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Transfer of antibiotic residues from digestate-amended soil to edible crops in a circular food production system☆

Katja C.W. van Dongen *, Weixin Huang, Esmer Jongedijk, Bram van de Kooi, Erik de Lange, H.J. van der Fels-Klerx

Wageningen Food Safety Research, Part of Wageningen University & Research, Akkermaalsbos 2, 6708 WB, Wageningen, the Netherlands

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ABSTRACT

Re-using residual streams can increase the circularity of food systems and therefore increase sustainability. However, besides the re-introduction of useful nutrients, chemical substances can possibly re-enter the food system as well, potentially impacting food and feed safety. For example, antibiotic residues may be present in animal manure or digestate used as fertilizer, and might be subsequently taken up by edible crops. This study investigated the plant uptake of four relatively persistent and immobile antibiotics from digestate-amended soil. Digestate was fortified with four antibiotics (doxycyclin, trimethoprim, flumequine, and tilmicosin), reaching respective soil concentrations of 30, 100 and 300 μg/kg. Radish and spinach plants were grown on the fortified soils in a pot system, and antibiotic concentrations in roots and shoots were quantified by liquid chromatography-tandem mass spectrometry. Trimethoprim, flumequine and tilmicosin were taken up by at least one of the plants tissues from both species, while doxycycline could not be quantified. In radish, trimethoprim and tilmicosin translocated more to shoots as compared to roots, while flumequine was only detected in radish roots. In spinach, trimethoprim translocated more to shoots as compared to roots, while this was the other way around for tilmicosin and flumequine. Plant uptake was simulated using three existing models, and results were compared to experimentally collected data. Predicted results overestimated plant uptake, but uptake patterns were comparable between experimental data and models. Overall, this study contributes to further understanding plant uptake of antibiotic residues, and thereby helping to ensure food safety of circular food production systems.

1. Introduction

An important strategy to improve the sustainability of the current food system is to increase circularity, as acknowledged by the European Union through its Green Deal with the Farm to Fork strategy (European Commission, 2020b) and New Circular Economy Action Plan (European Commission, 2020a). In a circular food system, nutrients that are present in by-products or residual streams are circulated back into the food system (Jurgilevich et al., 2016). Besides nutrients, this can also result in the (re)introduction or accumulation of food safety hazards in the food system, having a possible implication on food and feed safety (Focker et al., 2022; Thakali & MacRae, 2021; van Asselt et al., 2023; van der Fels-Klerx et al., 2024).

An example of circulating nutrients back into the food system is through the application of animal manure in terrestrial food production systems, either directly or upon anaerobic fermentation of the animal manure for biogas production, resulting in the so-called digestate as residual material (Ehmann et al., 2018; Shi et al., 2018; Tasho & Cho, 2016). Animal manure and derived products like digestate contain nutrients and are widely applied as fertilizer, amendment, or improver of agricultural soils (Köninger et al., 2021). However, besides nutrients, chemical hazards can be present in these streams as well, such as antimicrobial residues and heavy metals (Berendsen et al., 2015; Lehmann & Bloem, 2021; Tasho & Cho, 2016; Wolak et al., 2023; Zhen et al., 2020; Zhou et al., 2020). For antibiotics, it is known that the majority of the antibiotic applied to livestock is excreted as the parent compound or

Abbreviations: **BCF**, bioconcentration factor; **DOX**, doxycycline; **FLUM**, flumequine; **LOQ**, limit of quantification; **TF**, translocation factor; **TILM**, tilmicosin; **TMP**, trimethoprim.

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* Corresponding author.
E-mail address: katja.vandongen@wur.nl (K.C.W. van Dongen).

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active metabolite through urine or feces (Kim et al., 2011; Tasho & Cho, 2016). Application of animal manure or digestate to agricultural soils can thus result in possible (re)introduction of these hazards in the environment and food system, especially considering the relatively large volumes of animal manure being used as crop fertilizer. Multiple studies reported on the transfer or leaching of antimicrobials from animal manure to soil or water (Kim et al., 2011; Sun et al., 2017; Zhou et al., 2020) and, indeed, application of animal manure is one of the major sources of antibiotic residues present in the environment and surface- and groundwater (Shi et al., 2018; Tasho & Cho, 2016). Subsequently, these antibiotic residues can possibly be taken up by edible crops (Barra Caracciolo et al., 2022; Boxall et al., 2006; Chuang et al., 2019; Geng et al., 2022; Kumar et al., 2005; Pan & Chu, 2017b; Tasho & Cho, 2016; Wei et al., 2023), potentially affecting the safety of the crop for animal and human consumption. Multiple factors can influence these uptake and translocation processes, such as plant-specific factors (e.g., root composition) and physicochemical properties (Wei et al., 2023). Additionally, the spread of antibiotic residues through the environment and the food system through animal manure fertilization can possibly increase the emergence or spread of antimicrobial resistance (genes) (Bhattacharjee et al., 2024; Li et al., 2017).

To understand the potential impact of a circular food system on food safety, it is important to collect data on how relevant hazardous substances, such as antibiotics, behave and move through such a system (van Leeuwen et al., 2024). Multiple factors can influence the fate of antibiotics, such as physicochemical characteristics of the compound itself, but also (a)biotic characteristics of for example the type of animal manure or digestate, soil type, and crop species (Berendsen et al., 2021; Huang et al., 2024; Pan & Chu, 2017b).

This study aimed to investigate the transfer of antibiotic residues from digestate-amended soils to edible crops through plant pot experimental studies, and via simulation modelling to aid the understanding of the antibiotics plant uptake mechanisms and concepts (Trapp et al., 2023). Four different antibiotics were selected, being doxycycline (DOX), trimethoprim (TMP), tilmicosin (TILM) and flumequine (FLUM), because of: 1) their relatively persistent and immobile characteristics (Berendsen et al., 2021), 2) they are regularly found in animal-manure-based digestate (Berendsen et al., 2015; Zhou et al., 2020), 3) to date, limited studies on the plant uptake of these substances have been performed (Pan & Chu, 2017a; Wang et al., 2016; Zeng et al., 2022), and 4) they belong to different antibiotic classes (i.e., tetracyclines (DOX), diaminopyrimidines (TMP), macrolides (TILM), and fluroquinolones (FLUM)). By investigating the plant uptake of these four substances in a biosolid-amended soil system using two plant species (radish and spinach), and integrating these findings with modelling efforts, this study advances the current understanding of antibiotic residue transfer from soil to crop, in view of the food safety of circular food production systems.

2. Materials and methods

2.1. Chemicals and reagents

The reference standards doxycycline (DOX), trimethoprim (TMP), tilmicosin (TILM), and flumequine (FLUM) were purchased at Sigma Aldrich (St. Louis, MO, USA). The internal standards doxycycline-d3 (DOX-d3), trimethoprim-d9 (TMP-d9), tilmicosin-d3 (TILM-d3), and flumequine-13C2 (FLUM-13C2) were purchased at Toronto Research Chemicals (Toronto, ON, Canada), and the internal standard demeclocycline (DMC) was purchased at Sigma Aldrich (St. Louis, MO, USA). Acetonitrile (ACN), ammonium (25 %), ammonium acetate, citric acid monohydrate, disodium hydrogen phosphate, ethanol (EtOH), ethylenediaminetetraacetic acid (EDTA), formic acid (FA), and methanol (MeOH) were purchased at Merck (Kenilworth, NJ, USA). Ammonium formate (97 %), dimethyl sulfoxide (DMSO), lead acetate trihydrate, and trifluoro acetic acid (TFA) were purchased at Sigma-Aldrich Sigma Aldrich (St. Louis, MO, USA). Bondesil-PSA (40 μM) was purchased at Agilent (Santa Clara, CA, USA).

2.2. Preparation of solvents and stock solutions

McIlvain-EDTA buffer was prepared by adding 500 mL of 0.1 M citric acid, 280 mL 0.2 M disodium hydrogen phosphate, and 74.4 g sodium-EDTA to 1L water into a 2L volumetric flask. The pH was adjusted to 4.0 using the citric acid solution or di-sodium hydrogen phosphate solution. The solution was diluted with water up to 2 L. Stock solutions and internal standard solutions were prepared once at a concentration of 100 mg/L for TMP-d9 and at a concentration of 1000 mg/L for the other three compounds. All solutions were stored at −80 °C until further use. DOX, TMP, TILM, DOX-d3, TMP-d9, TILM-d3, and DMC were dissolved in MeOH. FLUM and FLUM-13C3 were dissolved in a 2 % M ammonium hydroxide solution in MeOH. For fortifying the digestate with antibiotics (see 2.3), a solution of reference standard was made in DMSO at a concentration of 30, 100 and 300 mg/L for FLUM, DOX, TMP, and TILM. In addition, a mixed solution of internal standards was made for the chemical analyses (see 2.4) in MeOH at a concentration of 1 mg/L for TMP-d9 and a concentration of 4 mg/L for DOX-d3, DMC, FLUM-13C3, and TILM-d3.

2.3. Plant uptake experiment

The soil was collected from an organically managed grassland in Wageningen, the Netherlands (51°59′28.6″N 5°40′07.3″E). A sandy soil was selected, as these soils tend to have relatively lower contaminant retention, leading to potentially greater plant uptake, representing a possible ‘worst case scenario’ in comparison to other soil types. A sandy soil is also suitable for a controlled pot experiment, as it can be homogenized well. The specific selected grassland was a research site that had not been fertilized for the last 10 years, so no recent input of antibiotic residues by fertilizer products was expected there, which was important for this research. The soil texture was classified as sand with 76 % sand, 13 % silt and 3 % clay. The soil had a pH of 4.8 and a carbon organic matter content of 3.7 %. The soil was collected from a depth of 0–30 cm after removal of the grass top layer. The soil was air-dried, sieved to 5 mm, and stored until the start of the experiment. Before use, the soil was thoroughly mixed. Digestate was collected from an anaerobic digester, in which solely bovine manure was digested anaerobically at 39 °C. The digestate was collected before separation between liquid and solid fractions and stored at 4 °C until further use. The digestate contained 4.57 g/kg nitrogen, and the amount of digestate mixed with the soil (225 g digestate to 5 kg soil) corresponded to the maximum amount of nitrogen derived from animal manure allowed to be used per hectare in Europe (91/676/EEC), i.e., 170 kg nitrogen per hectare (Rijksoverheid, 2022). DOX, FLUM, TILM, and TMP were selected as model compounds in the present study at concentrations ranging from 0.6 mg/kg to 6.0 mg/kg in digestate (see Table 1 for compound characteristics and properties) and were individually fortified to the digestate. These compounds and their concentrations were selected based on literature, analytical data from WFSR, antibiotic usage data, and expert opinions on their presence in animal manure, digestate, and antibiotic usage (Berendsen et al., 2015; Jansen et al., 2019). Although TMP is usually administered in combination with a sulfonamide (e.g., sulfamethoxazole), TMP was selected to fortify the digestate because sulfonamides degrade during anaerobic digestion processes while TMP persists (Yang et al., 2022). Digestate fortified with DOX, TMP, FLUM or TILM was mixed 15 min head-over-head, and subsequently thoroughly mixed with the soil. This resulted in final concentrations of 30, 100, and 300 μg/kg for each of DOX, TMP, FLUM, and TILM in digestate-amended soil, with a final concentration of 0.1 % DMSO. Besides these antibiotic treatments, a solvent control treatment (final concentration of 0.1 % DMSO in digestate-amended soil) and control treatment (non-fortified digestate added to soil) was prepared,

Table 1
Parameters, properties, and characteristics of doxycycline, flumequine, trimethoprim, and tilmicosin. Compound-specific parameters that were used as model input are specified. C_{soil} is experimental data of the present study. Underlined pK_a values were used in the standard model and PCPP model.

Parameter, property, or characteristic (unit)	Doxycycline	Flumequine	Trimethoprim	Tilmicosin
Molecular formula	$C_{22}H_{24}N_2O_8$	$C_{14}H_{12}FNO_3$	$C_{14}H_{18}N_4O_3$	$C_{46}H_{80}N_2O_{13}$
Molecular weight (g/mol)	444.4	261.3	290.3	869.1
Half-life in sandy soil (days) [a]	9 (Berendsen et al., 2021)	226 (Berendsen et al., 2021)	12 (Berendsen et al., 2021)	108 (Berendsen et al., 2021)
log K_{oc}: Logarithm of organic carbon-water partition coefficient ($L*kg^{-1}$) [a]	3.5 (Berendsen et al., 2021)	≥4.2 (Berendsen et al., 2021)	≥4.2 (Berendsen et al., 2021)	≥3.2 (Berendsen et al., 2021)
Compound-specific parameters used as model input				
pK_a: acid dissociation constant	3.0, <u>**8.0**</u>, 9.2 (Qiang & Adams, 2004)	6.35 (Zhao et al., 2022)	7.2 (Mikes & Trapp, 2010)	6.55, <u>**8.67**</u> (Xu et al., 2006)
log K_{ow_n}: Logarithm of octanol-water partition coefficient of the neutral form of a compound	−0.02 (Chabilan et al., 2022)	1.11 (Khandal et al., 1991)	0.91 (Kim et al., 2009; Naghdi et al., 2018)	3.8 (calculated) (McFarland et al., 1997)
K_{HSA}: Absorption coefficient to human serum albumin ($L*mol^{-1}$)	273000 (Hu et al., 2014)	2370000 (Skyrianou et al., 2010)	19100 (Deng et al., 2013)	36308 (Lemli et al., 2018)
log K_{aw}: Logarithm of air-water partition coefficient (L water*L^{-1} air)	−21.72 (ChemSpider, 2024a)	−12.92 (ChemSpider, 2024b)	−11.45 (ChemSpider, 2024c)	−27.45 (unit transformed) (Echemi, 2024)
z: Valency/charge number of a compound	−1	−1	1	1
C_{soil}: Concentration in soil ($mg*kg^{-1}$)	0.19228	0.15243	0.22654	0.31958

[a] In this cited study, all four antibiotics were included in the determination of the half-life and log K_{oc} and thus the values reported in this cited study were selected to include in this table, to illustrate differences amongst compounds. Reported literature values can differ substantially.

by applying the same ratios of digestate and soil as in the antibiotic treatments. Per treatment per plant type, 5 replicates were prepared, each containing 1 kg of the fortified digestate-amended soil, in pots of 11x11x12 cm. Each pot had an individual plate, and the pots were placed in the greenhouse in random order. The soil mixed with digestate was settled for 12–16 h, and each pot was sampled to analyze the soil start concentrations of the antibiotic residues. This was followed by seeding the radish and spinach seeds in separate pots. These two plant species were selected because of their different morphology (including a different root system), and radish as a vegetable of which mainly the root is consumed by humans, while for spinach this is the case for the shoots. Radish was seeded at three spots (triangle) in the pot each containing two seeds, and spinach was seeded at five spots (dice), each containing two seeds. For each plant species, this resulted in five replicates per treatment. The plants were randomly placed and watered as needed, when being grown in a semi-controlled greenhouse (with an average temperature of 20 °C and average relative humidity of 65.5 %; this ranged from 9 to 44 °C and from 18 to 94 %, respectively, from night to day). Within ten days, superfluous sprouted plants were weeded out randomly, aiming for three radish plants per pot and five spinach plants per pot. After a total of 31 days (radish) and 41 days (spinach), plants were harvested in the morning prior to watering the plants, resulting in relatively dry soils. Per pot, the plants were taken out, and the roots were separated from the shoots. Soil remains were removed from the plant material by brushing (shoots) or washing (roots), and the cleaned plant materials were weighed afterwards. The residual soil in the pot was mixed and subsequently sampled. Samples of soil and plant tissue were stored at −20 °C until further analyses. Due to the fewer available biomass (see Results 3.2), the harvested spinach roots and shoots of the five biological replicates were pooled per treatment, and after weighing stored at −20 °C until further analyses.

Average water content of the soil was determined by drying overnight (103 ± 3 °C) three representative soil samples collected at the start of the experiment and upon harvesting of the crops. Average water content was 9.1 ± 1.4 % and 6.0 ± 1.2 % at the start and harvest of the experiment respectively.

2.4. Chemical analyses

LC-MS/MS analyses of the antibiotic residues in soil and plant material were performed based on a previously published method for the detection of antibiotics (Jansen et al., 2019). This method included for measurement of the used soil and digestate, besides the antibiotic residues TILM, TMP, FLUM, and DOX, a range of other veterinary drug analytes belonging to the classes tetracyclines, sulfonamides, macrolides, and quinolones (Supplementary materials Table S1) in order to evaluate their occurrence as such, before fortification, in the used soil and digestate. For all sample types (i.e., plant tissues and soil samples), the sample clean-up was the same as previously described (Jansen et al., 2019). In short, 2 g of each sample was weighed, and an internal standard solution was added. For the extraction, 0.125 % TFA in ACN solution, McIlvain-EDTA buffer, and a lead acetate solution were added. Next, the samples were centrifuged followed by the evaporation of CAN. 0.2 M EDTA solution was added and subjected to solid phase extraction (SPE) with reversed-phase cartridges (Strata-X, Phenomenex, Torrance, CA, USA). The eluent was dried and dissolved in 100 μL of MeOH and 400 μL of water. The extracts were transferred into LC-MS/MS vials and immediately stored at −20 °C until further analysis.

To ensure correct and accurate quantification of the soil, roots, and shoots, matrix-fortified samples were used for each matrix. Moreover, isotopically labelled internal standards were added to all individual samples before sample preparation. All analytical methods applied were ISO 17025-accredited, and validated according to EC Regulation 2002/657 using certified reference materials.

The analysis was performed with a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) coupled to a Sciex Q-trap 6500+ mass spectrometer (Sciex, Framingham, MA, USA). The compounds of interest were separated using a Kinetex C18 column (2.1 × 100 mm, i.d. 1.7 μm, Phenomenex, Torrance, CA, USA). The mobile phases were 2 mM ammonium formate and 0.016 % FA in water (Solvent A), and 2 mM ammonium formate and 0.016 % FA in MeOH (Solvent B). The oven temperature was 40 °C, the injection volume was 5 μL, and the flow rate was 0.3 mL/min. The gradient elution profile was as follows: 0–0.5 min, 0 % B; 0.5–2.5 min, linear increase to 25 % B; 2.5–5.4 min, linear increase to 70 % B; 5.4–5.5 min, linear increase to 100 % B with a final hold of 1 min before returning to its initial conditions of 0 % B for 1 min. The operating parameters of the mass spectrometer including the ion transitions which are previously described (Berendsen et al., 2015). Data processing was done using Sciex OS 2.2 software (Sciex, Framingham, MA, USA). Limits of quantification (LOQ) in the used materials were determined with a signal-to-noise ratio of 10:1 (Vial & Jardy, 1999). The LOQs can be found in Supplementary Materials Table S2.

2.5. Data analysis

Data of the five replicates were averaged and standard deviations

(SD) were calculated. Differences in average biomass (g) between experimental conditions were evaluated with a one-way analysis of variance (ANOVA) combined with a Dunnett's multiple comparison test, using GraphPad Prism. Comparisons were made of the treatments with the control and with the solvent control. Results were considered statistically significant when P-values were <0.05. Concentrations of the antibiotics are expressed in μg/kg d.w., unless mentioned otherwise. These were calculated from the measured values in μg/kg w.w. using reported water content values, being 95.3 % for radish roots and shoots (FoodData Central, 2019), 85 % for spinach roots (Brunetti et al., 2019), and 92.5 % for spinach shoots (FoodData Central, 2021). For soil, measured values were used (as explained in 2.3). Bioconcentration factors (BCF) of the antibiotics, defined as the ratio of the concentration of the substance in the plant tissue to the concentration in the soil, were calculated by dividing the measured concentration in the plant tissue by the measured concentration in the soil at T0 (Equation (1)) (Pan & Chu, 2017b).

$$BCF = \frac{Concentration\ in\ plant\ tissue}{Concentration\ in\ soil} \quad \text{Equation 1}$$

Additionally, the translocation factor (TF) was determined to indicate the translocation of antibiotic residues from the roots to the shoots (Equation (2)) (Pan & Chu, 2017b).

$$TF = \frac{Concentration\ in\ shoot}{Concentration\ in\ root} \quad \text{Equation 2}$$

2.6. Simulation of antibiotic uptake by the crops

The two models developed by Trapp et al. (2023) were used to simulate the uptake of antibiotics in radish and spinach; the standard model assuming that the compounds stay in their neutral form during plant uptake processes, and the pharmaceuticals and personal care products (PPCP) model that considered the ionization of compounds and incorporated the transportation through plant cell membrane and plant phloem, as well as adsorption to proteins in plants (Supplementary materials Fig. S10). The model parameters for the plants (radish and spinach) and the antibiotics of interest needed for both models, could be classified as compound-related (Table 1) and compartment-related parameters (Supplementary materials Table S3). Some adaptations were made to the original model (Trapp et al., 2023): a) the growth scenario shifted from field farming to pot growth and b) the modeled crops were changed from maize into radish and spinach. Model parameter values were as much as possible retrieved from the original publication (Trapp et al., 2023) or from literature and databases, and in case not available, assumptions were made (see Table 1 and Supplementary materials Table S3).

The PPCP model considered that the substances exist in both the neutral and ionizable form during the uptake process. Trapp et al. (2023) calculated the fraction of the antibiotics in their different forms using single value of compound pKa. However (Trapp et al., 2023),This approach may not be suitable for compounds with multiple ionization functional groups in their molecular structure, such as for DOX and TILM (Jones et al., 2005; Kulshrestha et al., 2004), which may result in multiple pKa values for the compounds. Previous studies have estimated the speciation fraction of the substances with multiple pKa values (Supplementary Materials Table S4). Therefore, we replaced the single-pKa based estimation of compound fractions with the multiple-pKa based estimation. This is referred to as the PPCP-adjusted model in the present study. Overall, the simulation implemented three different models based on Trapp et al. (2023), namely the standard model, the PPCP model, and the PPCP-adjusted model.

3. Results

3.1. Presence of antibiotics in applied soil and digestate

Concentrations of TILM, TMP, DOX and FLUM in the used soil and digestate as such, before fortification, were below LOQ, except for DOX being found in digestate (4.8 ± 0.8 μg/kg). Other veterinary drugs were included in the analysis of used soil and digestate as well. In digestate, tetracycline (TC) (5.6 ± 0.3 μg/kg), oxytetracycline (OTC) (76 ± 4.1 μg/kg) and sulfadiazine (1 ± 0.0 μg/kg) were found, while the used soil did not contain any antibiotic residues above the LOQ (Supplementary Materials Fig. S1).

3.2. Growth and yield of the crops

The average biomass was 19.1 g for the control radish roots and 14.8 g for the control radish shoots, resulting in a total average radish control biomass of 33.9 g per pot. Average biomass of radish roots ranged from 15.0 g (TMP-300) to 25.6 g (TILM-300) and 28.3 g (TMP-30). Average biomass of radish shoots ranged from 12.1 g (FLUM-30) to 17.0 g (DOX-30) (Supplementary Materials Fig. S2). Biomass of the radish roots of TMP-30 were statistically increased with an average of 28.3 g, compared to the solvent control. For the other treatments, the average biomasses of both radish roots and shoots did not differ from the solvent control. The growth and biomass yield of the spinach plants was low. Total biomass of the five replicates (pooled together) ranged from 0.31 g (TILM-300) to 1.3 g (DOX-300) for spinach roots, and from 1.2 g (TILM-300) to 6 g (DOX-300) for spinach shoots (Supplementary Materials Fig. S3). Because of poor spinach growth, presumably due to non-optimal growing conditions (limited nutrients available in combination with high temperatures in the semi-controlled greenhouse), the further analysis of the antibiotics uptake in the spinach plants were deemed less reliable and will thus only be presented in the Supplementary Materials.

3.3. Concentrations of antibiotics in soil

The concentrations of TILM, TMP, DOX, and FLUM in the fortified soils (Supplementary Materials Fig. S4) were lower compared to the intended fortified soil concentrations. The determined concentrations relative to the intended concentration (%) in fortified soil ranged for the three treatment concentrations (30, 100, and 300 μg/kg fortified soil) between 48.9 and 50.8 % (TMP), 54.1–77.2 % (DOX), 63.4–106.5 % (TILM), 75.5 %–80 % (FLUM) for radish, and 45.4–62.3 % of TMP, 44.0–53.9 % (DOX), 72.11–104.1 % (TILM), 79.4–92.2 % (FLUM) for spinach. Although determined concentrations were generally lower than intended fortified soil concentrations, replicate values were in the same range and all relative SDs (%) of for example the fortified radish soil concentrations were <15 %, except for TMP-100 (21.9 %), DOX-100 (38.3 %), FLUM-30 (24.5 %). Determined concentrations in radish and spinach soil of the same treatment were in a similar range. For further calculations, the quantified fortified soil concentrations were used, instead of the intended fortified soil concentrations.

Dissipation of the antibiotics was assessed in the fortified soils by comparing measured concentrations prior to seeding (T0) with concentrations after harvesting (Tend). In the soil in which radish plants were grown, highest percentage of antibiotics remaining were for TILM and FLUM (69.8–82.2 % and 67.6–74.5 %, respectively), followed by TMP (47.8–75.5 %) and DOX (27.5–43.0 %) (Fig. 1). This indicates that the amount of antibiotic dissipation was highest for DOX, followed by TMP, and then TILM and FLUM. For the soil in which the spinach plants were grown, the antibiotic dissipation in the soil followed a similar pattern amongst the four antibiotics (Supplementary Materials Fig. S5).

3.4. Transfer of the antibiotics to the crops

Concentrations of the antibiotics were quantified in the roots and

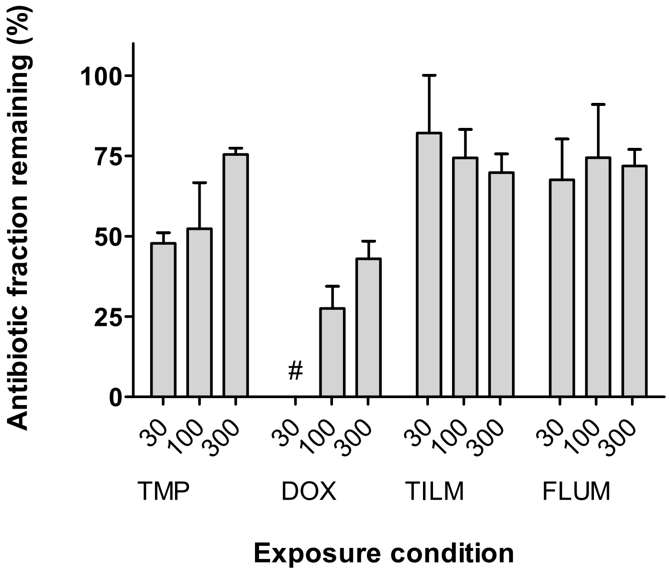

Fig. 1. Percentage of antibiotics remaining in soil, upon harvesting the radish plants after 31 days. The concentration in soil upon harvesting was compared to the concentration in fortified soil prior to seeding (μg/kg d.w.), which was set at 100 %. Data points represent the average ± SD of five replicates. TMP = trimethoprim; DOX = doxycycline; TILM = tilmicosin; FLUM = flumequine. The numbers on the x-axis indicate the treatment. # indicates < LOQ.

shoots of the radish plants per replicate, and in the pooled spinach plants (for qualitative confirmation only). Concentrations of all four antibiotics were below the LOQ in the radish roots and shoots cultivated in the control soils. This was also the case for the spinach roots and shoots. In radish roots cultivated on the fortified digestate-amended soils, uptake of some of the antibiotics was observed (Fig. 2A). TMP concentrations in radish roots were 1.5 and 1.7 μg/kg w.w. for the 100 and 300 μg/kg treatments, respectively. TILM and FLUM concentrations were found above the LOQ at the 300 μg/kg treatment (not at lower concentrations of the treatments), with concentrations in radish roots being 1.3 and 1.8 μg/kg w.w., respectively. In radish shoots, TMP concentration was 1.3, 4.9, and 6.7 μg/kg w.w. for the 30, 100, and 300 μg/kg treatment, respectively (Fig. 2B). TILM was present in radish shoots at concentrations of 1.3 and 4.5 μg/kg w.w. for the 100 and 300 μg/kg treatments, respectively. FLUM was not detected in concentrations above the LOQ in radish shoots, which was the same for DOX which was not present in radish shoots nor in roots in concentrations above the LOQ. Important to note is that the LOQ of DOX was higher compared to the other antibiotics (see Fig. 2).

In spinach roots, TILM and FLUM seemed to be mostly present, followed by TMP. In spinach shoots, TMP was present for all three treatment concentrations, while TILM was present in the two highest treatment concentrations and FLUM only in the highest treatment concentration (Supplementary Materials Fig. S6). This pattern is similar as observed in the radish shoots.

BCFs were determined for each treatment for radish roots and shoots, as well as for spinach roots and shoots (BCF_{root} and BCF_{shoot}), and together with the corresponding TF, values are shown in Table 2. When comparing between plant types and plant tissues per antibiotic, for TMP, BCF_{shoot} exceeded 1 and was higher than BCF_{root} in both radish and spinach. This corresponded to the TFs that also exceeded 1, indicating translocation of the antibiotic from root to shoot. For TILM, in radish, BCF_{shoot} was higher than BCF_{root}, and TF > 1. For spinach, this pattern was the other way around, indicating differences in TILM uptake and

Table 2
Bioconcentration factor (BCF) per plant tissue and translocation factor (TF) between roots and shoots, based on concentrations in soil and plant tissue in μg/kg d.w., when concentrations were above LOQ. Values represent the average ± SD of 5 replicates for the radish samples, and for spinach data based on the values for pooled samples of the 5 replicates was used.

Compound	Crop	Treatment concentration	BCF_{root}	BCF_{shoot}	TF
TMP	Radish	30	–	1.74 ± 0.25	–
		100	0.60 ± 0.09	1.92 ± 0.37	3.20 ± 0.25
		300	0.22 ± 0.05	0.85 ± 0.15	4.01 ± 0.95
	Spinach	30	0.42	1.11	2.67
		100	0.54	1.15	2.13
		300	0.78	1.40	1.79
TILM	Radish	30	–	–	–
		100	–	0.44[a]	–
		300	0.08 ± 0.02	0.28 ± 0.06	3.43 ± 0.64
	Spinach	30	0.85	–	–
		100	0.60	0.38	0.64
		300	1.17	0.82	0.71
FLUM	Radish	30	–	–	–
		100	–	–	–
		300	0.15 ± 0.03	–	–
	Spinach	30	1.61	–	–
		100	1.96	–	–
		300	1.41	0.10	0.07

BCF: bioconcentration factor; FLUM: flumequine; LOQ: limit of quantification; TF: translocation factor; TILM: tilmicosin; TMP: trimethoprim.
[a] Only one value of the 5 replicates was >LOQ and included in this table.
–measured concentrations in the roots and/or shoots could not be determined (values < LOQ), and thus the BCF and respectively TF could not be determined.

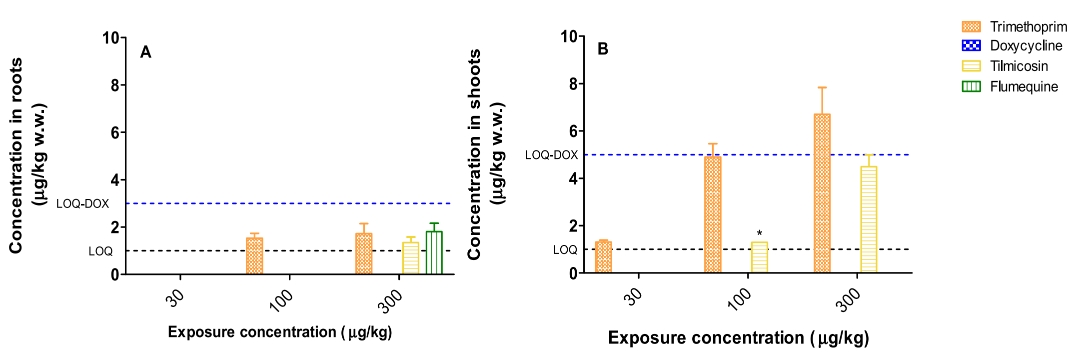

Fig. 2. Concentrations of the fortified antibiotics in radish roots (A) and radish shoots (B), per treatment. Values show the average ± SD of five replicates. The * indicates that only one of the five replicates' data points were shown, because only one replicate was >LOQ. For data points not shown, all measured values were <LOQ. LOQ for TMP, TILM and FLUM is indicated with the black dashed line, the LOQ for DOX is indicated in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

translocation between the two plants. For FLUM, a comparison between radish and spinach plants and between the plant tissues could not be made due to missing data points (because of concentrations in plant tissues being < LOQ).

3.5. Simulated uptake of the antibiotics by radish and spinach

In addition to determining the uptake of the antibiotics by radish and spinach using an experimental approach, the uptake of the antibiotics from soil was simulated by three different models based on (Trapp et al., 2023): the standard model, the PPCP model, and the PPCP-adjusted model. The simulated results overestimated the uptake of the investigated antibiotics with maximum six-orders of magnitude for both crops, as compared to the experimentally obtained data of the highest treatment concentration (Fig. 3 for radish, Supplementary Materials Fig. S7 for spinach). The simulated values can additionally be found in Supplementary materials Table S5. Although the models overestimated the uptake as determined via the plant pot experiment, the simulated uptake patterns corresponded at least in some extent to experimental observations. This was shown as 1) in both the simulated and experimental results, elevated TILM concentrations were found in both radish and spinach for both plant tissues; and 2) the simulation of the standard and PPCP model also showed an increased concentration of TMP in radish shoots compared to the roots, which was in line with experimental findings.

Sensitivity analysis of the simulation models demonstrated that the decrease of biomass of roots and shoots significantly increased the concentration of one or more of the four antibiotics, especially when the corresponding biomass of the input parameters was smaller than 1 kg (Supplementary Materials Figs. S8 and S9). This observation suggests that the models can overestimate predictions when the input plant biomass is lower than 1 kg, which was the case for both radish and spinach experimental-derived biomass values that were used as input parameter.

4. Discussion

In this study, it was shown that the antibiotics TMP, FLUM, and TILM were taken up by radish and spinach from fortified digestate-amended soils under the tested experimental conditions. In contrast to the other three antibiotics, DOX was below LOQ in the plant tissues. Because of the higher LOQ of DOX compared to the LOQs of the other antibiotics, no conclusions with regards to its plant uptake can be made based on the present study. The experimentally determined antibiotic uptake rates differed between the different antibiotics, as well as amongst the crop types. Uptake of antibiotics was observed, and in some cases also bioconcentration (BCF >1) in one or more plant tissues. This was the case for TMP in radish and spinach shoots, and FLUM in spinach roots. For radish, TMP and TILM translocated more in the shoots than in the roots, while DOX was not detected in the plant tissues, and FLUM only in roots at FLUM-300. In spinach, TMP translocated more in the shoots than in the roots, as was similar to the observations in radish. TILM, however, concentrated more in the roots as was indicated by BCF_{root} being on average 1.5-fold higher than BCF_{shoot}. FLUM also concentrated more in the roots as compared to the shoots. Although the quantification results of the spinach need to be interpreted with caution due to limited spinach growth, the results do indicate that there are differences between the two crops with regards to their uptake and translocation pattern of antibiotics.

To date, no or only a few studies reported on the uptake of (one of) the selected antibiotics by radish and spinach from amended soils. As such, for DOX, one study reported that DOX was not detected in radish upon treatment with fortified, manure-amended soil in a closed pot experiment (Wang et al., 2016). This is in line with the results of the present study, although LOQs of DOX were higher compared to these of the other antibiotics. For TMP, one study reported on the uptake of TMP by lettuce from fortified soil, of which the treatment concentration was around 3-fold higher as the highest treatment concentration of the present study. However, the reported concentration in lettuce leaves was over 2-fold lower as compared to the findings in spinach shoots and was

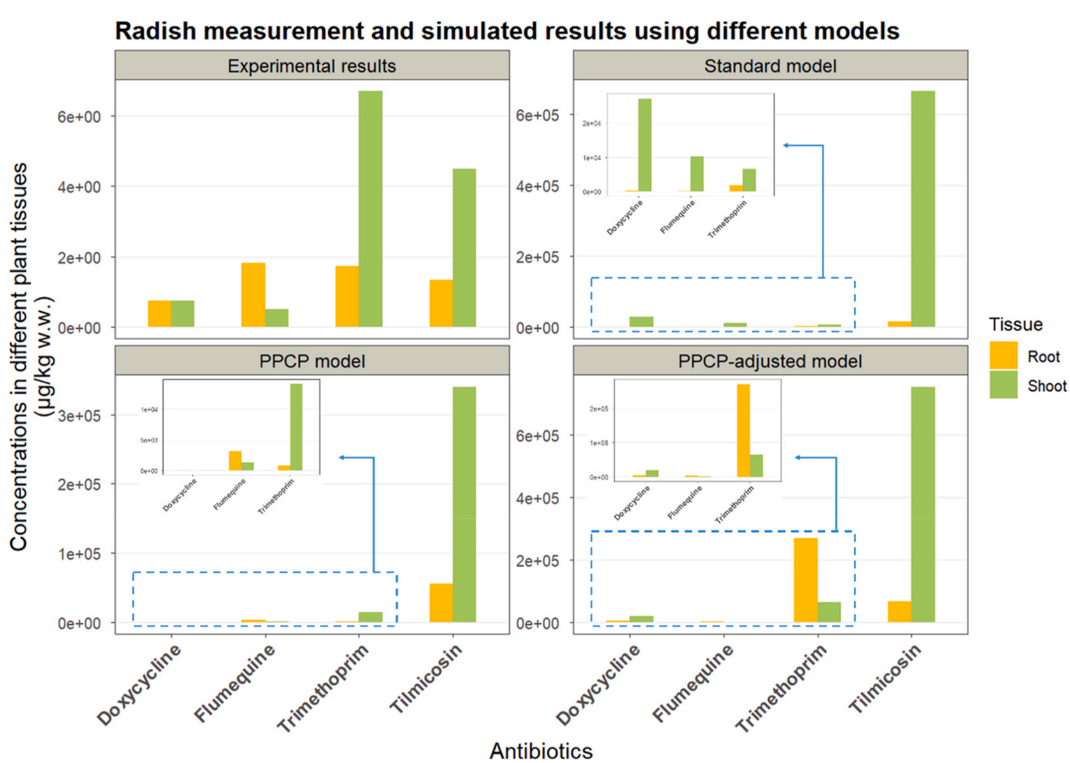

Fig. 3. Comparison of experimental and simulated concentration of antibiotics uptake in radish roots and shoots. Experimental results of doxycycline in plant tissues were below < LOQ and presented values are thus only indicative. Please note that the Y-axis of the experimental results differs from these of the model simulations.

in a similar range as the radish shoots concentrations in the present study (Boxall et al., 2006), suggesting an effect of differing experimental factors on the uptake rate such as the digestate influencing soil characteristics, as well as the different crop species tested. Another study reported on the uptake of TMP by lettuce in a hydroponic system and reported higher concentrations in roots as compared to shoots (around 5-fold difference) (Chuang et al., 2019), while this was similar in the spinach data of the present study. One study investigated the uptake of FLUM, a fluoroquinolone, by radish roots (45.3 ng/g d.w. with treatment concentration 0.5 ng/mL; 106.7 ng/g d.w. with treatment concentration 5 ng/mL), through watering with FLUM (5 ng/mL) (Zeng et al., 2022). Concentrations were higher in radish roots, compared to radish leaves. This is similar to the present study. Furthermore, the uptake of the antimicrobial class fluoroquinolones was recently reviewed by Chen et al. (2024), reporting that these antibiotics were taken up by a range of different crops (including lettuce, cucumber, and barley). However, no specific data on FLUM was described. Regarding TILM, a macrolide, no specific data on TILM uptake by plants was found to be reported in literature. Other studies reported only limitedly uptake of macrolides by plants (Pan & Chu, 2017a). For example, tylosin uptake was studied in lettuce and carrot, but was not detected in the plant material (Boxall et al., 2006).

In literature, several factors have been described to affect the uptake and translocation of antibiotics by plants from soil. For example, the uptake and translocation rate into leaves (shoots) has been reported to be influenced by the lipophilicity of the compound as well as the molecular weight (Pan & Chu, 2017a). This can again interact with plant physiology factors, such as the lipid content of roots, subsequently influencing the uptake of compounds as well (Christou et al., 2019). Additionally, cations and neutral compounds were reported to distribute similarly in roots and leaves, while anions often increasingly concentrate in roots (Chen et al., 2024; Dodgen et al., 2015). FLUM is anionic, and indeed higher levels were found in roots of both radish and spinach.

Upon the growing period, the antibiotics fortified to the soil were not fully recovered in the soil or the plants, indicating dissipation. This is in line with previous studies that reported on the degradation of antibiotics in soil (Berendsen et al., 2021; Gworek et al., 2021). In the present study, the amount of antibiotic dissipation was highest for DOX, followed by TMP, and then by TILM and FLUM. Indeed, an earlier study reported the lowest half-life in sandy soil for DOX, followed by TMP, TILM and FLUM (Berendsen et al., 2021). On the other hand, estimated half-lives in the present study did differ from reported half-lives in earlier studies (Berendsen et al., 2021; Cycoń et al., 2019; Dalkmann et al., 2014; Dong et al., 2023; Gworek et al., 2021) This could be due to many differing factors, for example but not limited to the experimental set-up, the soil and crop type that was used (Berendsen et al., 2021; Rietra et al., 2024; Wang et al., 2016), and the addition of digestate in the present study (Cycoń et al., 2019; Dalkmann et al., 2014; Gworek et al., 2021). Whether differences may be caused by for example the microbes present in the digestate warrants further investigation.

No specific maximum residue limits (MRLs) for the presence of antibiotic residues are available for vegetables in Europe. Therefore, the concentrations in radish roots and shoots and spinach shoots (the edible parts), were compared to regulation (EU) 37/2010, which describes MRLs for animal food products. The lowest available MRLs were selected for comparison, being 50 μg/kg for FLUM, TMP and TILM, and 100 μg/kg for DOX. The concentrations in the edible plant parts (i.e., the spinach shoots, radish roots, and radish shoots) detected in the present study, did not exceed these MRLs. It is however important to note that MRLs for vegetables, when being determined, are expected to differ from the available MRLs for animal food products, given differences in for example the amounts being consumed.

To better understand the impact of these antibiotic concentrations in plants when being cultivated on soil containing antibiotics, a full risk assessment would need to be employed, as the available European MRLs are not specified for vegetables. This was previously done for a range of antibiotics, and it was found that the daily consumption of antibiotic-contaminated crops resulted in a low to negligible human health risk (Geng et al., 2022; Pan & Chu, 2017a), although data on e.g., mixture health effects as well as chronic exposure remains to be further elucidated. Compared to radish and spinach, uptake by other crops can indeed be expected as well, although spinach and radish are being reported as crops with relatively high uptake rates of antibiotics compared to other crops such as tomatoes and eggplants (Christou et al., 2019). Besides the direct impact on food safety and human health, the impact on environmental health is important to assess as well (Patyra et al., 2023). Antibiotic resistance genes (ARGs) can be present in manure-based fertilizers and application can result in the direct introduction of ARGs in soil (Xie et al., 2018). Additionally, antibiotic residues in soil are potentially positively associated with ARGs (Huygens et al., 2022; Xie et al., 2018), although this warrants further investigation, just like assessing the impact of ARGs on food safety.

The antibiotics mass balances (i.e., amounts detected at the start compared to the end of the experiment) were incomplete, which potentially points towards the formation of degradation or transformation products. This has indeed been reported earlier for soil and plants (Geng et al., 2022; Klampfl, 2019; Nkoh et al., 2024). In further studies, it is important to evaluate the bioactivity and identity of these transformation products for further understanding of the potential impact on environmental and human safety (Berendsen et al., 2021).

In addition to the experimental approach, concentrations of the antibiotics in radish and spinach were simulated by application of multiple models that are based on different antibiotic plant uptake concepts and theories. For both spinach and radish, the estimated concentrations in shoots and roots were substantially higher, as compared to the experimentally obtained results. Overall, incorporating compound ionization into the model improved the alignment of the predicted uptake patterns of antibiotics with the obtained experimental results. However, the models did not consider other processes such as compound metabolism in plants during the growth period and the influence of the rhizosphere on uptake processes (Dong et al., 2023; Grilla et al., 2019; McCorquodale-Bauer et al., 2023). In-plant metabolism of substances can differ substantially between plant species (Kodešová et al., 2019) and between substances (Tian et al., 2019) and may thus be relevant to consider. Additionally, the models do not consider the degradation and transformation of the antibiotics in the soil itself. The lack of the inclusion of these processes in the models may also partially explain the overestimated simulation. Besides, the models applied in the present study were originally developed to simulate uptake in field conditions, which does not correspond to the pot experimental set-up of the present study, and for maize, having different characteristics compared to radish and spinach. Adjustments were made to these models; however, based on the results this was deemed insufficient to result in comparable values as the experimental conditions. The performed sensitivity analysis indicated that the implemented models require further verification for their applicability to radish and spinach, under plant pot experimental conditions, especially when it comes to plant-related parameters such as plant (tissue) biomass. The PPCP models were derived under the condition that the mass flow of antibiotics reached a steady-state situation between different compartments (e.g., soil pore water, root cytosol, root vacuole, plant xylem, phloem, and leaves). Given the morphological differences between maize, radish, and spinach, this probably shows the need for specific adjusted models per different crop types. Additionally, the original paper describing the models considered a constant plant growth rate, which may not be realistic for most plants as the logistic growth model describes a more realistic growth situation (Brunetti et al., 2019). Overall, the prediction models applied in this study can provide useful input in the order of plant uptake of antibiotics present in the soil but remain to be further optimized for accurate, quantitative predictions of concentrations in the studied plant tissues. Nevertheless, plant uptake patterns of the four different antibiotics were similar for the model estimations and the experimental obtained results.

This study provides valuable insights and data into the uptake of four different antibiotics comprising different classes of antibiotics by two edible crops. The fact that the experiments were performed simultaneously and under the same (plant growth) conditions, enables comparability of the results amongst the different antibiotics. However, the yield of spinach was insufficient, in contrast to the radish plants. For future studies, it is recommended to control for similar plant growth and yield to enhance comparability of the results. Although identifying the cause of the low spinach yield was beyond the scope of this study, suboptimal growing and sprouting conditions, such as limited nutrient availability, in combination with high temperatures may have played a role. Radish appeared to be more resilient under these conditions in this study. Fortified concentrations were realistic as these were based on findings in animal manure. Also, the fact that digestate was used to amend the soil contributes to a more realistic scenario, as digestate can also be applied directly as fertilizer on agricultural soils. In future studies, the nutrient content of the digestate in combination with the soil used should be further tested and optimized for optimal plant growth. It is important to mention that the closed pot system applied in the present study is not directly comparable to a field setting, since leaching of the (contaminated) ground water could not fully occur in the here-applied experimental conditions. To gain more mechanistic understanding and evaluation of plant uptake in future studies, assessment of the bioavailability of the antibiotics in the soils and their presence in the soil pore water could be investigated. To gain more insight in the formation of possible bioactive transformation products, future studies can proceed in the evaluation and identification of possible transformation products to further understand effects on environmental and human health.

To conclude, radish and spinach took up the antibiotics TMP, FLUM, and TILM, from fortified, digestate-amended soils to a different extent, dependent on the crop, antibiotic, and plant tissue type. DOX was below LOQ in plant tissues. Plant uptake of the antibiotics seemed to be relatively low. Model simulated uptake patterns differed with orders of magnitude from the experimentally obtained data, but uptake patterns were comparable. Overall, this study contributed to understanding the transfer of antibiotic residues in a circular food production system.

CRediT authorship contribution statement

Katja C.W. van Dongen: Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Weixin Huang:** Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Esmer Jongedijk:** Writing – review & editing, Investigation, Conceptualization. **Bram van de Kooi:** Writing – review & editing, Investigation, Conceptualization. **Erik de Lange:** Writing – original draft, Validation, Investigation, Conceptualization. **H.J. van der Fels-Klerx:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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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 A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.126915.

Data availability

Data will be made available on request.

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