



## Occurrence of 13 veterinary drugs in animal manure-amended soils in Eastern China



Ruicheng Wei<sup>a,b,1</sup>, Feng Ge<sup>c,2</sup>, Lili Zhang<sup>b,1</sup>, Xiang Hou<sup>b,1</sup>, Yinan Cao<sup>a,3</sup>, Lan Gong<sup>b,1</sup>, Ming Chen<sup>b,1</sup>, Ran Wang<sup>b,1</sup>, Endong Bao<sup>a,\*</sup>

<sup>a</sup> College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, PR China

<sup>b</sup> Key Lab of Agro-product Safety Risk Evaluation (Nanjing), Ministry of Agriculture, Nanjing 210014, PR China

<sup>c</sup> Nanjing Institute of Environmental Science, Ministry of Environmental Protection, Nanjing 210042, PR China

### HIGHLIGHTS

- Occurrence of veterinary compound residues were investigated in the study area.
- More than 80% of the sampled soils were contaminated by the selected compounds.
- The animal wastes were one of pollution sources of veterinary drugs in soil.
- The occurrence of selected veterinary drugs in soils was related to animal species.

### ARTICLE INFO

#### Article history:

Received 5 June 2015

Received in revised form 28 October 2015

Accepted 30 October 2015

Available online 22 November 2015

Handling editor: Klaus Kümmerer

#### Keywords:

Occurrence  
Veterinary drug  
Manure  
Soil  
HPLC–MS/MS

### ABSTRACT

The occurrence of 13 veterinary drugs were studied in soil fertilized with animal manures in Eastern China. The 69 soil samples were obtained from twenty-three vegetable fields in 2009 and analysed for selected veterinary drugs by HPLC–MS/MS at soil depths of 0–20, 20–40 and 40–60 cm, and two additional samples were re-analysed from an earlier study from November 2011. Results showed that animal wastes, especially those from poultry farms, were one of pollution sources of veterinary drugs in soil. The detection frequency of veterinary drugs in soil was 83%, 91% and 87% in the three soil depths, respectively. The detection rates for the five classes of drugs in soils followed the rank order cyromazine > tetracyclines > sulfonamides > fluoroquinolones > florfenicol. Veterinary drugs were detected in soil layers at 20–40 and 40–60 cm depth to a greater extent than at 0–20 cm depth. The results of the same point in years 2009 and 2011 indicated that veterinary drugs accumulate easily and persist in the deeper soil. In addition, residue levels of veterinary drugs in soil were related to the animal species the manure was derived from. Overall, the predominance of tetracyclines in sampled soils underscored the need to regulate their veterinary use in order to improve the management and treatment of associated releases.

© 2015 Elsevier Ltd. All rights reserved.

### 1. Introduction

Veterinary antimicrobial drugs are biologically active substances designed to kill microbes or inhibit their growth. In modern animal agriculture, most veterinary drugs fed to animals are poorly absorbed in the animal gut and up to 90% of them may be excreted either as a parent compound or in a partially metabolized form (Tolls, 2011). Veterinary drugs have the potential to enter the environment through animal manure fertilizers and can persist in soils for a long time (Förster et al., 2009). An increasing number

of reports show a rise in the occurrence and distribution of veterinary drugs in excrement and environment in various countries throughout the world (Manzetti and Ghisi, 2014). Moreover, veterinary drugs may be taken up by crop plants from manure-amended soils and can be transported to ditches, streams and rivers via runoff and drain flow to groundwater via leaching (Boonsaner and Hawker, 2015; Blackwell et al., 2009).

With the increase in use of veterinary antibacterial drugs in animal production, there is a growing concern that excreted drugs have potentials to encourage the selection of antimicrobial resistance and spread in the environment (Heuer et al., 2011). The applications of veterinary antibacterials as growth promoters in animal feeds are now strictly regulated in various countries and even totally prohibited in the European Union since 2006 and in the United States (Alban et al., 2008; Food and Drug Administration, 2003). But this practice still continues in other countries including

\* Corresponding author. Tel: +86 25 84395316

E-mail address: [endongbao43@126.com](mailto:endongbao43@126.com) (E. Bao).

<sup>1</sup> +86 25 84391617

<sup>2</sup> +86 25 85287298

<sup>3</sup> +86 25 84395316

China which is one of the world's largest producers and exporters of animal products. Large quantities of manure containing various antibacterials are produced and stored in manure pits for subsequent application or applied immediately to land (Boxall, 2008). However, only a few studies on occurrence and fate of veterinary drugs in manure-amended soil have been done in China (Hu et al., 2010; Ostermann et al., 2013; Li et al., 2014). Therefore, limited information is available on the presence and distribution of veterinary drugs in the various layers of manure-amended soil.

The present study was designed to investigate the occurrence of tetracyclines (TCs), sulfonamides (SAs), fluoroquinolones (FQs), cyromazine (CY) and florfenicol (FF) at three soil depths of 0–20, 20–40 and 40–60 cm collected from vegetable fields that used animal manure as fertilizer. To our knowledge, veterinary drugs as a source of environmental pollution have been largely ignored in China. This work embodies the first widespread investigation on the occurrence of important classes of veterinary compounds in the soil, especially in deeper soil in Eastern China. The results could provide useful information for assessing the impacts or potential risks of veterinary drugs to ecosystems and for proper handling of antibiotic-containing manure.

## 2. Materials and methods

### 2.1. Chemicals

Sulfadiazine (SDZ), sulfamethoxazole (SMX), sulfamethazine (SMZ), sulfaquinolaxine (SQX), sulfadoxine (SDX), chlortetracycline (CTC), oxytetracycline (OTC), tetracycline (TC), doxycycline (DOX), florfenicol (FF), enrofloxacin (ERFX), ciprofloxacin (CPFX) and cyromazine (CY) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Formic acid, methanol and acetonitrile (HPLC/MS-grade) were purchased from ROE Scientific INC (Newark, USA). Ultrapure water was prepared with a Milli-Q water purification system (Millipore, USA). Unless otherwise indicated, chemicals used were purer than the analytical grade. All the drugs were dissolved to create the 100 mg L<sup>-1</sup> stock solution in methanol with the exception for fluoroquinolone drugs, which were prepared in methanol containing 0.5% 1 M NaOH, from which a 7-point calibration curve was created. The stock solutions were stored at -20 °C and were stable for at least two months except for TCs and FQs. The working solutions were prepared freshly on the day of use.

### 2.2. Study site and sample collection

The study domain was located in Jiangsu Province, which is one of the most developed economic areas in China, and large-scale livestock and poultry production was initiated in this area earlier than other regions of the country. The study region covers an area of about 102 600 km<sup>2</sup>, and the plain area is 70 600 km<sup>2</sup>. Situated in a transition belt from a subtropical to temperate zone, it has a typical monsoon climate with moderate amounts of rainfall. The average organic matter content, pH and cation exchange capacity of soil in this region were 21 g kg<sup>-1</sup>, 7.2 and 13 cmol kg<sup>-1</sup>, with coefficients of variation of 35%, 17% and 41%, respectively.

The soils were collected from twenty-three sites (designated as S1 to S23 in Fig. 1) located in vegetable fields. All fields had a history of manure application from large-scale livestock and poultry operations. Soil samples were taken from 5 locations at each site as the S type for the different depths (0–20, 20–40 and 40–60 cm) and the soil samples from each depth were mixed. The samples were transported under cooled conditions to the laboratory and lyophilized. The ≤2 mm fraction was separated and stored in the dark at -20 °C before further treatment and analysis. The 69 soils sampled in April 2009 and two additional samples (S5 and

S13) re-analysed from an earlier study in November 2011, were extracted and the extracts analysed for selected veterinary drugs by high performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS).

### 2.3. Extraction

Extraction procedures for target compounds in soil were developed based on methods reported by Martínez-Carballo et al. (2007). The 3.0 g soil sample was added into 50 mL centrifuge tube with 9 mL methanol and then 1 mL of 0.1 M Na<sub>2</sub>EDTA-McIlvaine solution (pH 6) was added to prevent complexation between veterinary drugs and multivalent cations. The solution was homogenized for 30 s by vortex mixing and then ultrasonically extracted for 20 min in a bath sonicator. The supernatant was moved to a clean tube after centrifugation at 5000 r min<sup>-1</sup> (4 °C) for 10 min. This procedure was performed in triplicate and the supernatants were pooled.

The supernatant was applied to a polymeric Oasis HLB cartridge (6 mL, 500 mg), which had been preconditioned with 5 mL of methanol and 5 mL 0.1 M Na<sub>2</sub>EDTA-McIlvaine solution (pH 4). After the supernatant was loaded into the cartridge, the cartridge was rinsed with 10 mL of ultrapure water, and then eluted with 6 mL 0.01 M oxalic acid in methanol. The eluate was evaporated to near dryness under a gentle stream of nitrogen and the residue was dissolved with the HPLC mobile phase. The resulting solution was filtered through a 0.22 μm disposable nylon syringe filter and 10 μL was injected into the HPLC-MS/MS system.

### 2.4. Liquid chromatography/tandem mass spectrometry and quantification

Separation of veterinary drugs was performed by HPLC using an Agilent 1200 system with an Agilent Zorbax RX-C<sub>8</sub> column (2.1 × 150 mm, 5 μm). The mobile phase consisted of water (Solvent A) and acetonitrile (Solvent B) both acidified with 0.1% formic acid. The gradient was held at 10% B for 15 min, then run from 10% B to 30% B in 10 min, kept at 30% B for 8 min, then run from 30% B to 90% B in 1 min and kept 90% B to 40 min. The quantitation of target compounds was determined by Mass Spectroscopy using an Agilent 6410 triple quadrupole mass spectrometer with an electrospray ionization (ESI) source. Standard compounds (1 mg L<sup>-1</sup>) were infused through an integrated syringe pump to tune the mass spectrometer and to optimize capillary temperature, nebulizer pressure and drying gas flow rates. The source polarity was set in the positive ionization mode for CY, TCs, SAs and FQs, and in negative ionization mode for FF, respectively. The optimized conditions were as follows: capillary temperature of 350 °C, drying gas flow of 10 L min<sup>-1</sup>, nebulizer pressure of 40 psi (2.76 bar), capillary voltage of 4.0 kV. Nitrogen (≥99.995%) was used as the cone and collision gas. More detailed MS-MS parameters have been reported in previously published studies (Wei et al., 2011, 2012).

The recovery studies were carried out with control soils spiked at the 5, 25, and 100 μg kg<sup>-1</sup> levels. The extraction procedure was performed after 30 min equilibration. The control soil was prepared from 13 drug-free soil samples taken at depths of 0–20 cm, 20–40 cm, and 40–60 cm in the vicinity of the soil-monitoring site under investigation. The recovery rate was calculated as an average of eight experiments at each concentration. The quantification limit of the method (MQL) was defined as the lowest concentration of analytes that can be determined with acceptable precision and accuracy from the residue-free samples spiked at different levels (Martínez-Carballo et al., 2007; Boon et al., 2006). Table 1 summarized the results of MQL, recovery and relative standard deviation of the soils spiked with 13 veterinary drugs.

Download English Version:

<https://daneshyari.com/en/article/6307303>

Download Persian Version:

<https://daneshyari.com/article/6307303>

[Daneshyari.com](https://daneshyari.com)