

## REVIEW

# Fluoroquinolone Antibiotics and their Interactions in Agricultural Soils – A Review

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## Abstract:

Fluoroquinolones (FQs) are antibiotics widely used in human and veterinary medicine. The main sources of FQ contamination in agricultural soils result from the irrigation of fields with wastewater and the use of waste sludge and animal manure as fertilizer. Due to their physicochemical properties, these chemicals exhibit strong sorption in soils, presenting low mobility, high persistence and, therefore, long-term biological action in this matrix. This review addresses their main physicochemical and biological interactions in soils, as well as their main biotic and abiotic degradation pathways. In addition, we highlight the possible impacts of FQs on organisms that play an important role in the maintenance of biogeochemical cycles such as soil microbiota, earthworms, and plants. Routes of exposure to human beings through food grown in contaminated soils, and possible exposure to resistant genes derived from microbiota-soil-antibiotic interactions are also discussed. This review emphasizes the need for establishing regulatory limits on FQ contamination sources through fertilization with human and husbandry waste in agriculture soils.

**Keywords:** fluoroquinolones; ciprofloxacin; enrofloxacin; soil microbiota; environmental impact

## 1. Introduction

Quinolones are synthetic antibiotics, which are very effective in the treatment of Gram-negative bacterial infections. Their main mode of action is through the inhibition of the DNA gyrase enzyme that is responsible for preserving bacterial DNA [1]. Nalidixic acid is considered the first generation of commercialized quinolones (Figure 1A). After Nalidixic acid, a second generation of antibiotics were launched onto the market, called fluoroquinolones (FQs), which placed a fluorine atom in the C6 position of the quinolone ring [2]. Since then, FQs have had a broad spectrum of action with moderate efficiency in the treatment of Gram-positive bacteria, acting through inhibition of the Topoisomerase-IV enzyme [1, 2].

The class includes heavily prescribed

antibiotics, some of which are administered exclusively in human medicine (eg. enoxacin, fleroxacin, levofloxacin), others being employed in both human and veterinary medicine (Figure 1B-C) while some specific FQs are only for veterinary use (Figure 2A-C) [2]. The use of FQs have sharply increased in the global pharma market recently, reaching the third place as the most prescribed antibiotics nowadays, commanding 17% of global consumption [3]. Although antibiotics have a great medical advance against biologic infections, the problems related to bacterial resistance represent a huge challenge to maintain the desired effects of these molecules. After their administration to humans and animals, antibiotics, including FQs are not totally metabolized, and are partially excreted (30-70%) as microbiologically active compounds [3].

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Enrofloxacin, an FQ commonly used in Brazilian animal husbandry, undergoes a deethylation reaction in the liver, being metabolized in ciprofloxacin, an antibiotic commonly prescribed for human infections, for instance [2].

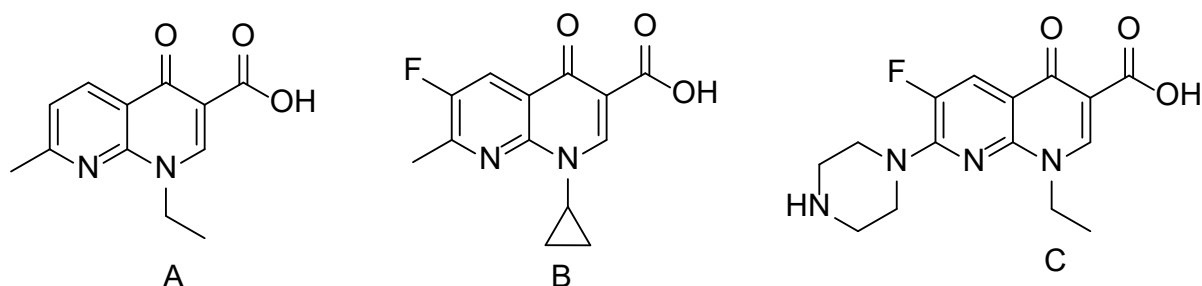
Parental chemicals or its active metabolites can contaminate abiotic and biotic matrices, resulting in diverse environmental impacts. The main sources of antibiotics in soil are the result of the application of urban waste sludge or animal manure as fertilizer [3-5]. These practices solve the problems of urban and animal husbandry waste disposal as well as providing the required nutrients for agriculture. However, widespread gene resistance to antibiotics in agriculture soils has already reported in literature especially when describes irrigated soils with contaminated wastewaters [6].

Many of the resistance genes that challenge human medicine have their origin in common, harmless bacteria from the human environment, whereas the environmental microbiome represents a much larger diversity with more complex resistance mechanisms [7]. In addition, some studies have cited FQ uptake by edible

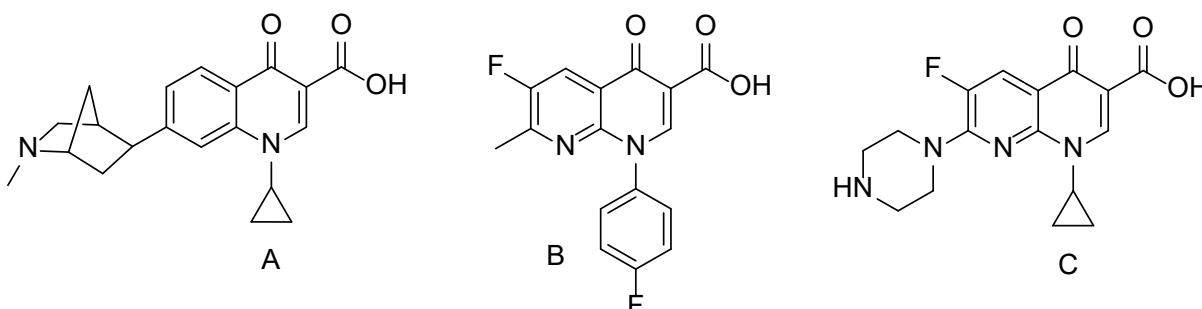
plants grown in contaminated soils [8-10]. In this context, food grown in contaminated soils with antibiotics may be a possible source of these compounds to human being as well as antibiotic resistant strains of microbiota communities.

Previous studies have considered researches on environmental impacts, regarding antibiotic applications in agriculture as a crucial importance issue [11]. In addition to the already described effects, synthetic antibiotics can also inhibit important environmental processes mediated by microorganisms, shifting biogeochemical cycles such as carbon and nitrogen likewise negatively impacts of pollutants biodegradation [12].

The ubiquitous occurrence of these compounds in the environment has caused great concern in the scientific community, thus stimulating multidisciplinary research in recent decades, regarding the risks associated with their potential mobility in contaminated soils, fate and toxicity [5, 13, 14]. The aim of this review is to join and evaluate studies that address the physicochemical and biological interactions of FQs in soils, as well as their effects on soil organisms and on human health.



**Figure 1.** Chemical structures of A) Nalidixic acid; B) Ciprofloxacin; C) Norfloxacin



**Figure 2.** Chemical structures of A) Danofloxacin; B) Difloxacin; C) Enrofloxacin

## 2. FQs Sources from Human and Veterinary Use

FQs are widely prescribed for treating human and veterinary infections, reaching environmental matrices through diverse sources. Among the sources of contamination, we can highlight wastewater from hospitals and domestic sewage [10], but also from domestic effluent without any treatment and from storm water systems. Additionally, some studies have been reported the FQ occurrence in Chinese [15-17], European [3, 18] and Brazilian rivers [19], which can be an antibiotic sources for the agricultural soils during flooding, or even through irrigation.

Due to FQ persistence and, in some cases, the lack of adequate technologies even in the wastewater treated, the antibiotics are only partially eliminated [20, 21]. This class of antibiotics may be heavily adsorbed into sludge, for instance. According to Picó and Andreu (2007), FQ concentrations in raw sewage sludge (1.40 - 2.03 mg kg<sup>-1</sup>) were similar to those found in digested sludge (2.13–2.42 mg kg<sup>-1</sup>), suggesting that effluent treatment is not usually adequate for safe disposal of these compounds [13].

Even in the face of challenges, concerning the large volume of domestic wastewater treatments, Van Doorslaer and co-workers [3] reported that the highest FQ environmental burden is due to their use in animal husbandry. The authors also suggest that irrigation of agricultural soils from treatment plant wastewaters result in lower levels of contamination (by this class of antibiotics) when observed in fertilized soils by animal manure [3].

Currently the overuse of antibiotics in animal husbandry is an important issue for scientific community and international organizations as Food and Agriculture Organization (FAO) and World Health Organization (WHO) [22]. It has been estimated that between 2010 and 2030, there will be an almost 70% increase of veterinary antibiotics consumption worldwide, reaching 105,500 tons/year at the end of the period [23]. According to the authors, 99% increase in antibiotic consumption was also estimated in the same period, through animal husbandry by BRICS (Brazil, Russia, India, China and South Africa).

The concentration of a wide variety of antibiotics, including FQs, in poultry, pig, cattle and horse manure range from 0.01 to 765 mg kg<sup>-1</sup> [5, 14, 24] and among them, poultry farming is the greatest FQ consumer. Enforcing this argue, studies have shown the presence of enrofloxacin (and ciprofloxacin, its main metabolite) in manure or poultry litter, and also in soils fertilized with this material [24, 25]. Antibiotics measurements were also carried out in São Paulo state farms (Brazil), where enrofloxacin was detected in 30% of poultry litter and 27% of soils, ranging from 6.7 mg kg<sup>-1</sup> to 22.9 µg kg<sup>-1</sup>, respectively [14]. Recently, was reported high concentrations of enrofloxacin + ciprofloxacin in poultry litter (100 mg kg<sup>-1</sup>) and in fertilized soils (> 6,300 µg kg<sup>-1</sup>) from Rio de Janeiro upland region [26]. The authors found plasmid-mediated quinolone resistance genes (*qnrS*) in long-term fertilized soils with poultry litter. Although poultry litter and chicken manure are important sources of micronutrients (e.g. copper, zinc, manganese) and macronutrients (e.g. nitrogen, phosphorous, potassium) to crops [27, 28], the input of antibiotics to environment should be reduced before soil application. Another study [31] detected FQs in all agricultural soil samples (n=100) in China, pointing out a wide occurrence of the main enrofloxacin metabolite, ciprofloxacin (104 µg kg<sup>-1</sup>).

Previous studies point out that after poultry litter application, soil and sediments can act as FQs integrating matrices [26]. Although previous studies have reported potential adverse effects on non-target organisms, there are a lack of knowledge about toxicological effects of FQs metabolites in these matrices [29, 30].

In addition, application of husbandry waste as fertilizer in agricultural soils can be a source of contamination to other environmental matrices such as groundwater, surface water and sediments [20, 32]. The high antibiotic concentrations detected in river sediments from agricultural areas, compared to areas without agricultural activities, give us a clearly evidence about the role of surface runoff of these contaminated areas to increase antibiotic levels into aquatic systems [20]. In a study conducted in China, which evaluated the presence of 22 antibiotics in water's rivers, were found higher antibiotic concentration in samples from suburban cultivated areas compared to urban areas, evidencing also a relevant contribution of

antibiotic contamination derived from animal husbandry [33].

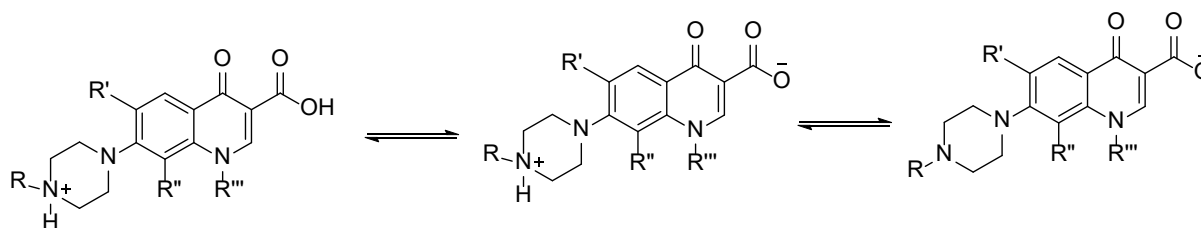
### 3. Physicochemical Interactions and Abiotic Degradation

The persistence and mobility of FQs in soils depends on factors such as degradability (biotic and abiotic) and soil constituent binding capacity. In the soil, FQs fixation occurs mainly through sorption process mediated by organic matter and clay minerals [34]. FQs have low octanol-water partition coefficients (median  $\log K_{ow} < 2.5$ ), which could influence a low sorption potential in soils and sediments, however, some authors reported high affinity and long half-life ( $> 200$  days) of these chemicals in soil/sediment matrices [3, 14].

Leal and co-workers (2013) reported high sorption of FQs in 13 tropical soils, reaching a distribution coefficient ( $K_d$ )  $\geq 544.2$  L  $\text{kg}^{-1}$  [35]. Moreover, high sorption values were observed even in soils with high sand and low organic carbon content (OC). According to the authors, the attributes that most influenced sorption were soil texture and total cation exchange capacity. Vasudevan et al (2009) previous pointed out that cation exchange capacity was the major soil factor

influencing sorption of ciprofloxacin in soils with a pH ranges from 3 to 8 in US agricultural soils ( $n=30$ ) [36].

Due to the high sorption potential of these compounds in soils, parameters such as the octanol-water partition coefficient seem to be limited in predicting their environmental behavior. In fact, according to Van Doorslaer et al. (2013) [3], in the case of ionizable chemicals, such as FQs, electrostatic interactions appear to have greater influence on adsorption processes than parameters related to hydrophobicity. Another property that has relevance on the interaction of these molecules in soils is its amphoteric nature. This group consists of molecules that have two environmentally relevant ionizable functional groups. The 3-carboxyl group ( $\text{pK}_a \sim 6$ ) and the *N*-4 piperazinyl ring ( $\text{pK}_a \sim 8$ ), which makes its sorption process pH dependent [13]. This approach appears relevant since the ionic speciation of FQs changes according to the pH of the water contained in the pores of the soil [36]. In Figure 3, the FQs acid-base balances are presented in three different scenarios according to the pH variation, where the molecule can be in cationic (Fig. 3 - left), zwitterionic (Fig. 3 - center) or anionic form (Fig. 3 - right).



**Figure 3.** Fluoroquinolone structure and acid-basic balance. Adapted from Turiel et al., 2006 [1].

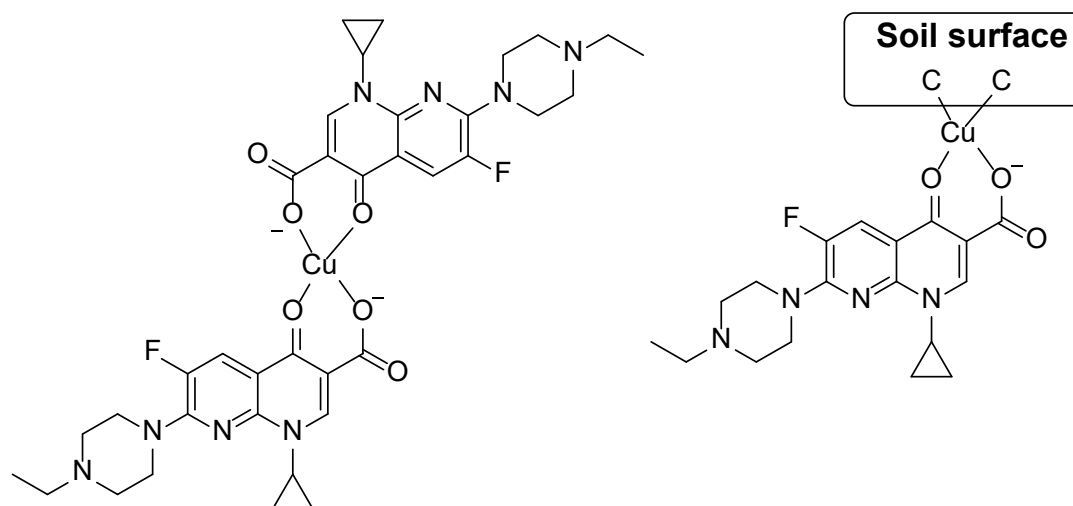
In an acid medium, with a  $\text{pH} < 6$  (Fig. 3 - left), the molecule is in a protonated state. This form is the most common one in acid soils. In this case, the cation exchange involves the electrostatic attraction of the cationic amine group to negatively charged surfaces (eg. clays and organic matter) [36]. According to the same authors, there may also be cation bridging by columbic attraction or complexation of the carboxylic group with cations bound to negatively charged sites and surface complexation of the carboxyl group with surface ions of Al and Fe. In environments close to neutral pH values - between 6 and 8 (Fig. 3 - center), the FQ molecules present both a cationic portion and

an anionic portion. Whereas in alkaline soils, with  $\text{pH} > 8$  (Fig. 3 - right), the molecules present negative charges. In these cases, FQs form complexes with divalent (eg.  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) and trivalent ( $\text{Al}^{3+}$ ) metal cations [1, 13]. Facing these characteristics, the physicochemical interactions of FQs in soils are affected by, among other factors, the nature (acid or alkaline) of the environment where these molecules are inserted.

On other study, Graouer-Bacart et al. (2013) [37] found that enrofloxacin preferentially adsorbed on the soil surface forming a cationic  $\text{Cu}^{2+}$  complex (Fig. 4 - right). According to these authors, the  $\text{Cu}^{2+}$  increased to up 35% the

antibiotic adsorption rate in the soil. They emphasized that the mobility of enrofloxacin was significantly reduced in the presence of the  $\text{Cu}^{2+}$  ion at an environmentally relevant pH and also

proposed the existence of chemical bonding of  $\text{Cu}^{2+} + (\text{enrofloxacin})^2 + (\text{H}_2\text{O})^2$  complex in the soil (Fig. 4 - left).



**Figure 4.** Copper in cationic divalent form in complex with  $(\text{ENR})^2 + (\text{H}_2\text{O})^2$  (left). Enrofloxacin complex with  $\text{Cu}^{2+}$  on soil surface (right); Adapted from Graouer-Bacart et al. (2013) [37].

Due to the high FQ attraction to solid matrices, abiotic degradation processes may be delayed, resulting in their persistence in sediments, soils and animal manure [25]. Among the main degradation processes, photolysis and oxidation by mineral oxides have relevance [3]. In soils, the occurrences of photodegradation processes are mainly expected in soil surface layers. In a study that evaluated the photodegradation of six FQs [38], it was observed that the breakdown of these chemicals in sunlight occurs in the following order: water > soil suspended water > soil. According to these authors, among the main expected photochemical processes, oxidative degradation of the amine side chain was the most relevant one. Similarly, Sturini et al (2012) also pointed out photodegradation as a relevant process for FQs depollution in soils [39]. The authors reported that sunlight exposure to soils contaminated with  $0.5 \text{ mg kg}^{-1}$  of enrofloxacin and marbofloxacin reduced both antibiotics by 80% after 50 h of irradiation. Another possible degradation route occurs through the FQ oxidation by mineral oxides naturally present in soils. According to Sukul et al. (2007) [40], the FQ degradation occurs due to their reaction with manganese oxide via dealkylation and hydroxylation of piperazine moiety.

## 4. Environmental Impacts and Biodegradation

### 4.1 Soil microbiota

Soil constitutes one of the most fascinating ecological systems, where the microorganisms, micro-, meso- and macro-fauna, microflora and plant roots interact among themselves and also with the abiotic part, modifying their surroundings although, usually on a perfect homeostasis. The soil is not an inexhaustible resource and if inappropriately used or mismanaged, be rapidly lost [41]. The main problems associated to the soil are its degradation and contamination. Soil degradation can be defined as a process that lowers the current and/or future capacity of the soil to produce goods or services [42]. Its deterioration are commonly associated to the changes in land use, such as, demographic pressure, deforestation and/or agriculture activities. The physical factors that can cause soil degradation are, for instance, waterlogging, subsidence, soil compaction, crusting and sealing. While, the chemical factors that can disturb the soil are the loss of nutrients and organic matter, soil salinization, acidification and pollution [42].

Highlighting the soil contamination topic, there

are a vast list of products and processes that can cause some damage to the soil's health, such as waste accumulation, excessive use of pesticides, excessive manuring, oil spills, deposition of airborne pollutants, etc. [43]. In the specific case of antibiotics, it can alter the structure and diversity of microbial communities in the soil, which can result in selection of more competitive groups and tolerant to antibiotics [6, 12, 48, 49]. According to Ding and He (2007) [48], there are two main effects of antibiotics on soil microbiota: (a) the first is related to microbial community disturbance, which in some cases may affect certain edaphic functions; and (b) the other considers the possible selection and transfer (vertical and horizontal) of resistant antibiotic genes.

The bioavailability and biodegradability of the antibiotics in soils depend on diverse factors, such as, the pH, temperature, humidity of the soil and nutrient and O<sub>2</sub> contents [10, 13]. Antibiotics such as FQs undergo secondary sorption and diffusion reactions in micro and nanopores of soil, making them unavailable to microbiological contact [51]. This process, called "sequestration" of antibiotics in the soil, reduces the biological contact and, therefore, its toxicity and biodegradability. However, the same phenomenon can increase its persistence and can release continuously small amounts of antibiotics, setting up chronic exposure to the microorganisms present in the soil solution. Moreover, it is known that bacteria are able to re-metabolize antibiotic metabolites into parent compounds, contributing to the persistence of microbiologically active compounds.

It was estimated that only 1g of agricultural soil shelters 10<sup>6</sup> bacteria, while in forest soils, the number is even higher (10<sup>9</sup>) [50]. However, these estimative usually are based on colony-forming unity (CFU) on a specific media and a methodology are not completely reliable. Since the last years of the last century methods based on the molecular fingerprinting have been used for evaluate the environmental biodiversity of samples instead cultivate approaches. Among the most applied techniques available, the terminal restriction fragment length polymorphism (TRFLP) [44] and denaturing gradient gel electrophoresis (DGGE) could be highlighted [45], such as others methods that allow to evaluate the functional genes present on the soils (eg. with a

clone library). However, since 2004, these methods have been replaced by the next-generation sequencing (NGS) approaches (e.g. the 454 pyrosequencer and MiSeq). From these, the MiSeq is one used for microbial biodiversity studies due the low price and the size of fragments sequenced (nearly 500 base pairs). Besides, since 2012, computational tools have emerged (such as, PICRUSt and Tax4Fun) [46, 47] made possible to do a metagenome prediction from 16S rRNA gene sequences. Then, nowadays it is possible inferring accurately the ecosystem health from DNA extracted directly from small environmental samples. The great vision of these micro-ecosystems allow a better evaluation of soil functions, such as organic matter mineralization and nutrient cycling. In these cases, some of the indicators monitored are microbial biomass, basal respiration and O<sub>2</sub> consumption induced by addition of substrate, nitrification through the production of nitrate, enzymatic activities, as well as other parameters [52]. On the specific case of factors that may influence FQ impacts on soil microbiota, we can highlight its bioavailability; the time of exposure to it; and the multiple presence of other compounds commonly found in agricultural soils (eg. antibiotics, pesticides and metals).

A study that evaluated ciprofloxacin effects (0-200 mg L<sup>-1</sup>) on sediments microbiota, have found a selective biomass increase favored by sulfate reducing bacteria and Gram-negative bacteria [53]. Follow them, in a stress situation, the functional soil microbiota can be maintained due to the functional redundancy that often occurs in the soil. For instance, the suppression of some organisms can favor the development of others, which may also to be important to maintain the soil edaphic function. In another study, a soil (haplic chernozem-type) artificially contaminated with 0.2 mg kg<sup>-1</sup> ciprofloxacin showed a reduction of soil respiration to less than 50% compared to a control whose effect was observed since the 12<sup>th</sup> day of incubation [12]. Additionally, they observed no difference in soil respiration when was used different antibiotic concentrations (0.2, 2 and 20 mg kg<sup>-1</sup>), suggesting that there is a selective suppression and adaptation independent of the doses used. In the same study, the authors also have reported a low ciprofloxacin mineralization (only 0.09%) after 93 days of incubation. These results suggest a low biodegradability due to the

negative effects on the microbiota, in addition to other physicochemical factors, such as a strong FQ adsorption on soil.

In another study, Parente et al. (2018) [54], observed in spiked soils (A horizon of a Haplic Arenosol) with ciprofloxacin that the soil respiration ( $O_2$  consumption) increased (> 100% respect to control) at lowest concentration ( $0.1 \text{ mg kg}^{-1}$ ) and had a significant reduction with  $1 \text{ mg kg}^{-1}$  concentration. Furthermore, a recovery respiratory function was also observed in 10, 100 and  $1,000 \text{ mg kg}^{-1}$  doses and inhibition in very high concentrations ( $5,000$  and  $10,000 \text{ mg kg}^{-1}$ ). In the same study, the authors observed that ciprofloxacin negatively affected the nitrification function only at very high doses ( $5,000$  and  $10,000 \text{ mg kg}^{-1}$ ). The same authors also evaluated the biodegradability of the same compound at the beginning of the experiment and at the end (28 days). At  $100 \text{ mg kg}^{-1}$  dose there was a 56% reduction of ciprofloxacin over the 28 days of incubation. Due to the limitation of the experiment, the authors could not state how much was due to biodegradation and how much was due to abiotic degradation.

According to another study, the application of swine manure contaminated with lomefloxacin, enrofloxacin, norfloxacin, ciprofloxacin, and ofloxacin increased bacterial resistance [55]. There was a positive relationship between a greater number of manure applications and the number of bacteria resistant to ciprofloxacin. The same relationship was also observed in the abundance of plasmid-mediated quinolone resistance. In addition, bacteria resistant to ciprofloxacin were detected in the control soil, evidencing the existence of native bacteria potentially resistant to FQs. It is important highlight that metagenomic data are revealing a large reservoir of bacteria or even resistant gene to antibiotics in the soil [56, 57], called "soil resistome". In fact, there is growing evidence that the occurrence of large genetic diversity occurring on small spatial scales facilitates horizontal transfer and the possible spread of antibiotic resistant genes, including bacteria pathogenic to humans and animals [57].

Concerning fungal activity on FQ degradation, detected compounds in soils, such as ciprofloxacin and enrofloxacin, showed remarkable biodegradation by enzymatic systems

mediated by hydroxyl radical's characteristic of brown rot fungi [40]. In another study, a non-pathogenic fungus *Pestalotiopsis guepini* was able to biodegrade norfloxacin in a poultry litter sample with substrates of rice hulls and corncobs in 20 days of incubation [58]. According to the authors, with rice hull substrate, the fungus produced four metabolites of norfloxacin (7-amino-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinolone-3-carboxylic acid, deethylene- *N*-acetylnorphloxacin, *N*-formylnorphloxacin and *N*-acetylnorloxacin), whereas with corncobs there were just two metabolites (*N*-formylnorphloxacin and *N*-acetylnorloxacin) detected. In another investigation, the biodegradation of enrofloxacin by the zygomycota fungus *Mucor ramannianus*, common in soil and decomposing organic matter, was evaluated detecting as final products: *N*-oxidation, *N*-dealkylation, *N*-acetylation and the breaking of the piperazine ring [59].

Another approach still to be taken comes from the fact that agricultural soils are regularly receiving massive loads of pesticides. According Kim and co-authors [60], although pesticides degradation through soil microbiota is an important metabolic pathway in soil, the presence of antibiotics may affect its microbial activity, and consequently may influence the biodegradation rate of both. Besides, a reduced degradation of these xenobiotics on the soil ecosystem can result in potential leaching and contamination of groundwater by pesticides and antibiotics.

## 4.2 Earthworms

Agricultural soils are common habitats for diverse groups of edaphic macroorganisms. Among the different groups, earthworms play an important role in the dynamics of organic matter, in the maintenance of soil structure, among other ecological and environmental functions that result in greater sustainability of agricultural soils. According Mougín et al. [61], the inclusion of two species of oligochaeta - *Aporrectodea caliginosa* and *Aporrectodea longa*, in an experiment with soil (Luvisol) fertilized with pig slurry and [ $^{14}C$ ]ciprofloxacin ( $25$  and  $250 \mu\text{g kg}^{-1}$ ) increased antibiotic mineralization by 5 to 8 times. In addition, the worm's displacement activity transferred 40% of the antibiotic from the upper layer to the lower layer of the soil, altering the

distribution pattern of the contaminant in the soil profile.

In tests with *Eisenia fetida* in farm soils with enrofloxacin ( $10 \text{ mg kg}^{-1}$ ), transient oxidative stress and decreased respiratory activity were observed after an eight-week exposure [62]. Over the same period, the authors noted a positive correlation between increasing enrofloxacin concentrations in soils ( $0.1$ ,  $1$  and  $10 \text{ mg kg}^{-1}$ ) and cadmium bioaccumulation (Cd is present as an impurity in phosphate fertilizers) in worms. In this case, the observed effects were: oxidative stress; decrease in the length of the wormhole and  $\text{CO}_2$  production. The authors also highlight the importance of studies about antibiotic contamination in agricultural soils, including the effects on exposure to multiple contaminants. Another study conducted by Huang et al. [63], the effects of different concentrations of divalent copper ( $\text{Cu}^{2+}$ ) + ciprofloxacin (pH 6) solutions in quartz sands in *Eisenia fetida* were tested. The authors observed that increasing antibiotic concentration reduced Cu ion concentration in the external solution and raised Cu concentration in worms. Then, although ciprofloxacin had no relevant toxic effects directly to the worms, its presence increased Cu absorption.

#### 4.3 Terrestrial plants

Undoubtly, the presence of antibiotics in agricultural soils affect plant growth. In addition, absorption of antibiotics by plants is a potential source of human exposure through food. After absorption by the root system, antibiotics are transported by passive diffusion through xylem (shoots and leaves) and phloem (fruits) [10]. According to Hu et al. [64], antibiotics are distributed by vegetative parts in the following sequence: leaf > stem > root.

A study evaluated the effect of ciprofloxacin contamination on Haplic Arenosol soil on the germination and root elongation of *Allium cepa* L. (onion), *Lolium perene* L. (ryegrass) and *Raphanus sativus* L. (radish) [54]. The elongation of the onion root, a monocotyledon, was more affected than leaf growth at all doses ( $0.1$ ,  $1$ ,  $10$ ,  $100$ ,  $1000$ ,  $5000$  and  $10,000 \text{ mg kg}^{-1}$ ). In addition, a significant reduction, respect to control, was observed only at the highest concentrations ( $5,000$  and  $10,000 \text{ mg kg}^{-1}$ ). Ryegrass was not

affected by ciprofloxacin, even at the highest dose. While root growth of radish showed an increase over control at  $1$  and  $100 \text{ mg kg}^{-1}$ , and inhibition of stem and root growth occurred only at high doses ( $5,000$  and  $10,000 \text{ mg kg}^{-1}$ ). The same response was also observed in soil microbiota and in other studies with plants [10, 54, 65]. The authors found that ciprofloxacin effects were selective in relation to the species studied. It was also possible to estimate the half maximal effective concentration ( $\text{EC}_{50}$ ) for onion root, established in  $10 \text{ mg kg}^{-1}$  of ciprofloxacin. At this dose, this compound is classified by the European Community Regulation N°. 1272/2008 as "Hazardous to terrestrial organisms" [66]. Although this regulation is not specific for antibiotics, it should be considered as a safety parameter for human and environmental health.

The absorption of the antibiotics ciprofloxacin, norfloxacin and ofloxacin were evaluated for the following species of edible plants: *Solanum tuberosum* L. (potato), *Daucus carota* L. (carrot) and *Triticum vulgare* L. (wheat) grown in loamy and loamy sand soil [67]. Some results of this study should be highlighted: 1) Accumulation of ofloxacin with  $500 \mu\text{g kg}^{-1}$  resulted in uptake of  $80 \mu\text{g kg}^{-1}$  in carrot and  $90 \mu\text{g kg}^{-1}$  in potato, both in loamy sand soil; and 2) The mean concentration of  $160 \mu\text{g kg}^{-1}$  in carrots cultivated with  $1,000 \mu\text{g kg}^{-1}$  of ofloxacin in loamy sand soil was higher than the maximum limit of residues (MLR) used as a parameter by the authors.

Lillenberg et al. (2010) [9], have cultivated three edible species - *Lactuca sativa* L. (lettuce), *Hordeum vulgare* L. (barley) and *Cucumis sativus* L. (cucumber) in soils contaminated with enrofloxacin and ciprofloxacin, following distinct concentrations:  $10$ ,  $20$ ,  $50$  and  $500 \text{ mg kg}^{-1}$ . According to the authors, there was absorption of enrofloxacin by all species. In addition, at the end of a treatment with  $10 \text{ mg kg}^{-1}$ , a lettuce sample had the maximum concentration of  $44 \text{ mg kg}^{-1}$ , evidencing bioaccumulation over the vegetative period. Similar results were also previous observed by Migliori et al. (2003) which reported uptake of enrofloxacin by edible plants [65]. Moreover, the authors pointed out that *Cucumis sativus*, *Lactuca sativa* and *Phaseolus vulgaris* biologically converted 25% of enrofloxacin absorbed into its main metabolite, ciprofloxacin.



## 5. From Soil to Human Exposure

The overuse and misuse of antimicrobials in veterinary medicine plays a key role in the spread of antibiotic resistant bacteria, especially enteric pathogens [68, 69]. The United States, the world's largest chicken producer, has banned enrofloxacin administration in its production system since 2005. Enrofloxacin is associated with the development of resistant bacteria of the genus *Campylobacter* in digestive tract of animals and the possible transfer to human microbiota [70]. However, health risks associated with the presence of antibiotic residues and bacterial resistance are not only related to food consumption. The authors point out that many resistant bacteria are present in agricultural environments and can affect humans through complex pathways of environmental exposure [71].

Human exposure to FQs present in soil can occur through two main pathways. The first refers to the unintentional absorption of antibiotics, or biologically active metabolites, through contaminated food and water. In fact, as described before, previous studies confirm the absorption and bioaccumulation of FQs by edible plants grown in contaminated soils [8, 9]. With regard to the contamination pathway in drinking water, the data suggest that the transfer of FQ from soil to groundwater is unlikely, mainly due to its high sorption in this matrix. Although there is an extensive occurrence of FQs in surface water [3, 20, 33], the limited reported occurrence in drinking water [72] suggests that this is not the most likely route for unintentional FQ intake.

A second pathway exposure is possible due to the transfer of viruses and antibiotics resistant bacteria through direct contact with the soil [73]. In these cases, antibiotic resistant strains exposure can occur via consumption of food and water, and exposure to contaminated areas. According to Pourcher et al. (2014) [74], strains of *E. coli* resistant to ciprofloxacin survived for at least three months in soil fertilized with chicken manure. The authors concluded that animal manure application has high microbial biomass and is an important route of potential pathogens to the soil, including bacteria (eg. *E. coli* O157:H7) and enteric viruses (eg. rotavirus). The persistence and mobility of these pathogens in environmental matrices is a big concern to the

maintenance of environmental quality, food safety and human health. However, Chang et al. (2015) [75], stated that limited studies are available for establishing causal mechanisms between the amount of antibiotics used in animal production, their impact on soil microbiota, specifically with regard to the selection of resistant bacteria, and their possible consequences to human health.

## 6. Conclusions

FQs are widely administered antibiotics in human and veterinary medicine. These compounds are biologically metabolized and products of their partial biodegradation (microbiologically active compounds) are excreted into environment. Their main path of abiotic degradation is photolysis, which is waited to occur only in upper soil layers. Their persistence in soil matrix and the complexity of biota-soil-antibiotic interactions opens up a vast field of research into possible impacts on terrestrial organisms and the mechanisms involved in their (bio)degradation. It is important to highlight the impacts that these compounds may cause, such as: selective pressure favoring the development of resistant bacterial strains, including human pathogens; antibiotic mobilization to other environmental compartments; uptake into biological systems, affecting environmental functions and potential toxicity to non-target organisms. In this context, regulatory limits must be set for antibiotic contamination, specifically FQs, in sewage sludge and animal waste applied as fertilizer in agricultural soils.

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