Doxycycline adsorption and toxicity in earthworms after manure application to soil

Bjorn Berendsen[1], Iris Schaap[2], Nico van den Brink[3], Marjolein Derks[2], Marta Baccaro[3]

Introduction

Chemical contaminants can end up in agricultural soils after manure application. The use of persistent chemicals is unwanted as they can accumulate and impact soil health, biodiversity and food and feed safety (figure 1). For many chemicals, the ecotoxicological effects and food and feed safety hazards are largely unknown.

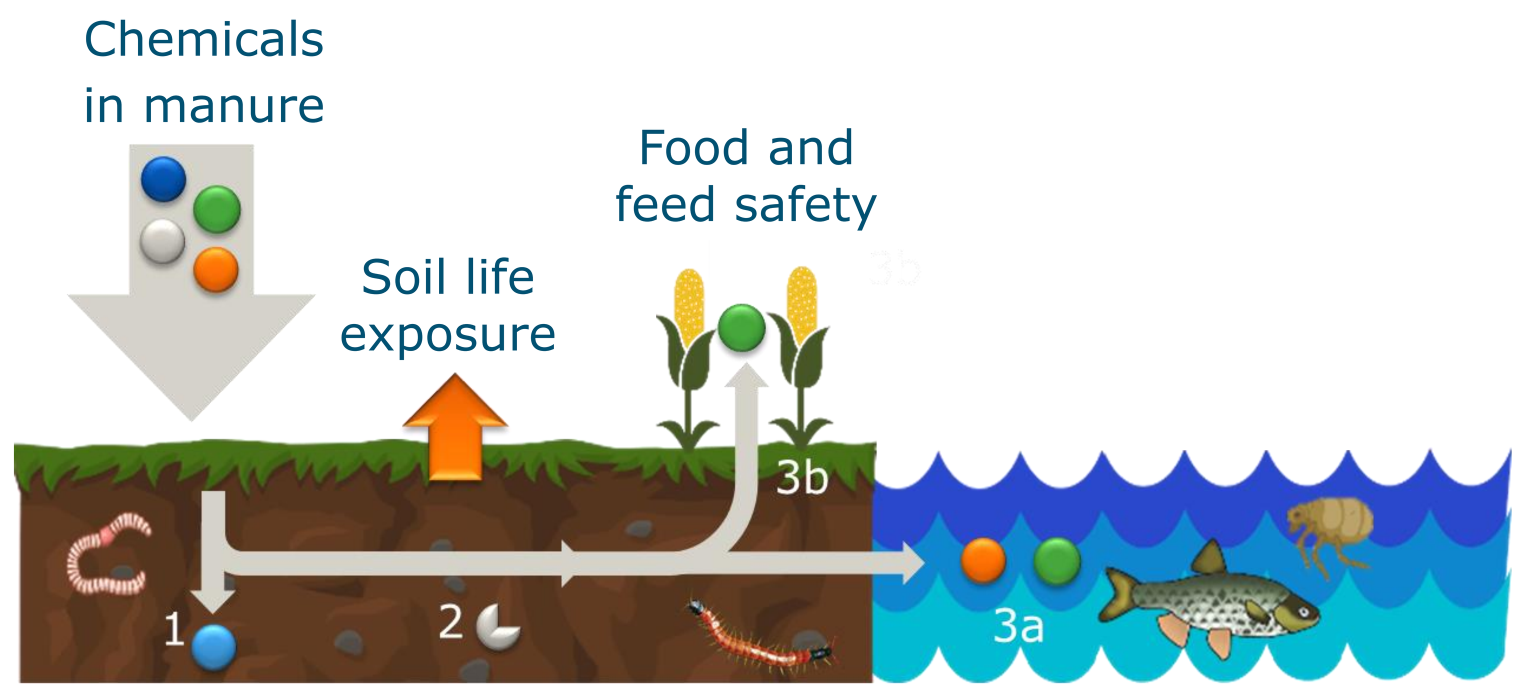

Figure 1. Exposure routes of chemicals after manure application.

To understand the ecotoxicological and food safety hazards of chemical residues, we aim to develop an in-silico tool to assess the potential accumulation and effects of chemicals for the bioindicator species earthworm. This will consist of (1) the development of a conceptual dynamic energy budget toxicology earthworm model and (2) the completion of experimental studies for model training and verification. We present a first pilot experiment using one of the most frequently applied antibiotics in animal husbandry: doxycycline (DOX).

Experimental

DOX was added to soil at seven concentrations ranging from 0 to 500 mg/kg (n=3). For concentration 0.5 and 5 mg/kg, additional jars were prepared (figure 2). To each jar, 7 adult earthworms (*Eisenia fetida*) were added. The jars were kept at 20 °C. At different days soil and earthworms were sampled. Earthworms were washed and allowed to purge their gut before analysis. At day 28, mortality and weight change in earthworms was recorded. DOX concentrations in soil and earthworms were determined by LC-MS/MS.

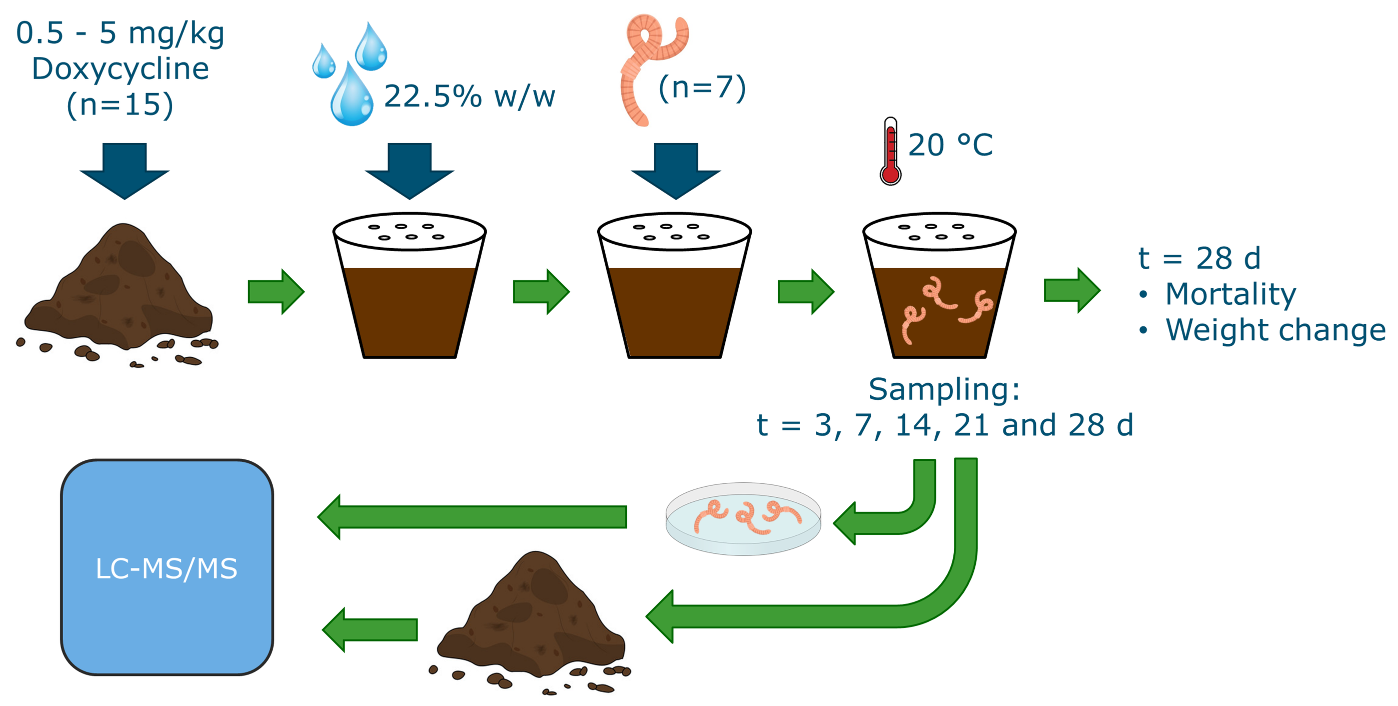

Figure 2. Representation of experimental set-up for soil concentrations 0.5 and 5 mg/kg.

The internal concentrations in *E. fetida* were fitted with a one compartment toxicokinetic model by the following equation:

$$c_{int} = k_1/k_2 * c_{exp} * (1-e^{-k_2 * t})$$

c_{int} = internal DOX concentration in earthworms, c_{exp} = measured DOX concentration in soil, k_1 = uptake rate constant, k_2 = elimination rate constant and t = time (d)

Results

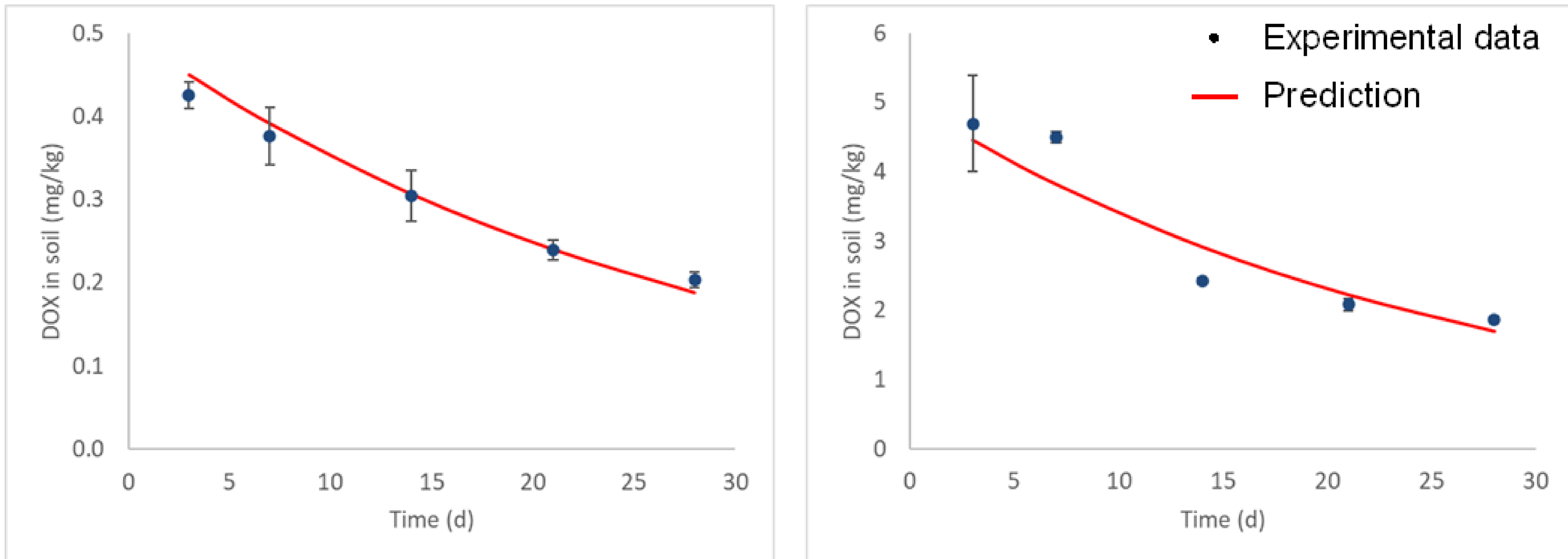

Figure 3. Degradation of DC in soil over time. The exposure concentration is 0.5 mg/kg (left panel) and 5 mg/kg (right panel). n=3, average and standard deviation.

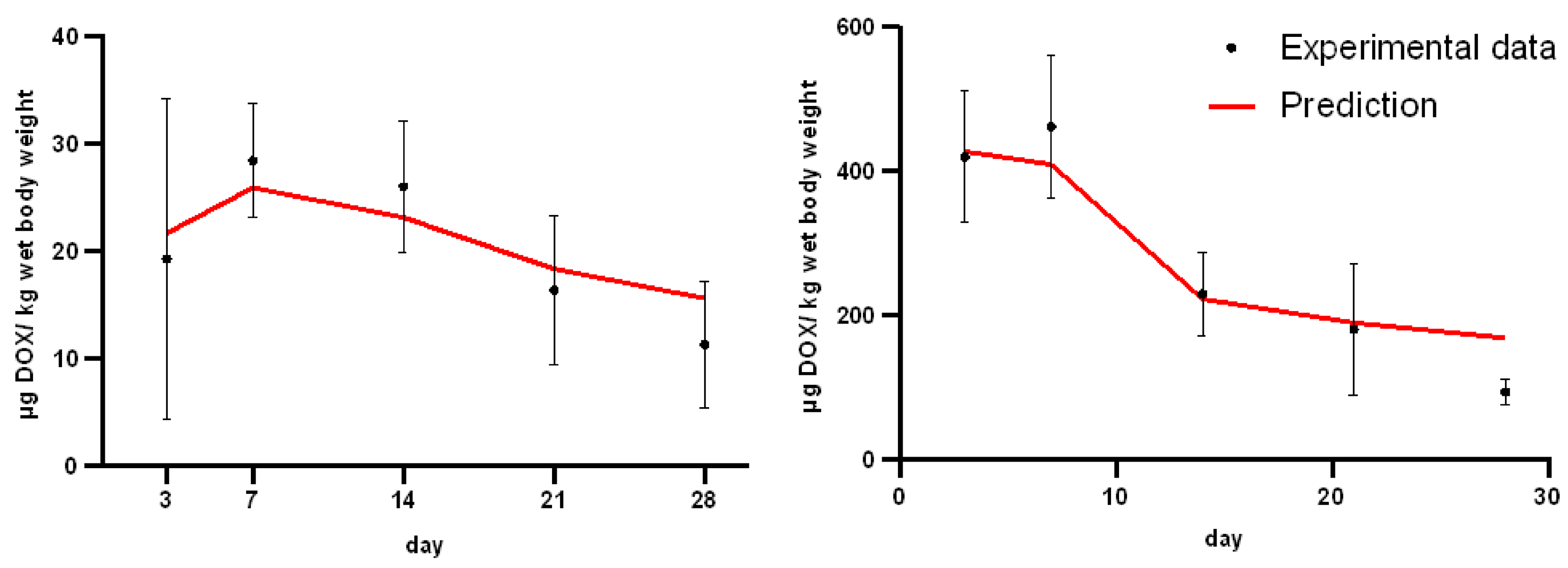

Figure 4. Kinetic of DOX accumulation in earthworms exposed to 0.5 mg/kg soil (left panel) and 5 mg/kg (right panel). n=3, average and standard deviation.

The estimated uptake and elimination kinetic rate constants are reported in table 1.

Table 1. Uptake and elimination kinetic constant rates estimated for each exposure concentration separately and for the joined concentrations.

	k_1	k_2
0.5 µg DOX kg^{-1} soil	0.025	0.333
5 µg DOX kg^{-1} soil	1.724	18.927

- Mortality and growth of earthworm were not affected at the tested concentrations.
- Degradation of DOX in the soil was determined (figure 3): the DT50 was estimated at 18 and 20 days for 0.5 and 5 mg/kg soil concentrations, respectively.
- Earthworms contained higher concentrations of DOX when exposed to 5 mg/kg compared to the 0.5 mg/kg treatment and eliminated residues at a higher rate (figure 4).

Conclusions

- The concentrations in the earthworms decreased along with the degradation of the compound in the soil. This showed that the earthworms successfully eliminated DOX either by excretion or biotransformation.
- Even at the tested unrealistically high DOX concentrations, the earthworms remain unaffected.

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Wageningen University & Research
P.O. Box 123, 6700 AB Wageningen
Contact: bjorn.Berendsen@wur.nl
T + 31 (0)317 14 32 30 29
www.wur.nl/wfsr

[1] Wageningen Food Safety Research
[2] Wageningen University, Farm Technology
[3] Wageningen University, Toxicology