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Fluoroquinolones in agricultural soils: Multi-temporal variation and risks in Rio de Janeiro upland region

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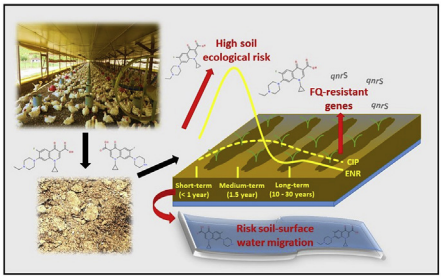
HIGHLIGHTS

- Short to medium-term fertilized soils accumulated enrofloxacin and ciprofloxacin.
- Fluoroquinolones (FQs) did not increase in soils over the years.
- Enrofloxacin and ciprofloxacin pose a high risk to soil organisms.
- FQ-resistant *qnr*S genes were detected in the less contaminated area.
- FQs occurrence and associated risks changed over the years.

GRAPHICAL ABSTRACT



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ABSTRACT

Our main goal was to investigate the potential accumulation of fluoroquinolones (FQs) in agricultural soils over extended periods of land use, predicting leaching and estimating risk quotients for soil microorganisms. Short to long-term of poultry litter fertilization (<1–30 years) were evaluated for enrofloxacin (ENR) and ciprofloxacin (CIP) input, in addition to the emergence of plasmid-mediated quinolone resistance (PMQR) genes. High FQs concentration (range 0.56–100 mg kg^{-1}) were measured in poultry litter samples. In soils, FQs occurrence and risks have changed over the years. An accumulation trend was observed between short and medium-term fertilized soils (ST and MT soils), reaching a range of 330–6138 μg kg^{-1} ENR and 170–960 μg kg^{-1} CIP in MT soil, followed by decreased concentrations in long-term fertilized soils (LT soils). The environmental risk assessment showed a high ENR risk quotient (RQ ≥ 1) in ST and MT soils ranging (7–226) and high CIP risk (9–53) in LT soils. The detection of *qnr*S genes in the area with the lowest FQs concentration emphasizes the importance of a broader approach to environmental assessment, in which not only target compounds are considered. FQs soil-water migration model pointed out a high leaching risk in ST soil. To reduce risks, management measures to decrease antibiotic environmental load should be taken before poultry litter application. In addition, the high

Abbreviations: short-term fertilized soil, ST soil; medium-term fertilized soil, MT soil; long-term fertilized soil, LT soil.

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weathering of tropical soils contributing to possible fate of antibiotics to water resources through drainage basins should be considered.

1. Introduction

The scientific community and international food safety and health monitoring agencies regard the overuse of antibiotics in animal husbandry as a great contemporary concern (FAO/WHO, 2015). Antibiotics are widely diffused in poultry farming as a prescription for therapeutic and prophylactic purposes as well as for growth promotion (Ho et al., 2012). Van Boeckel et al. (2015) estimated an increase of around 70% in veterinary antibiotics (VAs) use worldwide, reaching 105,500 tons/year in 2030. According to the authors, only BRICS countries (Brazil, Russia, India, China and South Africa) will be responsible to raise VAs consumption in 99% over the same period. Among VAs, enrofloxacin (ENR), a fluoroquinolone (FQ), is widespread in poultry farming due to its broad spectrum of antimicrobial activity (Gouvêa et al., 2015). It can be biotransformed into ciprofloxacin (CIP), its main metabolite, by a deethylation process. This is also an antibiotic commonly prescribed in human medicine (Morales-Gutiérrez et al., 2015; Van Doorslaer et al., 2004). However, considering the three major world poultry producers, the United States (U.S.A.), Brazil and China (ABPA, 2018), only the last two mentioned countries regard ENR usage in poultry farming.

On the last years, some studies have approached FQs environmental contamination and risk assessment in China, pointing out a widely occurrence of FQs in soils from greenhouses, organic and intensive farms (Li et al., 2011; Li et al., 2014; Li et al., 2015; Mu et al., 2015; Sun et al., 2017; Wu et al., 2014; Zhang et al., 2016). According to Yopasá-Arenas and Fostier (2018), studies on the fate of antibiotics in the Brazilian environment are still scarce so far. In addition, the authors estimated a high potential for leaching of VAs through soil to the groundwater in the central-west, south and southeast regions in Brazil. Due to the large volume of chicken production in the country (13 million tons in 2017), poultry litter is probably the main source of veterinary FQs to soils (ABPA, 2018; Leal et al., 2012). Poultry litter is a by-product of poultry farming, and is widely used as a fertilizer in agricultural soils. This practice helps to solve the disposal of poultry industry waste, in addition to recycling the required nutrients for good crop production (Bolan et al., 2010; Vollú et al., 2018). However, antibiotics have their main environmental input in agricultural soils because of the application of animal husbandry waste as fertilizer (Hou et al., 2015; Karci and Balcıoğlu, 2009; Li et al., 2015; Picó and Andreu, 2007).

Upon contact with soil, antibiotics can alter the structure of microbial communities, thus affecting the maintenance of important soil functions, such as nutrient cycling (Girardi et al., 2011). Furthermore, the selective pressure resulting in antibiotic-resistant bacteria and the horizontal transfer of resistant genes in agricultural soils increase the risk of human exposure to drug-resistant infections, mainly through water and contaminated food (Singer and Williams-Nguyen, 2014; World Bank, 2016). There are three main mechanisms of bacterial resistance to FQs: i) target-mediated FQs resistance; ii) active efflux from the cell; and iii) plasmid-mediated quinolone resistance (PMQR) (Rusu et al., 2015). Among the different mechanisms related to PMQR, the *qnr* genes code proteins that decrease antibiotics binding in target enzymes (DNA gyrase and topoisomerase IV), thus increasing FQs minimum inhibitory concentration (Grau et al., 2013; Rusu et al., 2015). The fact that FQs undergo strong sorption in soils, favors their diffusion and sorption in micro and nanopores reducing their biological contact and, therefore, their potential biodegradability and toxicity to soil organisms (Jechalke et al., 2014; Leal et al., 2013). However, the same phenomenon may maintain their continuous release through desorption at sub-inhibitory concentrations, implying a chronic selection pressure on soil microbiota (Riaz et al., 2018). Considering the long half-life of FQs in soils (varying from months to years), a trend of accumulation of these chemicals is expected due to poultry litter application over the years (Jechalke et al., 2014; Karci and Balcıoğlu, 2009). Since the tropical region is highly weathered (annual mean rainfall > 2500 mm) and the erosive process may lead to antibiotic dispersion through leaching and runoff, we chose an important pole of chicken production in Rio de Janeiro state as tropical environment study model (Dourado et al., 2012). In this context, the main goal of this study was to investigate FQs occurrence and their potential accumulation in soils with short-term (<1 year) to long-term (30 years) poultry litter fertilization. In addition we wanted to assess their fate and possible impacts on the environment, including the emergence of PMQR genes.

2. Material and methods

2.1. Study area and sampling

Agricultural areas and poultry farms are located in the São José do Vale do Rio Preto (SJVRP) municipality, situated in the upland region (220 km^2 and 615 m.a.s.l., mean elevation) of Rio de Janeiro state (RJ), in southeastern Brazil. SJVRP is the main poultry production pole at the regional level (around 100 poultry farms) and is surrounded by agricultural areas of great relevance for supplying fresh products (e.g. tomatoes, chayote, zucchini, eggplant, cucumber) to the RJ metropolitan region. In addition, the study area has fragments of Brazilian Atlantic rainforest, a tropical biome protected by national and state parks which have great relevance for biodiversity conservation and water resources. The sampling studies were conducted between October 2015 and December 2017. Poultry litter samples from 17 farms were collected at the end of a chicken production cycle (around 50 days). Fig. 1 presents the study area and the sampling points. Poultry house soils (soil samples from inside sheds) were collected from four poultry farms and, according to the farmers, the 50 agriculture soils were fertilized regularly with poultry litter over < 1–30 years. The soils were sampled in the surface mineral (Horizon A, depth 0–20 cm). Sample set characteristics and chemical parameters are described in Table 1.

2.2. Standards and reagents

ENR and CIP standards, both ≥98% purity (HPLC) were purchased from Sigma-Aldrich (Saint Louis, MO, U.S.). Acetonitrile (Tedia® - USA) and all other chemicals used - NH_4OH, $Mg(NO_3)_2$ and H_3PO_4 HCl, KCl, HNO_3, calcium acetate, ammonium molybdate and ascorbic acid were of analytical grade from Merck (Darmstadt, Germany) and Sigma-Aldrich (U.S.). Ultra-pure water Milli-Q® System (18.2 Ωm – high purity deionized water), Millipore/Merck

(Darmstadt, Germany) with 0.22 μm filter was used to prepare all aqueous solutions. FQs stock standard solutions (1000 mg L^{-1}) were prepared in acetonitrile with NH_4OH (2%) and stored at −18 °C.

2.3. Sample preparation and extraction

After sampling, poultry litter and soils were frozen (−80 °C), lyophilized, homogenized and stored, avoiding sunlight and humidity. Soil and poultry litter extraction was adapted from the method described by Turiel et al. (2006). Among selected extraction methods (Janusch et al., 2014; Uslu et al., 2008), the method of Turiel et al. (2006) was chosen for not using organic solvents and to be suitable for the purposes of the study. Briefly, 1 g of sample was extracted with 8 mL of an aqueous solution of $MgNO_3$ 50% (w/v) with 4% NH_4OH in ultrasonic bath. After that, the extracts were cleaned-up as described by Leal et al. (2012), with centrifugation (10 min with 3400 rpm) and filtration with Millipore™ syringe filter 40 μm (Darmstadt, Germany).

2.4. Analytical procedures and quality control

FQs (ENR and CIP) were determined using a HPLC system (CBM-20A) equipped with a quaternary pump (LC-10ATVP) and a fluorescence detector (RF-10AXL), all equipment modules from Shimadzu Corp., Japan. Manual injection was performed, using 20 μL for total injection volume. Columns used were: guard column C18, 10 × 4 mm, 5 μm and analytical column C18, 250 × 4.6 mm, 5 μm, both Kromasil® (Sweden). Isocratic elution was used with a flow rate of 1.0 mL min^{-1}. The mobile phase was composed of 0.02 M *o*-H_3PO_4:ACN (80:20) as previously described by Uslu et al. (2008). The fluorescence wavelengths were set at 280 and 450 nm for excitation and emission, respectively (Leal et al., 2012).

FQs quantification in poultry litter samples were made by two matrix-matched calibration curves: 10–250 μg L^{-1} (curve 1) and from 500 to 7000 μg L^{-1} (curve 2). The correlation coefficients were only accepted when ≥0.99. The recovery tests were performed in 5 replicates with 2 concentrations: 150 μg L^{-1} (curve 1) and 3500 μg L^{-1} (curve 2). Recoveries and coefficient of variation (CV) were: 81.7% (5.4) ENR and 75.4% (3.4) CIP (curve 1); 76.3% (6.0) ENR and 80.5% (3.0) CIP (curve 2).

The limit of quantification (LOQ) in poultry litter samples were: 171 μg kg^{-1} (ENR) and 271 μg kg^{-1} (CIP). For soil samples, matrix-matched calibration curve with six points ranged from 10 to 250 μg L^{-1}. Recoveries were: 77.2% (2.9) for ENR and 77.5% (2.6) for CIP. LOQ for soils were: 116 μg kg^{-1} (ENR) and 189 μg kg^{-1} (CIP). The chemical characterization methods are given in Topic S1 (Supplementary material).

2.5. Risk assessment in fertilized soils

According to the Technical Guidance on Environmental Risk Assessment (European Commission, 2003), the risk quotient (RQ) for specific contaminants is estimated from the ratio of measured or predicted environmental concentration (MEC or PEC) to the predicted no-effect concentrations on non-target organisms (*PNEC*). In the present study, we estimated the RQ with the MEC of soil samples. Due to the very few published studies (from acute and short-term assays) and the lack of a base set of quinolones terrestrial toxicity, $PNEC_{soil}$ was estimated with $PNEC_{water}$ data considering the equilibrium partitioning method (European Commission, 2003; Gao et al., 2008; Wu et al., 2014). The proposed equation by Wu et al. (2014) was:

$$\boldsymbol{PNEC_{soil} = PNEC_{water} \times K_d} \quad (1)$$

where: $PNEC_{water}$ is the effective concentration 50% (EC_{50}) in an acute or short-term assay. These results must be divided by an assessment factor (AF) which considers inter-species variations: AF = 1000 for acute-term assays; and AF = 100 if based on a chronic test (European Commission, 2003; Li et al., 2015). The soil-water partition coefficient (K_d) for each antibiotic was derived from previously published study (Wu et al., 2014). In this estimation it is considered that the antibiotic bioavailability, bioaccumulation and toxicity are related to its concentrations in soil solution (Chen et al., 2018; Li et al., 2015). Estimated $PNEC_{soil}$ for ENR and CIP are presented in Supplementary material (Table S1).

2.6. DNA extraction and qnr genes in soils

The total microbial community DNA was extracted directly from the soil samples (0.5 g of each sample) using DNeasy PowerSoil Kit (QIAGEN, USA). 2 Samples were then stored at −20 °C. The DNA

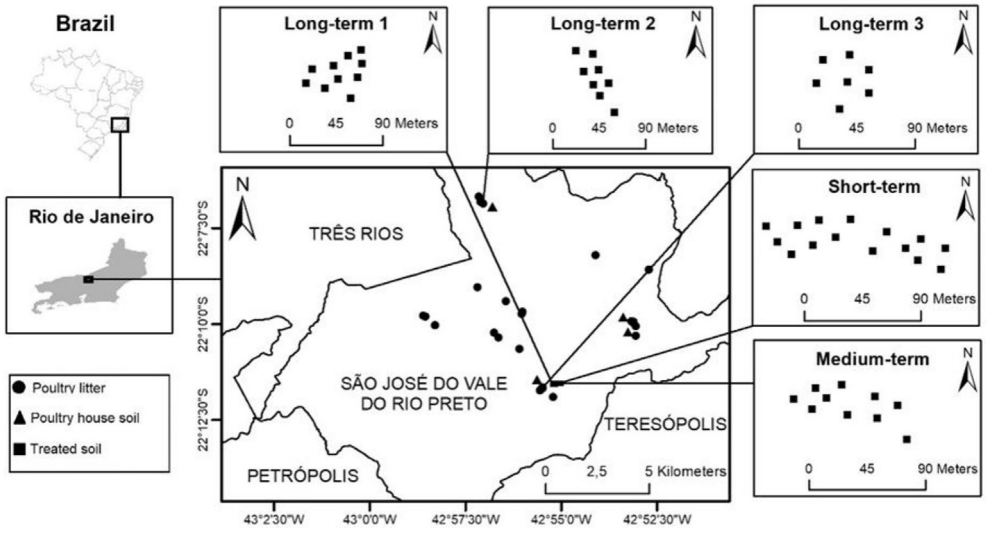

Fig. 1. Study area and sample points.

Table 1
Characteristics of the samples and chemical parameters.

Sample set	Samples (n)	Land use (yrs)[b]	Na	Ca	Mg	H + Al	CEC[c]	OC[d] %	pH	Soil classification[e]
			$Cmol_c\ dm^{-3}$							
Poultry litter (PLitter)	30	–	3.2	3.5	1.0	0.5	23	31.0	8.9	–
Poultry house soil (PH soil)	15	10	1.2	4.2	1.8	1.3	15	0.8	5.9	Typic Hapludox
Short-term soil (ST soil)[a]	15	<1	0.3	4.7	2.5	3.1	13	2.2	5.7	
Medium-term soil (MT soil)[a]	10	1.5	0.6	7.1	4.0	2.8	16	3.1	5.1	
Long-term soil 1 (LT soil 1)	15	10	1.1	11.5	4.0	4.1	23	6.5	5.5	
Long-term soil 2 (LT soil 2)	8	15	1.2	10.9	5.6	3.9	24	6.2	5.3	
Long-term soil 3 (LT soil 3)	7	30	1.5	12.5	4.3	4.9	25	6.8	6.1	

[a] ST and MT soil are from the same area with sampling intervals of 8 months.
[b] Years of continuous poultry litter application.
[c] Total cation exchange capacity.
[d] Organic carbon.
[e] USDA - Soil Survey Staff (1999).

extracted was used for PCR amplification of plasmid-mediated quinolone resistance (PMQR) genes - *qnrA*, *qnrB* and *qnrS*, in separate reactions. PCR was performed in 25 μL reactions containing 5 μL PCR buffer (50 mM Tris-HCl, pH 9.0; 50 mM NaCl), 2 U of *Taq* polymerase (Promega, SP, Brazil), 200 μM each deoxynucleotide triphosphate, 5 pmol of each forward and reverse primers (Kraychete et al., 2016), 1.5 mM $MgCl_2$ and 2 μL template DNA (20–40 ng). PCR conditions were: 10 min at 95 °C and 25 cycles of amplification consisting of 45 s at 95 °C, 45 s at 58 °C and 15 s at 72 °C and 3 min at 72 °C as previously described by Kraychete et al. (2016). DNA fragments were analyzed by electrophoresis in a 1.4% agarose gel at 80 V for 1 h in 1x TBE buffer and stained with ethidium bromide. PCR analysis was made in triplicates with the following samples: 1) soil control, from a forest surrounded by agricultural lands; 2) PH soil, due to its chronic poultry litter exposition; 3) ST soil, to assess the influence after short exposure; 4) LT soil 3, the sampling area with a longer exposure time.

2.7. Estimating FQs migration from soil to surface water

The risk of measured environmental concentration in soil reaching surface water can be estimated with a sequence of equations. The predicted environmental concentration in pore water - $PEC_{pore\ water}$ (μg L^{-1}) was calculated with the following equation (Zhang et al., 2016).

$$\boldsymbol{PEC_{porewater} = \frac{MEC_{soil}}{K_d}} \quad (2)$$

where: MEC_{soil} is the measured environmental concentration of each antibiotic (μg kg^{-1}) and K_d (L kg^{-1}) is calculated according to (EMA, 2016a; Zhang et al., 2016) in Equation (3).

$$\boldsymbol{K_d = K_{oc}\left(\frac{f_{oc}}{100}\right)} \quad (3)$$

where: K_{oc} (L kg^{-1}) is the organic carbon normalized octanol-water partition coefficients (Tolls, 2001) and f_{oc} (%) is the organic carbon content for each sample set – ST soil, MT soil and LT soils (Table 1). Finally, the predicted environmental concentration in surface water - $PEC_{surface\ water}$ (μg L^{-1}) was calculated following Equation (4) (EMA, 2016a).

$$\boldsymbol{PEC_{surface\ water} = \frac{PEC_{pore\ water}}{DF}} \quad (4)$$

where: $PEC_{pore\ water}$ was calculated with the result of Eq. (2) and the dilution factor (DF) of 3 was used. According to EMA (2016a), the DF = 3 consider that one part of run-off is diluted in two parts of receiving water.

2.8. Statistical analysis

For data analysis, measured values < LOQ were considered equal to Df * LOQ, where Df is the detection frequency in each sample set, as described by Das et al. (2017). Only quantified samples were considered for the estimates described in topics 2.5 and 2.7. Data normality was verified by a Shapiro-Wilk test. Due to the non-normal distribution, a Kruskal - Wallis test was chosen with Dunn's post-test that compares all pairs of columns. For statistics test, the adopted significance level was 5% ($p < 0.05$). Graphpad Prism 5.0® was utilized for graphics and statistical tests.

3. Results and discussion

3.1. FQs occurrence in poultry litter samples

The results demonstrate a widespread use of ENR in the study area. CIP was detected in 100% of poultry litter samples (n = 30), while ENR occurred in 53%. The median concentration for ENR was 1.03 mg kg^{-1}, with range between 0.44 and 84.2 mg kg^{-1}. While CIP median was 0.93 mg kg^{-1}, with range 0.47–16.2 mg kg^{-1}. In a monitoring study conducted in Brazil (São Paulo), Leal et al. (2012) found ENR in 30% of poultry litter samples with ENR median 4.33 mg kg^{-1} and range (0.39–31.0 mg kg^{-1}). While CIP frequency was only 4%, with median and range 1.37 mg kg^{-1} (0.65–2.13 mg kg^{-1}). Our results showed much higher FQs frequency, mainly for CIP. Although Brazilian poultry farms are the largest exporters in the world, there are few monitoring studies that reported VAs in poultry litter and soils (Hahn et al., 2012; Leal et al., 2012). On the other hand, this is a problem of worldwide concern. Several monitoring studies were carried out with poultry litter and animal manure with maximum concentrations ranging 0.06–2.8 mg kg^{-1} ENR in Turkey and Austria (Karci and Balcıoğlu, 2009; Martinez-Carballo et al., 2007). While in China, Zhang et al. (2014) reported ENR concentration ranging between 0.01 and 8.57 mg kg^{-1} and CIP ranging 0.02–4.90 mg kg^{-1}. The highest concentrations measured in the present study may be due to the widespread use of FQs in poultry farming, since previous studies analyzed manures of diverse origin, including cattle and pig. In fact, Martinez-Carballo et al. (2007) found ENR mainly in chicken and turkey manure and Zhao et al. (2010), measured higher ENR and CIP concentrations in chicken manure (geometric means 4.65 and 3.78 mg kg^{-1}, respectively) compared with cattle and pig dungs. Karci and Balcıoğlu (2009) reported a low occurrence of ENR in fresh manure from Turkey. However, the authors observed high tetracycline (TC) occurrence (88%), suggesting their alternative use

instead of FQs. In another study conducted in China, Hou et al. (2015) detected a high frequency of CIP (>80%) in poultry manure sampled in piles from agricultural areas. Nevertheless, the FQs mean concentration (0.41 ± 1.45 mg kg^{-1}) was much lower than the FQs (ENR + CIP) concentrations reported in our study (10.8 ± 23.3 mg kg^{-1}), with samples collected directly on poultry sheds. Selvam et al. (2012) observed CIP elimination from 1 to 0.31 mg kg^{-1} (69%) and 10 to 1.71 mg kg^{-1} (83%) during composting (>50 days). The authors reported a greater CIP persistence compared to other VAs - sulfonamides (SAs) and TCs. However, due to the high persistence also reported to both classes of antibiotics is possible that the observed elimination rates are related to their binding in non-extractable forms (EMA, 2016b; Junge et al., 2011).

3.2. FQs occurrence in soil samples

Among the sets of soil samples, the lowest ENR and CIP concentrations were measured inside poultry houses (PH soils). Some factors that may have influenced the results were: 1) high variability of contamination inside poultry houses, since the samples were randomly collected; 2) high poultry litter water holding capacity, reaching 30 L m^{-2} (Dunlop et al., 2015) and, therefore ENR accumulation in poultry litter, since FQs are administered through water; 3) and possible binding in the non-extractable fraction of the soil. The concentrations of ENR, CIP and $\sum$FQs in each set of samples, in addition to the basic statistics, are presented in Table 2. Among fertilized soils the $\sum$FQs range concentration were higher in MT soil samples: 560–6309 μg kg^{-1}.

Wu et al. (2014) pointed out a high ENR and CIP occurrence (100%) in Chinese soils from organic vegetable farms, with median concentration of 2.44 and 1.01 μg kg^{-1}, respectively. Although similar frequency was reported in MT soil, ENR and CIP median concentrations were extremely higher 893 and 339 μg kg^{-1}, respectively. In addition, high stability of both antibiotics ($\sum$FQs = 541 μg kg^{-1}) in the ST soil area, was observed, even 8 months after application. Karci and Balcıoğlu (2009) also measured ENR (50 μg kg^{-1}) in short-term treated soils (~7 months) from Turkey, reinforcing its persistence into agricultural soils. The median concentrations in all fertilized soils (ST, MT and LT soils) were ENR (263 μg kg^{-1}) and CIP (189 μg kg^{-1}). Our results were much higher than previously reported in fertilized soils for ENR 1.02–8.3 μg kg^{-1} (Li et al., 2014; Sun et al., 2017) and CIP 16.6–72.4 μg kg^{-1} (Li et al., 2014; Sun et al., 2017). FQs are widely used in Brazilian poultry farming, highlighting ENR-based products (Gouvêa et al., 2015). Between 2008 and 2018, the country imported more than 2100 tons of ENR (Comex Stat Brazil, 2018,). However, there is a lack of specific legislation about antibiotic contamination on organic fertilizers marketed and on agricultural soils (Conama n^{o} 420/2009; IN n^{o} 25/2009). This context, aggravated by the stronger affinity of FQs to Brazilian soils (Leal et al., 2013), can explain the high FQs concentration reported here.

3.3. FQs pattern in temporal land use

The ST soil to LT soil 3 areas, present a temporal variation (<1–30 years) related to the time of land use for agricultural production (Table 1). All areas had poultry litter applied regularly in crop production. In Fig. 2, boxplots with $\sum$FQs (ENR + CIP) concentration in relation to temporal land use are presented. It can be observed that there was an increase in FQs concentration between ST soil (<1 year) and MT soil (1.5 year).

In this context, our results demonstrated a trend of FQs accumulation over time and with new poultry litter applications for ST and MT soils in the same sampling area. On the other hand, a decrease of FQs concentration was observed in LT soils (10–30 years). Similar trend of antibiotic accumulation in soil was reported by Fang et al. (2014) in an experimental assay with chlortetracycline. Zhang et al. (2016) also reported a TCs and FQs accumulation in ST to MT soils, followed by decreased concentrations in LT soils. This accumulation pattern may be due to the high sorption rate in soils of both VA classes (Leal et al., 2013; Regitano and Leal, 2010; Uslu et al., 2008). In soils, the organic and mineral exchange sites without previous contact with antibiotics would have a greater accumulation capacity compared to exposed soils. This capability is related to the composition (e.g. organic matter content, clay minerals) and soil physico-chemical parameters (e.g. pH, CEC, divalent and trivalent metal complexes) (Riaz et al., 2018; Turiel et al., 2006; Vasudevan et al., 2009). Zhang et al. (2016) also did not observe the accumulation of SAs in ST and LT soils. This trend may be due to the low sorption potential reported in this matrix (Doretto et al., 2014; Leal et al., 2013), or by the also reported formation of non-extractable residues in soils (Goulas et al., 2019; Heise et al., 2006). On the other hand, high FQs sorption rates and long half-lives in soils suggest that the soil microbiota can be exposed for a long time to these biologically active molecules (Jechalke et al., 2014; Rosendhal et al., 2012). In this context, we observed a clear pattern between ENR and CIP degradation (red to yellow), in addition to the concentration ranges (circles size) in each sample set (Fig. 3). ENR showed dominance over its metabolite in ST and MT soils, and a CIP predominance with a significant difference ($p < 0.05$) was observed in LT soils (10–30 years). In addition, we found a gradual increase in the number of samples with concentration < LOQ in LT soils (10–30 years).

Li et al. (2014) also reported higher CIP concentrations and occurrence compared to ENR in soils within regions of intensive agriculture in China. According to the authors, the amount administered during chicken production, soil properties and crop characteristics may contribute to the observed trend. In fact, Lillenberg et al. (2010) reported ENR uptake by barley and cucumber over a wide range of concentrations (10–500 mg kg^{-1}), while CIP absorption occurred only at the highest concentration. In addition, antibiotics in soil can select competitive groups such as archaea, fungi and other antibiotic-tolerant microorganisms over target or more sensitive organisms (Brandt et al., 2015; Chen et al., 2014; Ding and He, 2010). Therefore, the observed temporal trend in the present study may also be due to the microbiota structural changes influencing a high ENR de-ethylation, resulting in higher CIP concentrations. Girardi et al. (2011) observed an inhibition effect on soil respiration with a low CIP concentration, followed by soil function recuperation over 80 days. According to Fang et al. (2014), there was an increase in tolerance to SAs and TCs by soil microbiota undergoing treatments with manure contaminated by both classes. In addition, it was previously shown that plants are able to metabolize ENR in CIP (Li et al., 2012). Thus it is possible that vegetative parts incorporated into the soil after harvesting may contribute to higher CIP concentrations in LT soils. In addition to the possibilities mentioned above, CIP enrichment should be investigated in the future, also considering the role of erosion processes.

3.4. Potential environmental risk and PMQR genes

Estimated risk quotient (RQ) values result in a range from low (<0.1), medium ($0.1 \leq RQ < 1$) to high (≥ 1) environmental risk (European Commission, 2003; Wu et al., 2014). In the present study, the high FQs concentration measured in soils resulted in increased ecological risks ($RQ \geq 1$) for non-target microorganisms. Extreme RQ values ranged from 5 to 226 for ENR and 8–53 for CIP, respectively (Fig. 4). ENR presented a higher RQ means in ST soil (29) and MT soil (49), while for CIP there was an increased risk of predominance in LT soils (mean RQ = 22).

Table 2
FQs concentration (μg kg^{-1}) in soils and basic statistics.

Samples	FQs	% Freq[a]	Mean	SD[b]	Median	Range
Poultry house soil (n = 15)	ENR	40	179	238	46.4	46.4[d] - 927
	CIP	7	37.6	96.8	12.6	12.6[d] - 388
	∑ FQs[c]	7	217	273	59.0	59.0–940
Short-term soil (n = 15)	ENR	53	453	529	178	83.5[d] - 1612
	CIP	33	184	133	106	189–555
	∑ FQs[c]	33	637	651	284	189 - 2167
Medium-term soil (n = 10)	ENR	100	1330	1718	893	330 - 6138
	CIP	90	381	229	339	170[d] - 960
	∑ FQs[c]	90	1710	1689	1250	560 - 6309
Long-term soil 1 (n = 15)	ENR	80	238	177	164	51.0[d] - 521
	CIP	70	415	417	315	83.2[d]–1371
	∑ FQs[c]	70	653	421	505	134–1563
Long-term soil 2 (n = 8)	ENR	38	164	220	51.0	51.0[d] - 686
	CIP	38	276	318	83.2	83.2[d] - 976
	∑ FQs[c]	38	440	536	134	134–1662
Long-term soil 3 (n = 7)	ENR	0	<LOD[e]	n.d.[f]	<LOD[e]	n.d.[f]
	CIP	14	151	178	83.2	83.2[d] - 555
	∑ FQs[c]	0	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]

[a] Frequency of quantification.
[b] Standard deviation.
[c] ∑FQs = ENR + CIP.
[d] Concentration in samples < LOQ, as described in topic 2.8.
[e] Below limit of detection.
[f] Not determined.

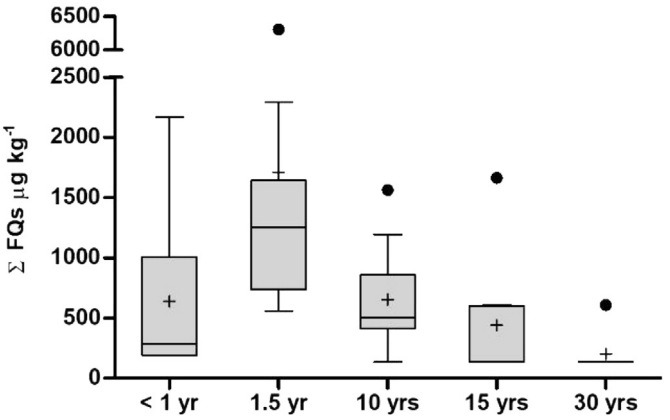

Fig. 2. Boxplots of ∑FQs concentration in fertilized soils over the years; Horizontal lines: Minimum and maximum; Box lines: Quartiles Q1, Q2 (median), Q3; + symbol: Means; Black circles: Outliers.

Using the same methodology employed in the present study (equilibrium partitioning method), previous studies reported low FQs risk (<0.1) to medium risk (0.1–1) in agriculture soils from China and Kenya (Wu et al., 2014; Yang et al., 2016). While Li et al. (2015), in a study with soil samples from greenhouses, estimated high risks to soil microorganisms derived from TCs and FQs, with ENR reaching a RQ > 10. However, even the highest values are low compared to the extreme RQs estimated here. It should also be considered that the model used considers exposure to a single contaminant; nevertheless, these soils receive a constant load of agrochemicals (e.g. fertilizers, veterinary products, pesticides). The results suggest that these soils are critically impacted, with possible effects on soil function, such as nutrient cycling, degradation of pollutants, as well as selective pressure and transfer of resistant antibiotic genes (Ding and He, 2010; Jechalke et al., 2014).

Here, we also studied the occurrence of PMQR genes, whose mainly determinants are *qnr* peptide encoding genes (*qnr*A, *qnr*B, *qnr*S), besides *qnr*C, *qnr*D, that were not investigated in the present study (Strahilevitz et al., 2009). Although our soils were highly impacted by FQs, we did not found *qnr* genes in poultry house and ST soils, nor in control soils. On the other hand, the presence of *qnr*S genes was observed in LT soil 3, the area with the lowest frequency of quantified samples, where we found only CIP (Supplementary material - Fig. S2). In an experimental study, low CIP concentrations (40–400 μg kg^{-1}) were able to increase CIP-resistant bacteria and delay PMQR genes dissipation in soils fertilized with manure (Huang et al., 2016). This concentration range associated with higher CIP-resistant bacteria communities, is in the same range of ENR and CIP concentrations found in LT soils. The persistence of resistance genes in soils depends on factors such as native reservoirs, the decay of manure-associated resistant bacteria, and the horizontal transfer of genes (Xu et al., 2015). Previous field studies reported a wide variety and occurrence of antibiotic resistant genes, including *qnr*S in animal manure and soils (Mu et al., 2015; Rusu et al., 2015). In addition, FQs delayed PMQR gene dissipation in manure-fertilized soils (Xiong et al., 2015), and increased the resistance of isolates and PMQR genes in LT soils, persisting for at least five months (Xu et al., 2015). Moreover, strains of CIP-resistant *Escherichia coli* were able to survive for at least three months in soil fertilized with chicken manure (Pourcher et al., 2014). Considering that the study area is an important supplier of fresh vegetables to more than 12 million inhabitants from the RJ metropolitan region, the presence of *qnr* genes in soil increases the risk of human exposure to PMQR genes through contaminated food.

3.5. Estimating FQs migration from soil to water

Although FQs show high sorption in solid matrices, studies have demonstrated their occurrence in urban rivers impacted by wastewater discharge and in rivers from agricultural areas (e.g. Brazil, China and Europe) (Locatelli et al., 2011; Van Doorslaer et al., 2014; Xu et al., 2009). In addition, high antibiotic concentrations measured in river sediments from agricultural areas, compared to non-agricultural zones, suggest an important contribution of antibiotic transfer between soil-water systems (Kim and Carlson, 2007). The sorption processes of ionizable compounds in soil are strongly influenced by pH and clay content (Riaz et al., 2018). In

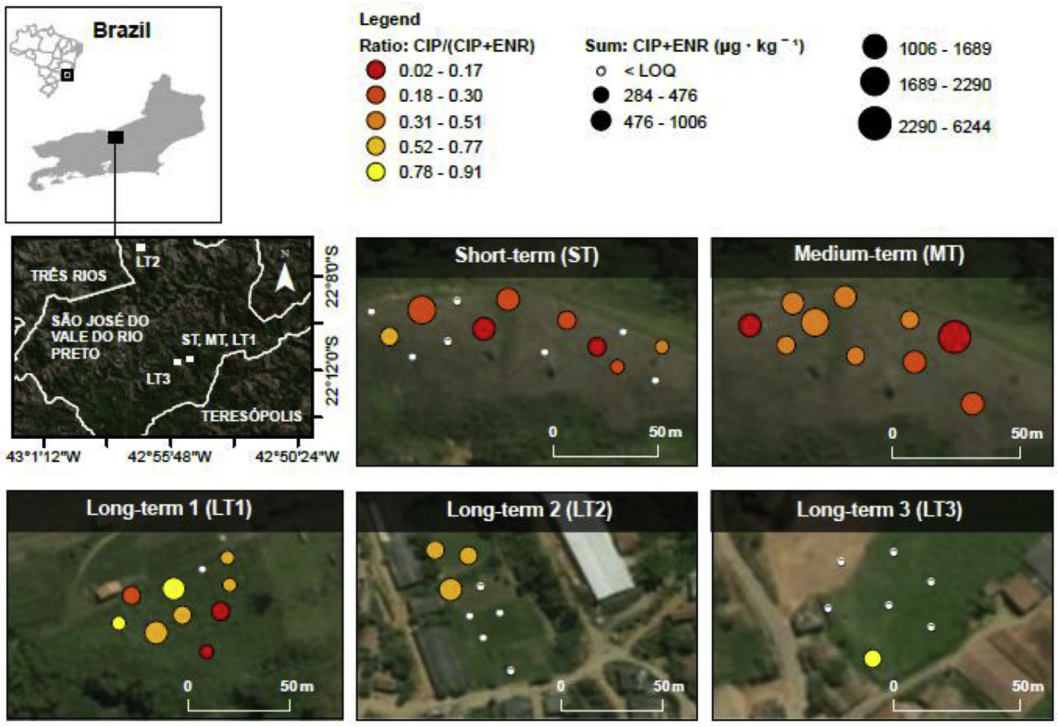

Fig. 3. Study area and soil ENR and CIP concentrations along short to long-term fertilized soils.

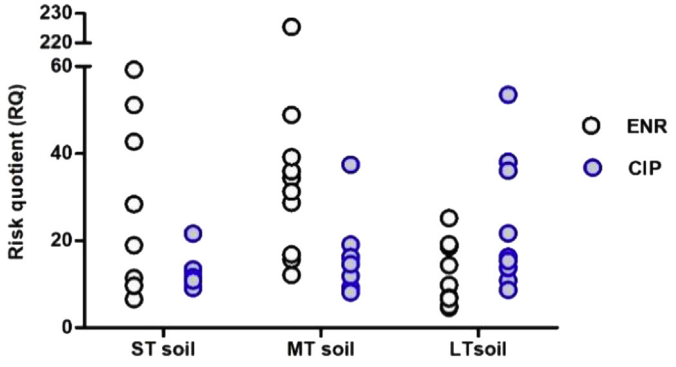

Fig. 4. Risk quotients in short (ST soil), medium (MT soil) and long term (LT soil) fertilized soils.

acidic soils (around pH 5), previous studies pointed out that CEC has an important role on sorption of cationic species of FQs, including ENR and CIP (Rath et al., 2018; Vasudevan et al., 2009; Yan et al., 2012).

The potential migration of ENR and CIP through soil to water was estimated as the predicted environmental concentration for surface water $PEC_{surface\ water}$ (Fig. 5). The limit of 0.1 µg L^{-1} (dashed line) was set to compare with the limits recommended by the Steering Committee of the Veterinary International Committee (VICH) and was established as the groundwater and drinking water standard by the EU (EMA, 2016a; Zhang et al., 2016).

According to Fig. 5, high $PEC_{surface\ water}$ is observed in ST soil, pointing out a risk of ENR leaching above the standard water quality. Followed by a decreased risk tendency over time of poultry litter fertilization. The estimative model considers organic carbon soil content (OC) to estimate equilibrium dissociation constant soil-water (K_d) value. Therefore, the increased soil OC over the years - 2.2% in ST soil to 6.8% in LT soil 3 - can explain the decreased risk of leaching, even with high FQs concentrations in MT soils. Although the influence of soil OC for ionizable compounds sorption is unclear, increased OC is associated with increased organic matter (OM) content (Leal et al., 2012). In turn, OM soil enrichment contributes to higher levels of cation exchange capacity (CEC) (Vasudevan et al., 2009). In the present study both parameters, OM (indirectly by OC content) and CEC were increased in fertilized soils due to continuous poultry litter application (Table 1). Considering that FQs have pK_a around 6–8, in soils with acidic pH (<6), these compounds are present as cationic species and can be attracted through columbic interaction to soil negatively charged sites, such as OM, metal oxides and aluminosilicates (Conkle et al., 2010; Riaz et al., 2018; Vasudevan et al., 2009). Although the loss tendency of SAs in soils was widely described (Doretto et al., 2014; Leal et al., 2013), Sukul et al. (2008) reported an increase in sorption by sulfadiazine in soils with manure, thus reducing its risk of leaching. On the other hand, even with the expected low mobility of FQs in soils, Hu et al. (2010) found CIP concentrations (31.8–42.5 ng L^{-1}) in groundwater from wells surrounded by organic farms reaching 40 m depth. Therefore, the high $PEC_{surface\ water}$ estimated in the present study highlights the concern and need for monitoring aquatic environments in agricultural areas. Zhang et al. (2016) also estimated high $PEC_{surface\ water}$ (>0.1 µg L^{-1}) for CIP in conventional and organic farms with manure application between 4 and 30 years. In this case, there was little variation of fertilization time in soil parameters, such as OM and CEC. In tropical environments summer storm waters are common, which can result in erosion processes, thus increasing leaching and runoff that can mobilize antibiotics, as well as antibiotic resistant genes through drainage basins (Kümmerer, 2009; Su et al., 2018). In this case, water resources used for animal watering, fish farming and as raw water for treatment plants can be the final destination.

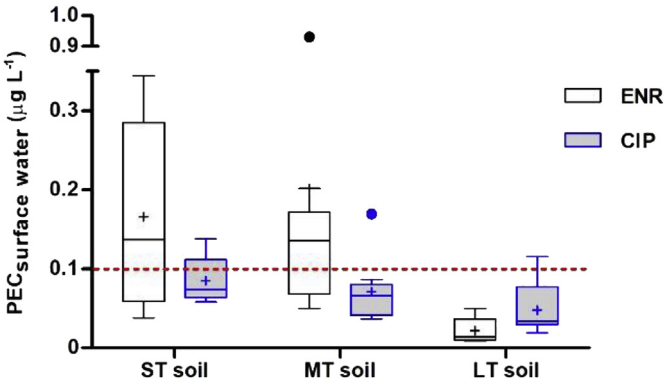

Fig. 5. Boxplots of $PEC_{surfacewater}$ for ENR and CIP from soil sample sets (0.1 μg L^{-1} limit value of water quality standard; + = means).

4. Conclusions

The study results demonstrate that FQs occurrence and risks have changed over years of land use. The FQs accumulation trend associated with increased ecological risks, pointing out the need for management actions to reduce antibiotic levels prior to poultry litter use as fertilizer in soils. The detection of *qnr*S genes in the area with the lowest frequency of antibiotic quantified samples emphasizes the importance of a broader approach to environmental assessment. In this context, not only the target compounds should be considered, but also their biomolecular impacts and changes. Although soil chemical attributes have decreased potential leaching over the years, predicted concentrations above established limit for groundwater and drinking water quality (0.1 μg L^{-1}) highlight the risk of antibiotic contamination through drainage basins. Furthermore, considering the high weathering characteristic in tropical soils and the prediction of increasing extreme climatic events, the possible impacts of antibiotic contamination and PMQR genes transfer to water resources should be monitored. Given the extreme measured antibiotic concentrations, risk assessment models can be important tools for preliminary investigations and plans for further action in critical areas.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2018.11.184.

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