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# Effect of different oxytetracycline addition methods on its degradation behavior in soil

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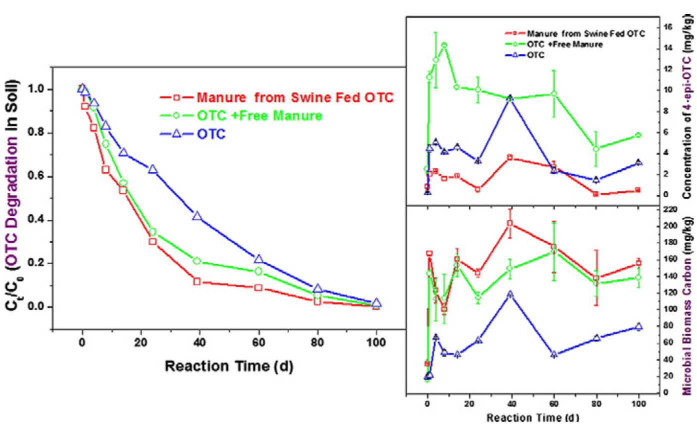
## HIGHLIGHTS

- We compare the different oxytetracycline (OTC) addition method on its degradation behavior in soil.
- OTC degradation half-lives show the following differences: manure from swine fed OTC < antibiotic-free manure + OTC < OTC.
- Differences could be caused by distinct chemical reaction equilibria.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The degradation behavior of veterinary antibiotics in soil is commonly studied using the following methods of adding antibiotics to the soil: (i) adding manure collected from animals fed with a diet containing antibiotics, (ii) adding antibiotic-free animal manure spiked with antibiotics and (iii) directly adding antibiotics. No research simultaneously comparing different antibiotic addition methods was found. Oxytetracycline (OTC) was used as a model antibiotic to compare the effect of the three commonly used antibiotic addition methods on OTC degradation behavior in soil. The three treatment methods have similar trends, though OTC degradation half-lives show the following significant differences ($P < 0.05$): manure from swine fed OTC (treatment A) < antibiotic-free manure + OTC (treatment B) < OTC (treatment C). Differences could be caused by distinct chemical reaction equilibria due to dissimilar concentrations of 4-epi-OTC and α-apo-OTC. The pH could also have affected the concentration of 4-epi-OTC and α-apo-OTC, thus influencing OTC degradation. The treatments presenting manure (A and B) significantly enhanced EC, enzyme activity, microbial biomass carbon and nitrogen when compared to the treatment without manure (C), thus increasing degradation of OTC in the soil. Because the main entry route for veterinary antibiotics into soil is via the manure of animals given with antibiotics, the most appropriate method to study the degradation and ecotoxicity of antibiotic residues in soil may be to use manure from animals that are given a particular antibiotic, rather than by adding it directly to the soil.



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## 1. Introduction

Veterinary antibiotics (VAs) are widely used in animal husbandry to promote animal growth and control disease. However, 40 to 90% of the administered VAs are excreted to the environment in the form of parent compounds and/or their metabolites (Kemper, 2008; Kumar et al., 2005; Phillips et al., 2004; Winckler and Grafe, 2001).

The ecotoxicological effects of broad-spectrum VAs have generated considerable concerns and research. The three most common methods by which VAs are added to the soil to study their degradation are (i) adding manure collected from animals fed with a diet containing VAs, (ii) supplementing antibiotic-free manure with VAs before adding it to the soil and (iii) directly adding VAs to the soil. The primary entry route of VAs into soil is via animal excreta, which contains not only parent materials but also their metabolites. Therefore, the degradation of VA residue in soil is complex because of the presence of VA metabolites and the potential interactions among parent compounds, metabolites and the physicochemical components of the animal manure. However, most studies have only used methods (ii) and (iii) in their research (Braschi et al., 2013; Lin and Gan, 2011; Srinivasan et al., 2014). Similarly, the physical and chemical characteristics of the soil, for example soil pH, can influence antibiotic degradation. Antibiotics are more stable under acidic conditions (pH 3.0), and degradation is relatively faster under alkaline conditions (Chrysi and Bruce, 2002); furthermore, antibiotic degradation can also change the pH value of the soil.

Information is scarce on whether the addition method affects degradation behavior of VAs in soil, which is the focus of this study. Oxytetracycline (OTC) is a broad-spectrum VA in the tetracycline (TC) family that inhibits many gram-positive and gram-negative bacteria, including *Spirochaeta*, *Rickettsia*, *Mycoplasma*, *Chlamydia* and *Protozoa*, and has been widely detected in farm animal manure, water and soil (Bao et al., 2009; De liguoro et al., 2003; Qiao et al., 2012; Zhao et al., 2010). OTC was used as a model antibiotic to examine the effect of three VA addition methods on the degradation behavior of OTC in soil by determining the changes in concentration of OTC and its metabolites. Additionally, the chemical and biological properties of the soil were monitored to explain the observed changes because these properties can affect antibiotic degradation.

## 2. Materials and methods

### 2.1. Collection of swine manure

Thirty growing-swine of approximately 30 kg that were fed an antibiotic-free diet for 28 days were used to collect the manure for this study. The swine were equally divided (n = 15) into the following two groups: the control (C) group, which continued to receive an antibiotic-free diet, and another group that was fed with a diet containing 200 g OTC/ton feed; these diets were given for 7 days. On each of these 7 days, approximately 2 kg of the manure produced by the swine in each group was sampled and stored at −20 °C for subsequent use. Daily samples from each group were analyzed for OTC concentration, and those with intermediate levels of OTC were selected for the study. The chemical properties of the selected manure samples are presented in Table 1.

### 2.2. Collection of soil

The soil was collected within 24 cm of the top-soil in a vegetable field in Guangzhou, south China, which had not been fertilized with organic manure for 6 months prior to collection. These samples were later confirmed to be free of OTC by LC–MS analysis (Agilent 1200 and Agilent G6410B, USA). Part of the air-dried soil was sieved (2 mm) for the culture experiment, whereas the remaining soil was more finely sieved (1 mm) to determine the physical and chemical properties of the soil samples (maximum field moisture content capacity = 74.09%, total carbon = 9.21 g/kg, total nitrogen = 0.86 g/kg, electrical conductivity = 160 μs/cm and pH = 7.30).

### 2.3. Experimental design

The experiment was a completely randomized design (CRD) consisting of three groups of OTC addition methods (Table S1). The 3 addition methods were (i) addition of manure collected from swine fed with diets containing OTC (A), (ii) addition of antibiotic-free manure spiked with OTC (B) and (iii) direct addition of OTC to antibiotic-free soil (C). The initial concentration of OTC was 101.54 mg/kg. Treatment A also contained 1.09 mg/kg 4-epi-OTC and 2.47 mg/kg α-apo-OTC.

Each treatment was replicated 3 times. A total of 2.5 kg of soil was placed in a 3 L open beaker, and deionized water was added to adjust the moisture content of the soil to 40% of the maximum field capacity. The beaker was then placed in a biochemical incubator at 23.2 ± 1 °C with intermittent light (12 h light and 12 h dark) for a week to stabilize fermentation before the test. On the first day of the test period, depending on the designated treatment, a certain concentration of OTC and/or 0.5 kg of swine manure were added to the reactor, along with deionized water, to achieve a moisture content of 50% of the maximum field capacity of the soil; the soil was thereafter adjusted every 2 days to maintain this moisture content. Soil samples (100 g) were taken from each replicate on days 0, 1, 4, 8, 14, 24, 39, 60, 80 and 100 to determine the OTC, 4-epi-OTC, and α-apo-OTC concentrations; pH; electrical conductivity (EC); total nitrogen; total carbon; catalase, urease and alkaline phosphatase activities; microbial biomass carbon; and microbial biomass nitrogen.

### 2.4. Detection of OTC and its metabolites

A total of 10 g of the soil samples were extracted three times and mixed with 2.5 g citric acid, 1.1 g oxalic acid, and 15 mL methanol: water (90:10, V/V). The samples were placed in a 50 mL polypropylene centrifuge tube and shaken for 30 min at 300 rpm, followed by centrifugation at 12,000 rpm for 10 min at 20 °C. The supernatants from the above process were pooled in a 50 mL flask and diluted to 50 mL with ultra-pure water. Then, 1 mL of the diluted supernatant was taken from the flask and transferred to a 1.5 mL centrifuge tube and centrifuged at 14,000 rpm for 10 min. The supernatant was filtered through a 0.22 μm organic membrane before determining OTC and its metabolites by LC–MS (Agilent 1200–Agilent G6410B, USA) with an Agilent Zorbax SB-C18 chromatographic column (250 × 4.6 mm, 5 μm) at 35 °C. The injection volume was 5 μL. The mobile phase consisted of a methanol–water mixture (20:80 V/V for A and 95:5 V/V for B); 3% formic acid was added to the mobile phase for A and B. The flow rate

**Table 1**
Chemical properties of the manure from swine fed diets with and without OTC.

| Group | OTC (mg/kg) | 4-epi-OTC (mg/kg soil) | α-apo-OTC (mg/kg soil) | Total carbon (mg/kg) | Total nitrogen (mg/kg) | EC (μs/cm) | pH |
|---|---|---|---|---|---|---|---|
| OTC free manure | 0 | 0 | 0 | 698.21 | 36.10 | 2666.67 | 5.56 |
| Manure from swine fed OTC | 1829.94 | 1.09 | 2.47 | 716.35 | 37.17 | 2333.33 | 5.78 |