

This study outlined a simple method to assess the occurrence of selected micropollutants on a catchment scale through different environmental compartments, showing that PhAC emission pathways in the receiving water body may consistently differ also depending on their specific source, such as human and veterinary consumption.

In the specific case study, the human antiepileptic CBZ is discharged into the river mainly by WWTP effluent. Concerning SMX, according to theoretical considerations and available knowledge, it was expected that it may reach the receiving water bodies mainly *via* surface runoff, tile drainage and interflow from arable land amended with animal manure, but the results of a monitoring campaign suggested that, in this case study, the WWTP effluent may be the main emission pathway for this substance as well. The contribution of the CSOs to the release into surface water body is low compared to the other two emission pathways for both substances. This situation might however be different in other river catchments.

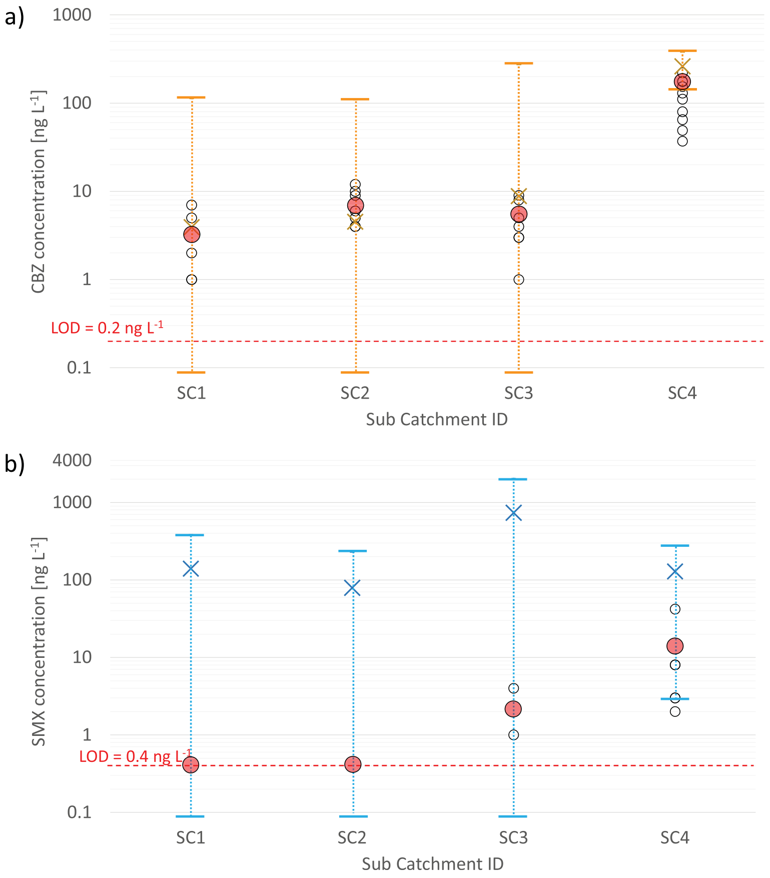

Fig. 5. Comparison between the modeled and measured concentrations of CBZ (a) and SMX (b) at the river endpoint of each SC. Measured concentrations and the average measured concentration are represented by empty black dots and red dots, respectively, while the average modeled concentrations and uncertainty range are represented by crosses and scatters respectively.

However, the sensitivity analysis remarked that the release of CBZ into surface water is consistently influenced by degradation in WWTP, while the release of SMX mainly depends on the amount of manure applied onto the soil, and consecutively, on the soil characteristics.

These findings highlight the importance of appropriate wastewater treatment and suggest that animal consumption may become an important source of PhACs in the receiving water bodies, especially when manure treatment is inadequate or totally absent.

On the other hand, the uncertainty analysis revealed the strong inaccuracy of some methodological steps, such as the evaluation of the PhAC fate in the soil compartment and surface waters, and the difficulty of properly estimating the contribution of soil vadose zone flows from arable land amended with sewage sludge and manure.

The same uncertainty was thus reflected in the SMX monitoring campaign results in which, also because of uncertainty about SMX degradation in the river, it was not possible to strictly outline the correlation between the predicted and measured results.

To sum up, in order to make a comprehensive analysis of the pathways a PhAC may take in the environment, future research could focus on:

- PhAC fate and mobilization in arable land, depending on the physical and chemical properties of the PhAC, the soil characteristics and the hydrology of the system;
- PhAC fate in surface waters depending on water conditions, such as turbidity, salinity, temperature and other physical and chemical properties;
- monitoring of PhAC occurrence in different environmental compartments of a catchment in different weather conditions, by means of exhaustive and comprehensive sampling campaigns;
- discrepancies between modeled and measured concentrations found for SMX should be better investigated with on field studies in order to better understand the fate of this key compound with regard to the different emission pathways.

Nevertheless, SFA proved to be a valuable tool for the estimation of micropollutant occurrence in the water compartments of a catchment, especially when a large and reliable set of data is available.

CRediT authorship contribution statement

Authors equally contributed to the conceptualization, developing of the study, investigations, data processing, writing and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix 1. List of the parameters requested by SFA with their corresponding value and the section explaining how they were evaluated

Parameter description	ID in STAN	CBZ (unit of measurement)		SMX (unit of measurement)		Section
PhAC load released into the SC1 sewer system with domestic wastewater	SC1_F1	2559.8	(g year^{-1})	102.4	(g year^{-1})	Section 2.3
PhAC load released into the SC2 sewer system with domestic wastewater	SC2_F1	5067.3	(g year^{-1})	202.7	(g year^{-1})	Section 2.3
PhAC load released into the SC3 sewer system with domestic wastewater	SC3_F1	2440.1	(g year^{-1})	97.6	(g year^{-1})	Section 2.3
PhAC load released into the SC4 sewer system with domestic wastewater	SC4_F1	2848.5	(g year^{-1})	113.9	(g year^{-1})	Section 2.3
PhAC load in pig manure in SC1	SC1_F2.1	–	–	2682.1	(g year^{-1})	Section 2.4
PhAC load in poultry manure in SC1	SC1_F2.2	–	–	167.3	(g year^{-1})	Section 2.4
PhAC load in cattle manure in SC1	SC1_F2.3	–	–	912.5	(g year^{-1})	Section 2.4
PhAC load in pig manure in SC2	SC2_F2.1	–	–	14,719.1	(g year^{-1})	Section 2.4
PhAC load in poultry manure in SC2	SC2_F2.2	–	–	486.6	(g year^{-1})	Section 2.4
PhAC load in cattle manure in SC2	SC2_F2.3	–	–	678.1	(g year^{-1})	Section 2.4
PhAC load in pig manure in SC3	SC3_F2.1	–	–	6584.2	(g year^{-1})	Section 2.4
PhAC load in poultry manure in SC3	SC3_F2.2	–	–	390.0	(g year^{-1})	Section 2.4
PhAC load in cattle manure in SC3	SC3_F2.3	–	–	777.7	(g year^{-1})	Section 2.4
PhAC load in pig manure in SC4	SC4_F2.1	–	–	8891.4	(g year^{-1})	Section 2.4
PhAC load in poultry manure in SC4	SC4_F2.2	–	–	290.1	(g year^{-1})	Section 2.4
PhAC load in cattle manure in SC4	SC4_F2.3	–	–	2123.3	(g year^{-1})	Section 2.4
Percentage of PhAC discharged into the CSO in SC1	SC1_F3	1.1%	(%)	1.1%	(%)	Section 2.5
Percentage of PhAC conveyed into the treatment in SC1	SC1_F4	98.9%	(%)	98.9%	(%)	Section 2.5
Percentage of PhAC discharged into the CSO in SC2	SC2_F3	0.9%	(%)	0.9%	(%)	Section 2.5
Percentage of PhAC conveyed into the treatment in SC2	SC2_F4	99.1%	(%)	99.1%	(%)	Section 2.5
Percentage of PhAC discharged into the CSO in SC3	SC3_F3	1.0%	(%)	1.0%	(%)	Section 2.5
Percentage of PhAC conveyed into the treatment in SC3	SC3_F4	99.0%	(%)	99.0%	(%)	Section 2.5
Percentage of PhAC discharged into the CSO in SC4	SC4_F3	1.2%	(%)	1.2%	(%)	Section 2.5
Percentage of PhAC conveyed into the treatment in SC4	SC4_F4	98.8%	(%)	98.8%	(%)	Section 2.5
Percentage of PhAC released with the treated effluent of the WWTP	SC4_S·F1	89.4%	(%)	54.4%	(%)	Section 2.5
Percentage of PhAC absorbed into primary and secondary sludges	SC4_S·F2	1.6%	(%)	0.5%	(%)	Section 2.5
Percentage of PhAC degraded or transformed in the WWTP	SC4_F5	10.5%	(%)	45.2%	(%)	Section 2.5
Percentage of the treated effluent released outside the catchment	SC4_F6	17.6%	(%)	17.6%	(%)	Section 2.5
Percentage of treated effluent discharged into the surface water	SC4_F7	82.4%	(%)	82.4%	(%)	Section 2.5
Percentage of PhAC degraded during sludge treatment	SC4_F8	13.3%	(%)	88.9%	(%)	Section 2.5
Percentage of PhAC not degraded during sludge treatment	SC4_F9	86.7%	(%)	11.1%	(%)	Section 2.5

(continued on next page)

(*continued*)

Parameter description	ID in STAN	CBZ (unit of measurement)		SMX (unit of measurement)		Section
Percentage of sludge not used in agriculture	SC4_F10	40.1%	(%)	40.1%	(%)	Section 2.5
Percentage of sludge used in agriculture	SC4_S·F3	59.9%	(%)	59.9%	(%)	Section 2.5
Percentage of sludge applied onto SC1 arable land	SC1_F11	9.7%	(%)	9.7%	(%)	Section 2.5
Percentage of sludge applied onto SC2 arable land	SC2_F11	41.0%	(%)	41.0%	(%)	Section 2.5
Percentage of sludge applied onto SC3 arable land	SC3_F11	20.0%	(%)	20.0%	(%)	Section 2.5
Percentage of sludge applied onto SC4 arable land	SC4_F11	29.3%	(%)	29.3%	(%)	Section 2.4
PhAC fraction sequestered in soil solid phase	SC#_S·F4	95.0%	(%)	73.0%	(%)	Section 2.6
PhAC fraction moved in soil aqueous phase	SC#_F12	5.0%	(%)	27.0%	(%)	Section 2.6
Percentage of surface water used in arable land irrigation in SC1	SC1_F13	0.0%	(%)	0.0%	(%)	Section 2.7
Percentage of PhAC degraded in surface water in SC1	SC1_F14	–	(%)	–	(%)	Section 2.7
Percentage of PhAC not degraded in surface water in SC1	SC1_F15	100.0%	(%)	100.0%	(%)	Section 2.7
Percentage of surface water used in arable land irrigation in SC2	SC2_F13	0.0%	(%)	0.0%	(%)	Section 2.7
Percentage of PhAC degraded in surface water in SC2	SC2_F14	0.0%	(%)	24.9%	(%)	Section 2.7
Percentage of PhAC not degraded in surface water in SC2	SC2_F15	100.0%	(%)	74.1%	(%)	Section 2.7
Percentage of surface water used in arable land irrigation in SC3	SC3_F13	0.0%	(%)	0.0%	(%)	Section 2.7
Percentage of PhAC degraded in surface water in SC3	SC3_F14	–	(%)	–	(%)	Section 2.7
Percentage of PhAC not degraded in surface water in SC3	SC3_F15	100.0%	(%)	100.0%	(%)	Section 2.7
Percentage of surface water used in arable land irrigation in SC4	SC4_F13	0.0%	(%)	0.0%	(%)	Section 2.7
Percentage of PhAC degraded in surface water in SC4	SC4_F14	0.0%	(%)	50.1%	(%)	Section 2.6
Percentage of PhAC not degraded in surface water in SC4	SC4_F15	100.0%	(%)	49.9%	(%)	Section 2.7

Appendix 2. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.142328.

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