Science of the Total Environment 605–606 (2017) 322–331

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Uptake of the veterinary antibiotics chlortetracycline, enrofloxacin, and sulphathiazole from soil by radish

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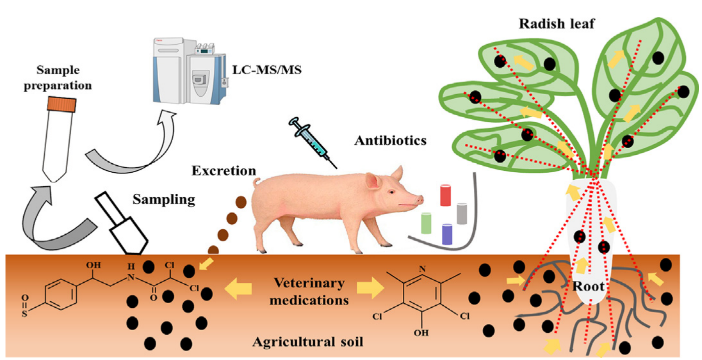
HIGHLIGHTS

- A method to estimate the half-life of veterinary antibiotics in soil was developed.
- Veterinary antibiotic uptake rate by radish from contaminated soil was determined.
- The half-lives of antibiotics in soil with and without radishes did not differ.
- Trace antibiotic residue levels moved to roots and leaves from contaminated soil.
- We elucidate residual antibiotic transplantation and antibiotic tolerance in soil.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 May 2017
Received in revised form 26 June 2017
Accepted 26 June 2017
Available online xxxx

Editor: Jay Gan

Keywords:
Veterinary antibiotics
Chlortetracycline, Enrofloxacin, Sulphathiazole,
Half-life
Uptake rate
Movement

ABSTRACT

Veterinary antibiotics are available for uptake by the plants through sources such as manure, irrigation, and atmospheric interaction. The present study was conducted to estimate the half-lives of three veterinary antibiotics, chlortetracycline (CTC), enrofloxacin (ENR), and sulphathiazole (STZ), in soil and experimentally explore their uptake from contaminated soil to radish roots and leaves. Samples were extracted using a modified citrate-buffered version of the quick, easy, cheap, effective, rugged, and safe "QuEChERS" method followed by liquid chromatography coupled with tandem mass spectrometric analysis (LC–MS/MS) in the positive ion mode. Good linearity was observed for the three tested antibiotics in soil and plants (roots and leaves) with high coefficients of determination ($R^2 \geq 0.9922$). The average recovery rates at two spiking levels with three replicates per level ranged between 77.1 and 114.8%, with a relative standard deviation (RSD) ≤ 19.9% for all tested drugs. In a batch incubation experiment (*in vitro* study), the half-lives of CTC, ENR, and STZ ranged from 2.0–6.1, 2.2–4.5, and 1.1–2.2 days, respectively. Under greenhouse conditions, the half-lives of the three target antibiotics in soil with and without radishes were 2.5–6.9 and 2.7–7.4; 4.7–16.7 and 10.3–14.6; and 4.4–4.9 and 2.5–2.8 days,

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http://dx.doi.org/10.1016/j.scitotenv.2017.06.231

Environment
QuEChERS
LC–MS/MS

respectively. Trace amounts of the target antibiotics (CTC, ENR, and STZ) were taken up from soil *via* roots and entered the leaves of radishes. The concentration of CTC was lower than 2.73%, ENR was 0.08–3.90%, and <1.64% STZ was uptaken. In conclusion, the concentrations of the tested antibiotics decreased with time and consequently lower residues were observed in the radishes. The rapid degradation of the tested antibiotics in the present study might have only little impact on soil microorganisms, fauna, and plants.

1. Introduction

In livestock farming, antibiotics are widely used for treatment and prevention of infectious diseases and are also administered at sub-therapeutic levels to increase the feed efficiency and growth rate in food-producing animals (Schwarz et al., 2001; Wassenaar, 2005). After being metabolised, veterinary antibiotics are normally excreted through urine and faeces. Hence, excreta from treated animals may contain high concentrations of either the parent compound or its metabolites (Arikan et al., 2009; Broekaert et al., 2011). The livestock faeces containing antibiotic residues are often used in the preparation of farming materials, such as solid and liquid fertilisers. These contaminated fertilisers are used in the agricultural lands to increase crop productivity. In addition, faeces containing residual antibiotics may directly enter rivers with run-off rainfall, and this water may be used in agricultural irrigation and thus introduced to the crops (Jiemba, 2002; Sarmah et al., 2006; Kim et al., 2011). Therefore, there is a considerable possibility for crops to take up antibiotics from contaminated soil and water, which may later be introduced to humans through the food chain (Broekaert et al., 2012). It is therefore of vital importance to know the half-lives of these antibiotics in the environment and their uptake rate by plants, the route by which these antibiotics enter the human food chain.

Veterinary antibiotics that are frequently used in livestock are tetracyclines (TCs), sulphonamides (SAs), and fluoroquinolones (FQs), which are resistant to gram-positive and gram-negative bacteria (Bauditz, 1990; Oka et al., 2000; Baran et al., 2011). In particular, penicillins (PCs) and tetracycline (TC) antibiotics administered to livestock were reported to have excretion rates *in vitro* of 30–60% and 70–80%, respectively (Anadon and Martınez-Larranaga, 1999; Wassenaar, 2005; Seo et al., 2007; Broekaert et al., 2011; Kim et al., 2011; Kwon et al., 2011; Kim et al., 2012). When crops are cultivated in contaminated soils containing high concentrations of veterinary antibiotics, they can take up antibiotics from the soil, which may ultimately affect crop growth (Dolliver et al., 2007; Broekaert et al., 2012; Azanu et al., 2016). For instance, antibiotic residues were detected at high levels in the roots and leaves of cucumbers and tomatoes grown in antibiotic contaminated soil, and the antibiotics negatively affect their growth (Ahmed et al., 2015). Antibiotics may accelerate the development of resistant bacteria, which may pose as a threat for human and livestock health (Ferguson et al., 2005; Son et al., 2008; Ding and He, 2010; Kim et al., 2010; Lau and Lennon, 2011; Liu et al., 2011).

Many factors could be responsible for the trace amounts of antibiotic residues in plants. Metabolism, soil characteristics, plant type, and the antibiotic's octanol water partition co-efficient (Log *K*ow) may influence antibiotic uptake by plants from soil (Burken and Schnoor, 1998; Boxall et al., 2006; Dolliver et al., 2007; Seo et al., 2010). Antibiotics may degrade and form metabolites, which can be less, similarly, or more harmful than the parent compounds (Roberts and Hutson, 1999; Seo et al., 2010). Therefore, transformation products should be taken into account for total residue analysis in plants. However, unlike for pesticides, regulation criteria are not present for monitoring the levels of antibiotics in crops, and it is difficult to quantify their hazardous nature. Soil acidity and soil organic matter may influence the binding of antibiotics with soil and make them less available for plant uptake (Jiemba, 2002; Kang et al., 2013). Plant root uptake of compounds is usually affected by the log Kow of the compound (Burken and Schnoor, 1998). A compound with a low log Kow has greater polarity, and therefore is easier to take up by the roots because of higher water miscibility. Conversely, higher log Kow values are indicative of the lipophilicity of the compound, which makes them less soluble in water and therefore not easily taken up by plant roots.

The regulatory authorities of several countries are attempting to control the use of antibiotics in livestock. In this context, the Korean Rural Development Authorities (RDA) prohibited the addition of all antibiotics, with the exception of nine, to livestock feed in 2011 to reduce the risk of contaminated livestock products and high antibiotic resistance rates. Tetracyclines (chlortetracycline), tetracycline, sulphonamides (sulphathiazole and sulphamethoxazole), and fluoroquinolones (enrofloxacin) are three classes of veterinary antibiotics that are frequently used to combat animal diseases. Because contaminated faeces are applied to agricultural fields, it is important to

Table 1
Recovery rate of CTC, ENR, and STZ in radishes (root and leaf) using different methods.

No.	Veterinary antibiotics	Recovery (mean ± RSD)%, n = 3											
		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9	Trial 10	Trial 11	Trial 12
		M1[a] + PSA[e]	M1 + C18[f]	M1 + GCB[g]	M2[b] + PSA	M2 + C18	M2 + GCB	M3[c] + PSA	M3 + C18	M3 + GCB	M4[d] + PSA	M4 + C18	M4 + GCB
1	CTC	7.2 ± 11.6	N.D[h]	N.D	N.D	2.3 ± 55.8	N.D	64.9 ± 16.7	66.6 ± 18.1	42.9 ± 10.5	20.5 ± 18.4	31.5 ± 12.7	19.4 ± 19.6
2	ENR	93.1 ± 29.4	92.1 ± 8.8	12.2 ± 35.1	33.1 ± 1.9	46.5 ± 33.8	14.2 ± 14.9	96.3 ± 5.1	79.4 ± 12.8	18.5 ± 7.9	141.8 ± 3.5	97.1 ± 12.4	32.8 ± 46.0
3	STZ	60.2 ± 8.1	62.8 ± 11.4	52.7 ± 3.1	50.1 ± 0.4	43.3 ± 2.2	49.2 ± 2.4	74.1 ± 4.2	70.0 ± 4.5	64.3 ± 3.1	63.8 ± 1.9	58.6 ± 4.5	55.0 ± 3.1

[a] M1, HAc(0.2 mL) + NaOAc(1 g).
[b] M2, HAc(0.2 mL) + NaOAc(1 g) + $MgSO_4$(4 g).
[c] M3, HAc(0.2 mL) + $Na_3Cit.2H_2O$(1 g) + $Na_2Cit.5H_2O$(0.5 g).
[d] M4, HAc(0.2 mL) + $Na_3Cit.2H_2O$(1 g) + $Na_2Cit.5H_2O$(0.5 g) + $MgSO_4$(4 g).
[e] PSA, Primary secondary amine (30 mg).
[f] C_{18}, C_{18} Endcapped QuEChERS Bulk Sorbent (30 mg).
[g] GCB, Graphite carbon black (30 mg).
[h] N.D, Not detected.