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Occurrence and sorption of fluoroquinolones in poultry litters and soils from São Paulo State, Brazil

Rafael Marques Pereira Leal [a], Rafael Fernandes Figueira [a], Valdemar Luiz Tornisielo [a], Jussara Borges Regitano [b,*]

[a] Laboratory of Ecotoxicology, Center of Nuclear Energy in Agriculture, University of São Paulo — CENA/USP, P.O. Box 96, 13400-970, Piracicaba (SP), Brazil
[b] Department of Soil Science, Superior School of Agriculture "Luiz de Queiroz", University of São Paulo — ESALQ/USP, P.O. Box 9, 13418-900, Piracicaba (SP), Brazil

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ABSTRACT

Animal production is one of the most expressive sectors of Brazilian agro-economy. Although antibiotics are routinely used in this activity, their occurrence, fate, and potential impacts to the local environment are largely unknown. This research evaluated sorption–desorption and occurrence of four commonly used fluoroquinolones (norfloxacin, ciprofloxacin, danofloxacin, and enrofloxacin) in poultry litter and soil samples from São Paulo State, Brazil. The sorption–desorption studies involved batch equilibration technique and followed the OECD guideline for pesticides. All compounds were analyzed by HPLC, using fluorescence detector. Fluoroquinolones' sorption potential to the poultry litters ($K_d \leq 65$ L kg^{-1}) was lower than to the soil (K_d ~40,000 L kg^{-1}), but was always high (≥69% of applied amount) indicating a higher specificity of fluoroquinolones interaction with soils. The addition of poultry litter (5%) to the soil had not affected sorption or desorption of these compounds. Desorption was negligible in the soil (≤0.5% of sorbed amount), but not in the poultry litters (up to 42% of sorbed amount). Fluoroquinolones' mean concentrations found in the poultry litters (1.37 to 6.68 mg kg^{-1}) and soils (22.93 μg kg^{-1}) were compatible to those found elsewhere (Austria, China, and Turkey). Enrofloxacin was the most often detected compound (30% of poultry litters and 27% of soils) at the highest mean concentrations (6.68 mg kg^{-1} for poultry litters and 22.93 μg kg^{-1} for soils). These results show that antibiotics are routinely used in poultry production and might represent one potential source of pollution to the environment that has been largely ignored and should be further investigated in Brazil.

1. Introduction

Brazil is a worldwide leader in the production and exportation of animal products, such as poultry and cattle meat (USDA — United States Department Of Agriculture, 2011). Massive quantities of antibiotics agents are used in local animal production units, but we are unaware of any official statistics on the amounts used and sold in the country. Several overseas studies reported the occurrence of veterinary antibiotic residues in animal manures (typically in mg kg^{-1}) (Martínez-Carballo et al., 2007; Xian-Gang et al., 2008; Zhao et al., 2010) and also in manure fertilized agricultural soils (typically in μg kg^{-1}) (Karci and Balcioğlu, 2009; Uslu et al., 2008). These residues may negatively affect the environment, mainly through chronic toxic effects to terrestrial organisms or due to the controversial but worrisome potential influence on microorganism's resistance (Kemper, 2008).

The fluoroquinolones are one of the most used class of compounds for veterinary purposes in the world (Picó and Andreu, 2007) and have limited metabolism in the animal body (20–80%) (Boxall et al., 2004). In addition, they are strongly sorbed (K_d = 260 to 5012 L kg^{-1}, Sarmah et al., 2006) and slowly degradable ($t_{1/2}$ ~1000 days in biosolid amended soils, Walters et al., 2010) compounds. Soil fertilization with contaminated animal manure resulted in continuous soil accumulation of these residues (Picó and Andreu, 2007). Concentrations as high as 225 and 1420 mg kg^{-1} of norfloxacin and enrofloxacin, respectively, were found in chicken manures from China (Zhao et al., 2010), whereas 20 to 50 μg kg^{-1} of enrofloxacin were found in Turkish soils sampled seven months after they were fertilized with contaminated manure (Karci and Balcioğlu, 2009).

The fluoroquinolones are amphoteric compounds with two relevant ionizable functional groups, the 3-carboxyl group (pK_a ~6) and the N-4 in the piperazine substituent (pK_a ~8), which makes their sorption a pH-dependent process (Picó and Andreu, 2007). Sorption to solid matrices is one of the main mechanisms of fluoroquinolone dissipation in the environment. Concentrations as high as 2 mg kg^{-1} of norfloxacin and ciprofloxacin in raw sewage sludge (Golet et al., 2003) and 1.56 mg kg^{-1} of ofloxacin in river sediments (Yang et al., 2010) were reported. The polar nature and the high sorption potential of the fluoroquinolones suggest that mechanisms other

* Corresponding author. Tel./fax: +55 19 34172126.
E-mail address: regitano@usp.br (J.B. Regitano).

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than hydrophobic partitioning play an important role on their sorption (Uslu et al., 2008).

A good understanding of antibiotic concentration in the environment as well as the main mechanisms responsible for their retention is a crucial aspect for an appropriate evaluation of potential microbial exposure and all the associated risks these contaminants may represent to other organisms and also to human health (Vasudevan et al., 2009). Almost all of the currently available data were acquired under temperate conditions, while very little is known about the dynamics and impacts of major veterinary antibiotics in tropical environments, such as in Brazil, where climatic conditions and soil types are very distinct, as well as poultry farm management and manure application, among others.

This research evaluated the sorption–desorption potential of three fluoroquinolone antibiotics (norfloxacin, ciprofloxacin, and enrofloxacin) to two poultry litter types (rice straw and pine dust), as well as the effect of poultry litter addition (rice straw) on the sorption–desorption of these compounds to a typical Brazilian soil. It also provided natural environmental concentrations of norfloxacin, ciprofloxacin, danofloxacin, and enrofloxacin in poultry litters and soils collected at different production areas of São Paulo State, Brazil.

2. Materials and methods

2.1. Reagents and standards

Norfloxacin (NOR), ciprofloxacin (CIP), danofloxacin (DAN), and enrofloxacin (ENR) were purchased from Sigma-Aldrich, with purity higher than 98%. The stock standard solutions (1.0 mg mL^{-1}) were prepared in methanol containing 1% of acetic acid, stored at −18 °C in the dark, and used within 90 days. Ultra-pure water was used in order to prepare all aqueous solutions. Magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$), ammonium hydroxide (NH_4OH), calcium chloride ($CaCl_2$), and oxalic acid ($C_2H_2O_4 \cdot 2H_2O$) were all analytical grade. The organic solvents used (methanol and acetonitrile) were all HPLC grade.

2.2. Animal manure and agricultural soil samples

Two poultry litters from poultry houses already containing rice straw and pine dust as substrates and a typical Brazilian soil (Typic Haplustox) were selected for the sorption–desorption studies. Their main physicochemical properties are presented in Table 1.

In order to determine fluoroquinolone environmental concentrations, 46 poultry litters and 11 soils (0–20 cm) were collected from different producing regions of São Paulo State, Brazil. All soils had a history of poultry litter application. Each sample (~500 g of either poultry litter or soil) was attained by homogeneously mixing 12 subsamples randomly collected inside a poultry house or an homogeneous agricultural field, respectively. After collection, the samples were placed into plastic bags and transported under refrigerated conditions within the same day. The samples were homogenized, sieved, lyophilized within 48 h, and analyzed within a week after collection (stored at −18 °C until extraction).

2.3. Extraction procedures

Fluoroquinolones' extraction was based on the method proposed by Turiel et al. (2006). However, a centrifugation step (10 min, 3560 g) was added after the ultrasonic bath, as proposed by Karci and Balcioğlu (2009). Briefly, 1 g of either the soil or the poultry litter and 8 mL of an aqueous solution of $Mg(NO_3)_2$ (29% w/v, adjusted to pH 8.1 with 4% of ammonia) were placed into Teflon tubes (50 mL), mixed in a vortex for 60 s, and let in ultrasonic bath for 30 min at room temperature. Samples were then centrifuged and the supernatants were filtered (0.45 μm syringe filter) prior to HPLC analysis. Samples were all analyzed in duplicate.

2.4. HPLC analysis

The instrument (HPLC from Agilent, 1200 series) was equipped with a quaternary pump, DAD and FLD detectors, and an automated injection system. The column (ACE C18, 250×4.6 mm, 5 μm) was kept at 25 °C and the injection volume was equal to 50 μL. An isocratic elution was used and the mobile phase was composed of 72% of oxalic acid 0.01 mol L^{-1} adjusted to pH 4.0 (solvent A) and 28% of methanol (solvent B). The flow rate was 1.0 mL min^{-1} and fluorescence wavelengths were set at 280 and 450 nm for excitation and emission, respectively.

In order to built the calibration curves and to validate the method, triplicates of a residue-free poultry litter and soil were spiked with different concentrations of fluoroquinolones (50, 150, 500, 1000, 2000 and 4000 μg kg^{-1}) and kept in the dark overnight at room temperature for sample equilibration prior to extraction. The correlation coefficients were ≥0.99. The limits of quantification for all compounds ranged from 3.35 to 27.38 μg kg^{-1} for soil and from 78.12 to 208.10 μg kg^{-1} for poultry litter based on seven replicates of the matrix blank. The recovery tests were performed at three fortification levels (500, 1000 and 4000 μg kg^{-1}) and five replicates, and ranged from 68.6 to 108.6% (standard deviation ≤7%) for the soil and from 67.4 to 99.2% (standard deviation ≤15%) for the poultry litter.

2.5. Sorption and desorption

The batch experiments followed the OECD guideline for pesticides (OECD, 2000), with a pre-test performed to define solid:solution ratio (2 g:30 mL), equilibration period (24 h), compounds stability, and tube walls sorption. In all cases compound losses were lower than 5% (data not shown). When needed, the poultry litter (rice straw) was added to the soil according to fertilization recommendations (5% w/w). It was thoroughly mixed (15 min) prior the addition of antibiotic solutions. Triplicates of 2 g of the samples (poultry litter, soil or soil amended with poultry litter) and 30 mL of the fluoroquinolone (NOR, CIP and ENR) solutions at different concentrations (170; 340;

Table 1
Characterization of poultry litters and a typical Brazilian soil used in the sorption studies.

Sample	pH-$CaCl_2$	CEC	Sand	Clay	Silt	OC	TN	P	K	Ca	Mg
		$mmol_c\ dm^{-3}$	g kg^{-1}			%		g kg^{-1}			
Poultry litter (rice straw)	7.4	–	–	–	–	39.90	4.19	32.40	32.90	31.90	4.60
Poultry litter (pine dust)	7.1	–	–	–	–	37.09	4.50	20.30	23.90	21.40	3.80
Soil	5.9	127.8	122	754	116	1.62	–	0.02	0.12	0.56	0.32

OC = organic carbon content; TN = total nitrogen.