EUROPEAN MASTER IN QUALITY IN ANALYTICAL LABORATORIES

ENROFLOXACIN IN SOILS: DETERMINATION OF SORPTION PARAMETERS BY LIQUID CHROMATOGRAPHY

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Barcelona, February 2010

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Barcelona, February 2010

ACKNOWLEDGEMENTS

En primer lugar, quiero agradecer a la Dra. Mercè Granados y al Dr. José Luís Beltrán su interés, orientación y dedicación en este trabajo y a todos los profesores que forman parte del grupo de Fluorescència.

Secondly, I would like to thank the EMQAL coordinators Dr. Esteban and Dr. Cavaco for the opportunity to participate in this master program.

A Alba por su paciencia y ayuda desde el primer día. También a Vanesa, Kumar, Mauricio, Dani y Fernando por la compañía y buenos momentos en el laboratorio. A Marc, compañero de suelos, muchas gracias por ayudarme a pesar de la distancia.

A Sílvia le agradezco todo su apoyo y el haber hecho estos 6 meses tan divertidos, dentro y fuera del laboratorio. Nunca me voy a olvidar de nuestras "aventuras" y risas constantes.

Also, to all my EMQAL colleagues for the good times we spent together in this journey. It was an honor to work and learn so much from each and every one of you. Even though we will be spread around the world, I hope we meet again somewhere.

Aos portugueses em Barcelona: Xana, Inês, Mé, Mary e Zé Maria por nunca me deixarem sentir sozinha e por todos os momentos divertidos que partilhámos.

À Mãe e ao Pai por serem os melhores pais de sempre, por todo o amor e preocupação e pelo exemplo de vida que são. Muito obrigada por tudo o que me ensinaram e proporcionaram, e por me apoiarem, incondicionalmente, nos bons e maus momentos.

Agradeço aos meus avós todo o carinho e conselhos experientes, que guardarei comigo para sempre. A todos os tios e primos um muito obrigada por nunca se esquecerem de mim, mesmo à distância.

Por fim, quero agradecer a todos os meus amigos pela paciência e apoio que me têm dado ao longo dos anos, e por fazerem de mim uma pessoa mais completa.

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SUMMARY

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There are several parameters influencing the sorption of analytes to soils. In this Master project, the sorption behavior of enrofloxacin (ENR) was characterized in six soils of basic pH and different properties, through the study of sorption isotherms. The Freundlich model fitted all the data, while the Langmuir equation was adjusted only to the soils showing an L-type isotherm. The values of experimental distribution coefficients (K_d) obtained for ENR ranged from 0.69 to 2.04 L/g, in agreement with the ones found in the literature. Principal component analysis was performed in the set of soils, and the properties that appeared to have a greater influence in ENR sorption were the content in Fe, Al and Mg oxides. In order to determine the role of organic matter (OM) in the ENR sorption process, one of the soil samples was amended with different amounts of humic acids. The values of K_d obtained seem to indicate an increase in sorption with the increase in OM. Further investigation is needed to confirm these findings.

1. INTRODUCTION

1.1. PHARMACEUTICALS IN THE ENVIRONMENT

With the developments in science, new analytical methods have the ability to detect smaller amounts of chemicals in environmental matrices. As a result, recent studies are revealing the presence of drugs, personal care products, and common everyday use substances, generally referred to as “emerging contaminants". These can be broadly defined as any synthetic, naturally occurring chemical, or any microorganism that is not commonly monitored in the environment, but has the potential to enter the environment and cause known or suspected adverse ecological and/or human health effects [1, 2]. Like the name suggests, “emerging contaminants” are newly recognized and their fate and effects are less well-known, so it is important to provide more information for the evaluation of their potential threat to the environment and human health. Detection of these contaminants in the environment is particularly challenging because of the low detection limits required, the complex nature of the samples, and the difficulty in separating these compounds from interferences [3].

In the 1970’s, for the first time, pharmaceuticals were detected in the environment. During the 80’s not much interest was shown towards this issue, and it was only in the 1990’s when the presence of pharmaceuticals in the environment, and the potential adverse effects these may have, was investigated to a greater extent [4].

Pharmaceuticals can enter the environment through two major sources: from human and veterinary use (Figure 1).

Human pharmaceuticals can go into the sewer system as excreted non-metabolized drugs in urine and feces and attend sewage treatment plants, or enter directly the sewer system as surplus medical substances, considered waste.

There are four main possible fates for human pharmaceutical residues going through a sewage treatment plant [5]:

- Complete mineralization to CO_2 and water
- Partial degradation of the substance, being the rest retained in the sludge or soluble in the treated water
- Degradation to a persistent form that passes the treatment plant, and ends up in the aquatic environment or is retained in the sludge

- The pharmaceutical isn't degraded and passes the treatment plant unaltered or is retained in the sludge.

Unfortunately the complete mineralization is quite unusual, meaning they are not, or are only incompletely, broken down to water, carbon dioxide and inorganic salts [6]. Depending on their physico-chemical behavior, the pharmaceuticals and/or metabolites may end up in the treated waste-water, and finally in the surface waters, or sludge. This resulting sludge is classified according to its composition, and its final fate will depend on it. Often it is used as fertilizer in agricultural soils, enclosing serious consequences.

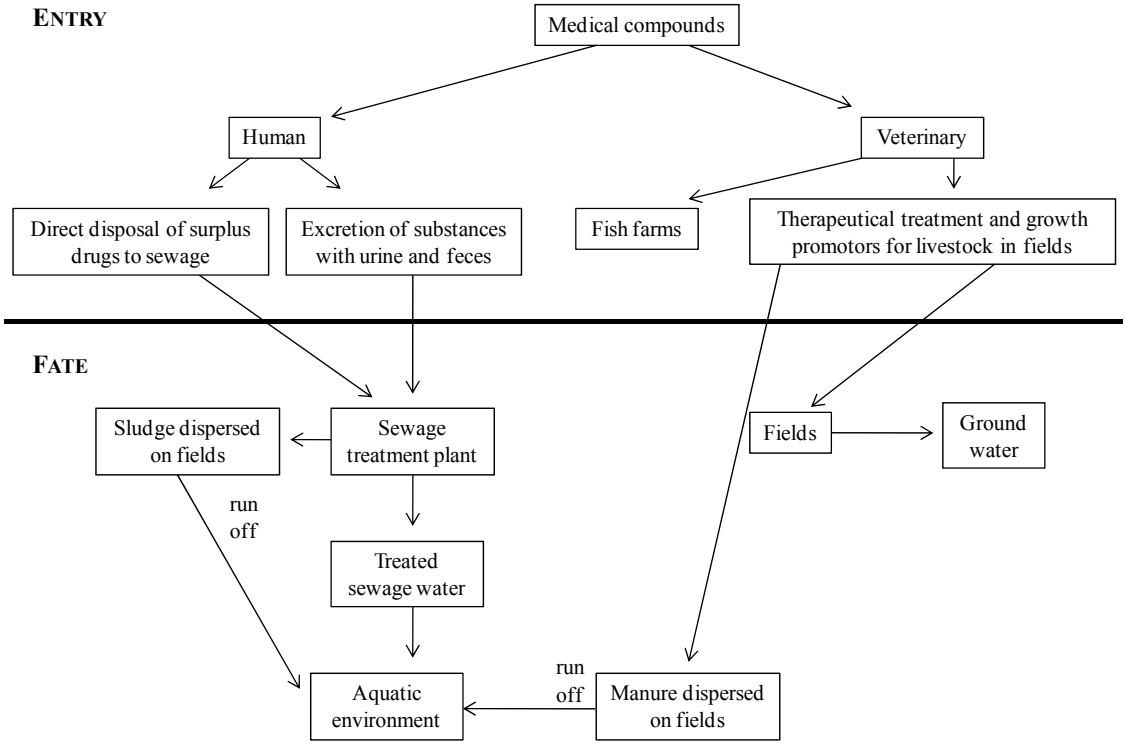

Figure 1 – Entry and fate of both human and veterinary drugs in the environment (adapted from [5]).

Veterinary pharmaceuticals are used in two main situations: as therapeutic treatment or growth promoters used in livestock, and in fish farms. In the first case, the drug residues will be directly excreted onto fields through urine and feces, or end up in manure spread on fields as fertilizer, affecting soil organisms. Furthermore, it is possible that runoff from the fields occurs, due to the rain, contaminating the aquatic environment and reaching the ground waters. In the particular case of veterinary

pharmaceuticals in fish farms, there is direct contamination of the environment, since the most employed method to avoid or treat fish diseases is to use the pharmaceuticals as feed additives. Even though the aquatic environment is very wide and will dilute the drug levels, a large portion of the medicated feed is deposited in the sea bed, affecting the aquatic organisms.

Summarizing, pharmaceuticals can enter the environment into surface waters, or into the soil compartment. In this process, soils function as chemical and biological "filters" that lessen the environmental impact of chemicals introduced into the biosphere by design or accident. Thereby, soils form a first line of defense against leakage of these compounds into surface and ground waters [7, 8]. However, depending on the interactions of the pharmaceuticals with the soil, and thus on their mobility, runoff to the aquatic environment can take place.

The chemical properties of pharmaceuticals, engineered to resist rapid metabolism in the body to ensure adequate pharmacological effect, may also be responsible for their environmental persistence. Although acute toxic effects are unlikely, continual exposure to low doses of pharmaceuticals may produce subtle, long-term effects on all species [9]. Moreover, the continuous discharge of generally persistent xenobiotics in the environment will result in their accumulation, increasing their concentration in time [10].

As to attenuate the effects of pharmaceuticals in the environment, work has been done in the fields of risk assessment and risk management, for instance [11]:

- The proposal of strategies to eliminate pharmaceuticals from wastewater or from the effluent of sewage treatment plants
- The employment of technical management measures such as oxidative or photolytic effluent treatment
- The introduction of the concept of "green pharmacy", which consists in developing new pharmaceuticals that are "benign-by-design" and have less impact on the environment. However, creating these new biodegradable drugs, while ensuring their stability in the pharmacy shelf, is not an easy task and therefore not many compounds are available so far [12].

There are several types of pharmaceuticals present in the environment, such as hormones, analgesics, antibiotics, antiepileptic drugs, beta-blockers, blood lipid regulators, contrast media, cytostatic drugs, anxiolytics, anti-depressants, and diuretics [13]. In Table 1 some of the different types of drugs found in water environments are represented, along with their concentration levels.

Table 1 – Concentration of different drugs (in µg L^{-1}) as measured in waste-water, surface water, ground water (GW), and drinking water (DW) [14].

Active substance/ Group	Waste-water	Surface water	Ground water/ Drinking water
Analgesics/ Antirheumatic agents	2.4 - 20	≤ 0.5	0.006 (DW)
Antibiotics	0.1 - 1.7	≤ 6	
Lipid lowering agents	≤ 1.7	0.55	0.07 - 0.17 (DW) 7.5 (GW)
Psychopharmacological agents	≤ 6.1		
Cytostatic agents	≤ 5	≤ 4	
X-ray contrast media		9 - 100	

Even though antibiotics are not the most common pharmaceuticals in the environment and are only found in low concentrations, they are a major threat for inducing resistance in bacterial strains, and thus their presence in the environment is a major concern [15].

1.1.1. ANTIBIOTICS

Antibiotics are chemical compounds with pharmaceutical activity, which are used as active principle in several drugs [16]. They have the ability to kill bacteria and other microorganisms (bactericidal activity), or to inhibit their growth and proliferation, allowing the body's natural defenses to eliminate them (bacteriostatic activity) [17].

According to their chemical structure, antibiotics can be classified as:

- Beta-lactams: penicillins, cephalosporins (1st generation, 2nd generation and 3rd generation) monobactams and carbapenems
- Aminoglycosides
- Tetracyclines

- Macrolides
- Sulfonamides
- Quinolones
- Azoles.

Additionally, antibiotics can be grouped by mechanism of action. The three main mechanisms of action for antibiotics are [18]:

- Inhibition of cell wall synthesis: beta-lactams
- Inhibition of protein synthesis: aminoglycosides, tetracyclines and macrolides
- Inhibition of nucleic acid synthesis: sulfonamides, quinolones and azoles.

Because of the widespread use (from human or veterinary to horticulture) and misuse of antibiotics, bacteria are constantly exposed to these drugs. While many bacteria die with antibiotics, this continuous exposure contributes to the development of bacterial resistance to the drugs' effects. Resistant microorganisms are the ones able to multiply in the presence of drug concentrations higher than the concentrations in humans receiving therapeutic doses. Resistance to an antibiotic may be inherent in a particular bacterial species, or may be acquired through mutations or acquisition of genes for antibiotic resistance that are obtained from another organism. These resistance genes encode several mechanisms that allow bacteria to resist the inhibitory effects of specific antibiotics. This is a main concern because if bacteria become resistant and don’t respond to particular antibiotics, the treatment of humans will be compromised. In order to control resistance there must be a reinforcement on the appropriate use of antibiotics, which would maximize the clinical therapeutic effect, and minimize both drug-related toxicity and resistance itself [17, 19, 20].

One of the most widely prescribed groups of antibiotics in human medicine, and to a lesser extent in veterinary medicine, is the quinolones due to their safety with good tolerance and broad antibacterial spectrum [21]. Quinolones are classified as synthetic antibiotics and were discovered in the 1960s while anti-malaria pharmaceuticals were being developed. There is a basic structure common to all quinolones, shown in Figure 2.

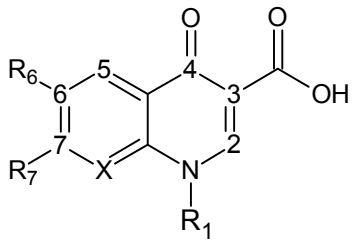

Figure 2 – Basic structure of a quinolone.

The first quinolone discovered was nalidixic acid (in 1962), composing the first generation of quinolones, along with other compounds similar in antimicrobial range and pharmacokinetics.

So far four generations of quinolones have been developed, and more compounds are being studied. Within the diversity of their various ring structures, the quinolones have common functional groups that are essential for their antimicrobial activity, and should not be altered in order to keep the basic mode of action of the drug. These are positions 2, 3 and 4 [22]. Despite this, modifications in other positions have been produced, resulting in compounds with different physical, chemical, pharmacokinetic, and antimicrobial properties. The present work is focused on the fluoroquinolones (FQs) – distinguished by a fluorine atom at the 6-position, enhancing the activity against both gram-negative and gram-positive bacteria, as well as mycoplasmas and chlamydiae [17]. Their mechanism of action is to inhibit the activity of bacterial (but not human) DNA gyrase, preventing the supercoiling of DNA, a process that is necessary for compacting chromosomes into the bacterial cell [18]. They are bactericidal, exhibiting concentration-dependent activity, which is ideal around 0.1-10 μg/mL [17]. Figure 3 represents some examples of FQs.

The primary mechanisms of degradation of FQs are photodegradation, sorption and biodegradation. Because administered FQs are excreted largely unchanged (generally <25% metabolized), they are expected to enter the environment mainly via human and animal excretion in urine or feces [23, 24].

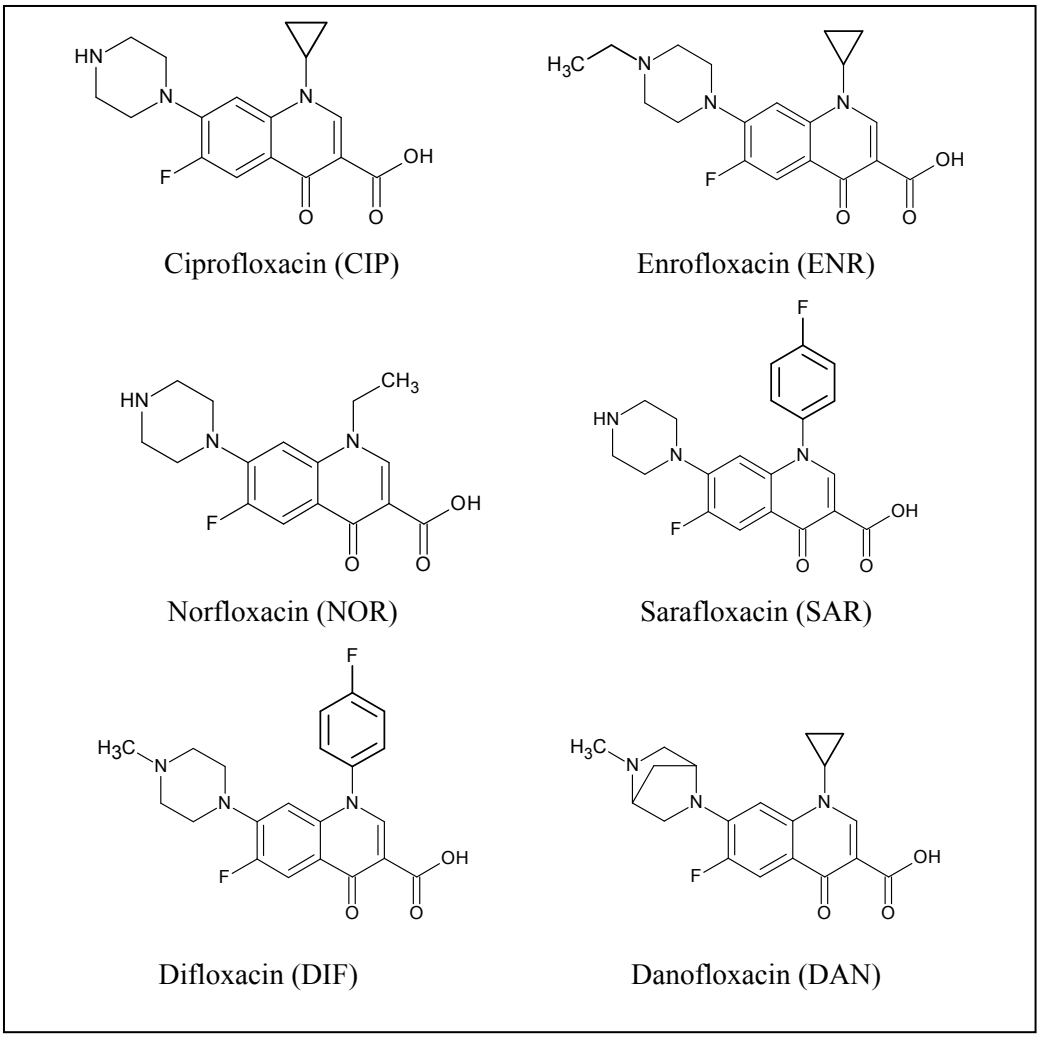

Figure 3 – Examples of fluoroquinolones.

FQs have an acidic carboxylic group, with reported pKa values in the 5.5–6.6 range, and an amino group with pKa values for the protonated amino form in the 7.2–8.9 range. Due to the zwitterionic character of FQs, the deprotonated carboxylic group prevails at typical soil pH values of 5–9, and it is assumed to be responsible for the high distribution coefficients of these compounds in soils [25-29]. Figure 4 shows the protolytic equilibria for the FQ enrofloxacin (ENR).

This study is focused on the sorption to soil of ENR (Figure 3), a FQ widely employed as a veterinary antibiotic. Its primary degradation metabolite is ciprofloxacin (Figure 3), which has a wide application in human medicine, and is produced by N-deethylation of the ethylpiperazine ring [30]. ENR, like ciprofloxacin, is highly photodegradable – with half-lives from 5 minutes to 5 hours, depending on pH, light

intensity, organic matter (OM), FQ level and phosphorous level – and readily adsorbs onto soils, delaying its biodegradation [8, 23].

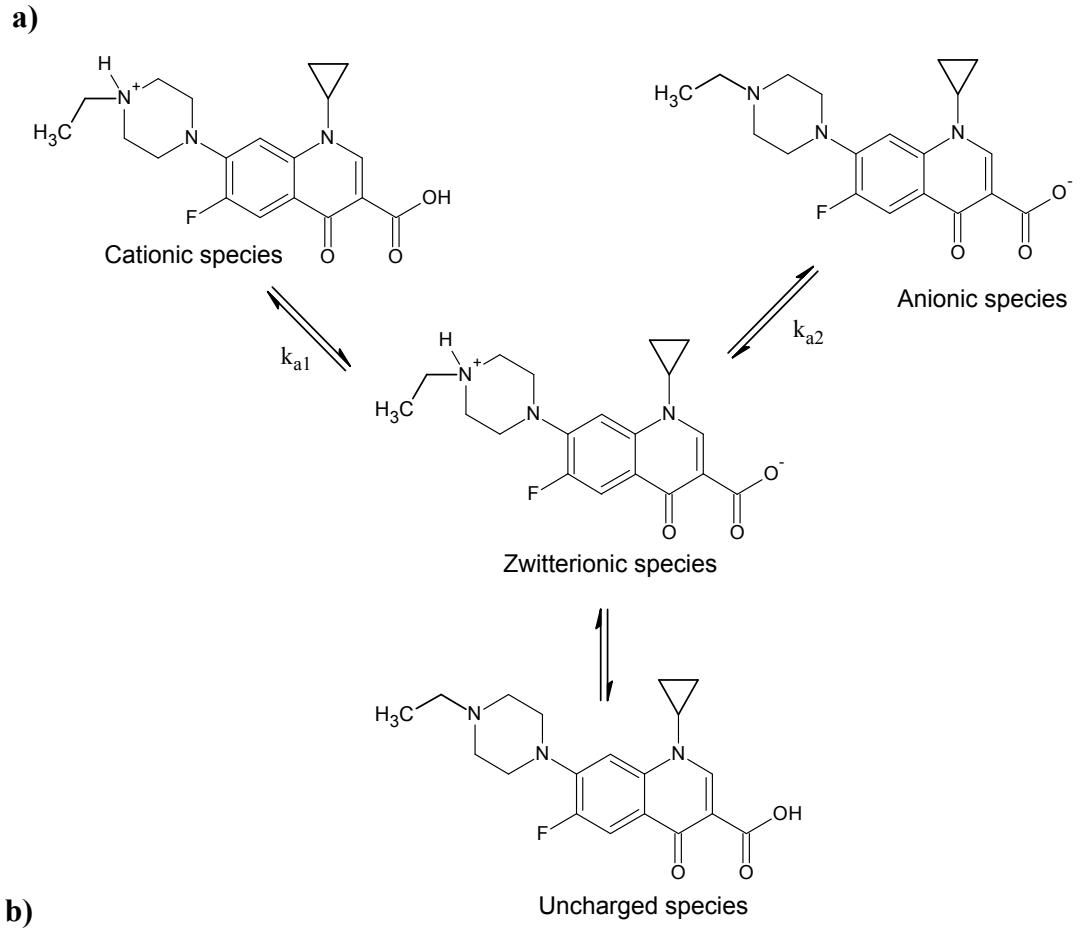

Figure 4 – a) Protolytic equilibria of ENR analogues, where k_{a1} and k_{a2} represent the dissociation constants; b) Distribution of ENR species

1.2. THE WATER-SOIL SYSTEM: SORPTION STUDIES

When a contaminant is present in the soil, the study of the sorption process is essential to estimate the environmental risk, based on the bioavailability of the contaminants, since it will be affected by the way it interacts with the soil.

Sorption is defined as the removal of solution chemical species from water by surfaces (e. g. metal oxides, clays, and soils) through processes such as adsorption, hydrophobic interactions, ion-exchange, and precipitation [31].

Sorption is an important process for deciding the ultimate fate of chemicals in soils. The extent of sorption is related to various soil properties, including organic matter content, texture, cation exchange capacity (CEC), and pH [10]. Experiments have shown that sorption reactions in soils are typically rapid, operating on time scales of minutes or hours, but they can also undergo a slower process called aging. Aging occurs when the transfer process from the aqueous phase to the soil exhibits long-time "tails" that extend over days or even weeks [32].

Since sorption is a process involving a two-phase system, it has a distribution coefficient, which represents the reversible sorptive exchange of chemicals between the water phase and a solid-phase sorbent, either soil or sediment [27]. The sorption process can be characterized by determining its distribution coefficient (K_d). It is defined as the ratio between the analyte's concentration in the solid phase (C_s) and its concentration in the aqueous phase (C_w), at equilibrium, as shown in equation 1.

$$K_d = \frac{C_s}{C_w} \tag{1}$$

The K_d is a key parameter in risk analysis since it determines the mobility of a compound in a medium. According to its definition (equation 1) a high value of K_d indicates that the compound is highly adsorbed to the soil, decreasing its mobility, and also its bioavailability. On the other hand, low K_d values indicate a weak sorption, increasing the risk of contamination in aquatic environments.

Because sorption involves a variety of interactions between the substance and the soil matrix, its magnitude depends on the soil and the solution composition. The range of K_d for ENR found in the literature is of 0.27–0.97 L/g [29].

A sorption isotherm is a graph of the equilibrium concentration of a compound adsorbed, plotted against the equilibrium solution concentration of the compound at fixed temperature, pressure, and solution conditions [33]. Isotherms allow for an evaluation of the environmental risks that a certain substance in soil causes. There are four general categories of isotherm curves, shown in Figure 5.

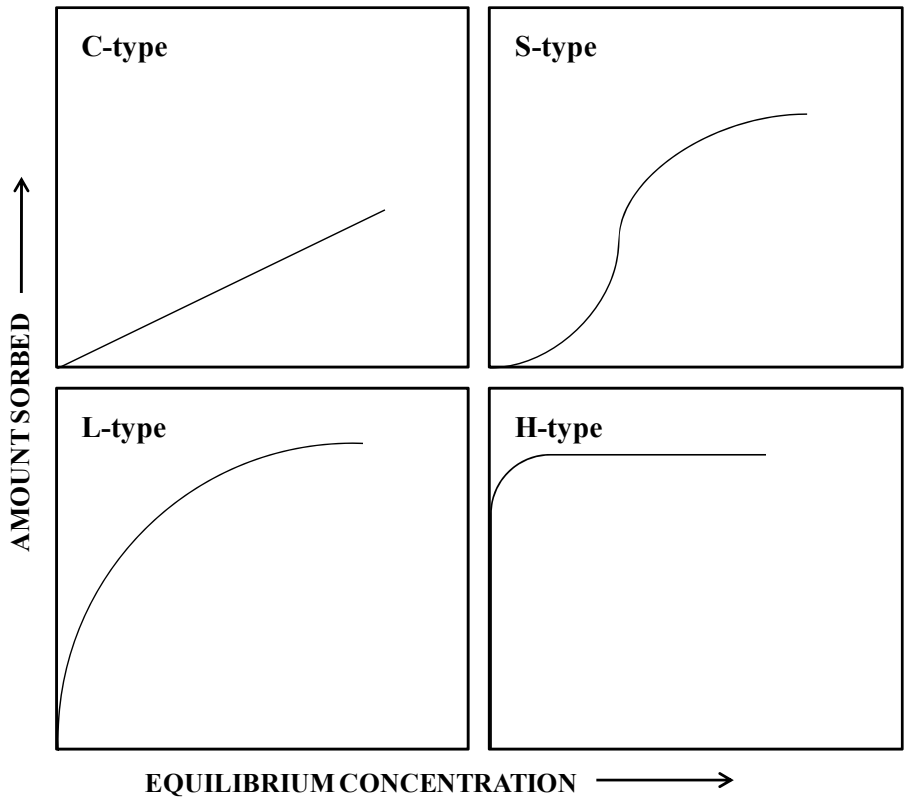

Figure 5 – General categories of sorption isotherms commonly observed in environmental science (adapted from [31]).

The C-curve (constant-partitioning) isotherm describes an initial slope that remains independent of adsorptive concentration until the maximum possible sorption is achieved. This type of curve indicates a partitioning mechanism whereby adsorptive ions or molecules are distributed between the interfacial phase and the solution phase, without any specific bonding between the adsorbent and adsorbate. It is usually observed at the low range of sorption, being commonly associated with the sorption of nonionic and hydrophobic organic compounds [7, 32, 34, 35].

The S-curve isotherm is characterized by an initially small slope that increases with adsorptive concentration, and eventually decreases and becomes zero, as vacant

adsorbent sites are filled. This type of curve suggests that the affinity of the soil for the compound is less than that of the aqueous solution, favoring the "clustering" of compound molecules at the surface, as they bond more strongly with one another than with the surface. When saturation is reached, sorption is improved [7, 31, 32, 34, 35].

The L-shaped (Langmuir) isotherm is characterized by a decreasing slope as concentration increases. It reflects a relatively high affinity between the compound and adsorbent which decreases as the vacant sorption sites decrease, and the adsorbent becomes covered [7, 32, 35].

The H-curve (high-affinity) isotherm is an extreme version of the L-curve isotherm, characterized by a large initial slope that suggests a very high affinity for the soil surface. It is associated with strong sorptive interactions, such as inner-sphere surface complexation or significant van der Waals interactions in the adsorptive process [32, 34, 35].

There are a number of models built to describe the sorption on soil surfaces. These include the widely used Freundlich and Langmuir equations.

The Freundlich equation is an empirical model that was firstly used to describe gas phase sorption and solute sorption, and is given in equation 2 [34].

$$C_s = K_f \, C_w^n \tag{2},$$

where C_s is the equilibrium concentration of the analyte in soil, C_w is the equilibrium concentration of the analyte in the aqueous phase, K_f is the distribution coefficient, and n is a correction factor.

The Langmuir equation was developed to describe the sorption of gas molecules in a planar surface, following some assumptions: (a) sorption occurs on planar surfaces that have a fixed number of sites that are identical, and the sites can hold only one molecule; (b) sorption is reversible; (c) there is no lateral movement of molecules on the surface; (d) the sorption energy is the same for all sites and independent of surface coverage, and there is no interaction between the compound molecules [35]. It is defined as shown in equation 3.

$$C_s = b \frac{K_L \, C_w}{1 + K_L \, C_w} \tag{3},$$

where C_s and C_w are the equilibrium concentration of the analyte in soil and aqueous phase, respectively, K_L is the distribution factor, and b is the sorption maxima.

As mentioned before, sorption depends on the physico-chemical properties of the soil, such as pH, organic matter content, and soil texture. Therefore, the sorption parameters, like K_d, are characteristic of a certain compound in a particular soil and can differ greatly from other soils.

The pH influences the sorption process depending on the ionization state of the molecule. ENR can exist in four possible forms: as an acidic cation, as a neutral un-ionized species, as a zwitterion, and as a basic anion (Figure 4) [36]. The pKa values for ENR are 5.9 and 7.8 [37] and, thus, the cationic species is more abundant at low pH (≤5), the anion species prevails at basic pH values (≥10), whereas between pH 6 and 9 the prevalent species is the zwitterion. Generally, sorption decreases when the soil pH increases. More specifically, it was shown that for ciprofloxacin the key soil factor influencing the extent of sorption was the cation exchange capacity at pH values from 3 to 8, with soil metal oxide content playing a smaller role at higher pH [38].

There are several methods already described to determine FQs in a great variety of samples, mostly biological fluids and food samples, but methods for environmental samples are still scarce, especially for soil samples. An important part of this determination is the sample treatment required. The first step of the analysis is extraction (when necessary). For the combination of sample and analyte used in this study, the extraction methods found were accelerated solvent extraction [39], dynamic microwave-assisted extraction [40], ultrasonic-assisted extraction [41] and mechanical extraction [29]. Before the analysis itself, sometimes clean-up steps are performed in order to purify the sample, such as solid-phase extraction [39, 42]. Concerning the analytical determination of FQs, the most common methods are based on chromatographic techniques, mainly liquid chromatography (LC). The detection system employed more frequently is fluorescence (FLD) [29, 39, 42, 43], since FQs have intrinsic fluorescent properties, and there is no need for derivatization. The excitation and emission spectra characteristic of ENR is shown in Figure 6. Other than FLD, the other detection methods used are diode array (DAD) [40, 43], ultraviolet-visible (UV) [41], and mass spectrometry [44].

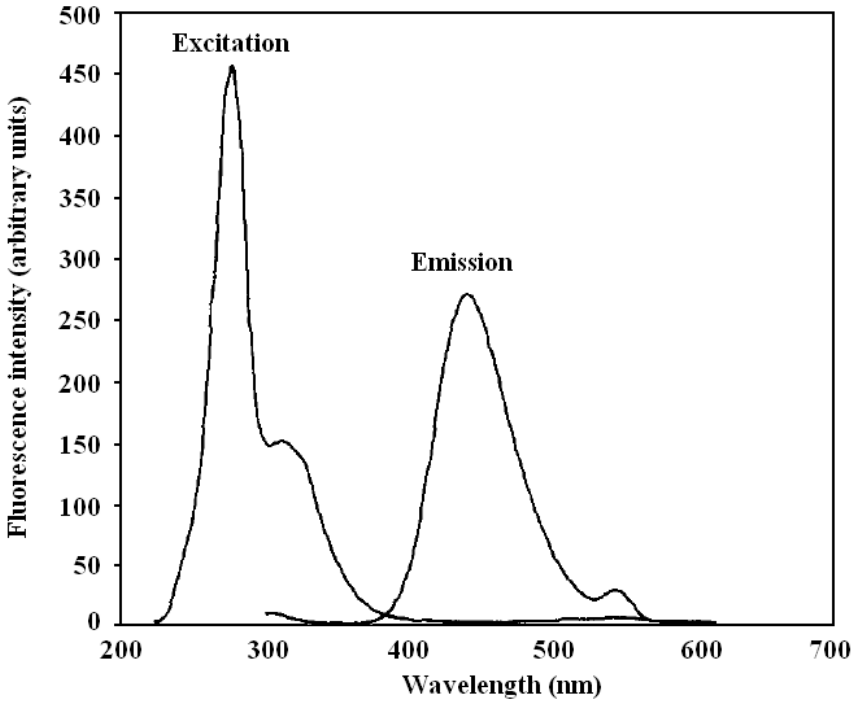

Figure 6 – Excitation and emission spectra of ENR (adapted from [36]).

The method chosen for this work was HPLC-FLD that allows a very sensitive measurement of the quinolone in the aqueous phase.

1.3. OBJECTIVES

The main objective of this work was to study the sorption and mobility of the fluoroquinolone ENR in soils with basic pH values, and different characteristics. In order to accomplish this, it was proposed:

- To characterize the sorption isotherms and calculate the distribution coefficients for ENR in the set of soils

- To investigate the effect of organic matter in the sorption process of ENR by amending one of the soils with different amounts of humic acids (HAs).

2. EXPERIMENTAL

2.1. EQUIPMENT

2.1.1. CHROMATOGRAPHIC SYSTEM

Agilent Series 1100 with the following components:

- Degasser with a vacuum system for the eluents
- Quaternary pump with a gradient elution system
- Autosampler that holds up to 100 vials of 2 mL, and allows an adjustable injection volume from 0.1 to 100 µL
- Chromatographic column: Reverse phase silica column Inertsil C8, from GL Sciences, 250 mm long, 4.6 mm of diameter and particle size of 5 µm. The pre-column used is 7.5x4.6 mm with the same material as the column.
- Fluorescence Detector

This system is controlled with the Agilent ChemStation for LC-systems software, where the results obtained are analyzed.

2.1.2. OTHER EQUIPMENT

- Classic Precision Balance: Mettler Toledo PB1502-L
- Roller mixer: Ovan Rollermix, RM120-D E
- Rotatory Mixer: Breda 34526
- Centrifuge: Heraeus Labofuge 400, with an 8 tube rotor and a range from 600 to 6000 rpm
- Heating magnetic stirrer: SBS A-06
- pH meter: Crison GLP 21, with a precision of ± 0.01 pH units, a combined glass electrode of pH Crison 52-02, and a reference system of Ag/AgCl
- Solid sample splitter: Sepor Micro Riffle Splitter (Jones Type), with 14 channels of 32 mm each, and a collector of 11.4x15.2 cm
- Bead preparation system for X-ray fluorescence: Perl'X 3, PANalytical

2.2. REAGENTS AND DISSOLUTIONS

- Anhydrous calcium chloride 95%, Panreac
- Enrofloxacin analytical standard, Fluka
- ortho-Phosphoric acid 85% PA-ACS-ISO, Panreac
- Oxalic acid Dihydrate ≥99.5%, Fluka
- LiChrosolv® Methanol gradient grade for liquid chromatography, Merck
- Acetonitrile (HPLC-gradient grade) PAI-ACS, Panreac
- Ultrapure water obtained from MilliQ-Plus, Millipore
- Humic acid technical, Aldrich

The **calcium chloride solution** 0.01 M used for sorption experiments was prepared by dissolving anhydrous calcium chloride in milliQ water.

The **stock solution of ENR** at 200 ppm was prepared by dissolving the ENR in a phosphoric acid solution 0.02 M, pH 2.

The **working ENR standard solutions** (between 0.5 ppb and 1500 ppb) were prepared daily from the 200 ppm stock solution, in calcium chloride 0.01 M.

The **oxalic acid solution 0.01 M** as a mobile phase for the HPLC analysis was prepared daily by dissolving the salt in milliQ water using a magnetic stirrer, and then the pH was adjusted to 2.2 with sodium hydroxide 3 M. This solution was subsequently filtered and placed in the HPLC solvent bottle.

2.4. SAMPLES

Six soils were used in this study, and its properties are collected in Table 2. The soil samples were sent to *Aragogamma S.A*. to be irradiated with γ-rays (^{60}Co source), in order to avoid the interference of microbial activity. The soil was then homogenized in a roller mix at the *Laboratori de Preparació de Materials pel Control de Qualitat* (MAT Control), sieved through a 2 mm sieve, split into several fractions (of ≈ 100 g) with a solid sample splitter to assure its representativity, and stored in polyethylene flasks.

Table 2 – Physico-chemical properties of the six soils studied.

SOIL	Osca	Papiol secà	Papiol regadiu	Lleida-2	Lleida-1	St. Joan
pH	8,2	8,2	8,0	8,3	8,4	7.9
OM (%)	1,96	2,5	2,58	1,7	12,8	4.5
Sand (%)	13,6	38,6	37,3	7,7	30,2	51.5
Clay (%)	30,9	23,7	18,9	34,0	23,4	19.6
CEC (mEq/100g)	17,1	10,6	8,9	*	21,5	*
Fe_2O_3	4,01	5,26	3,74	5,29	3,36	4,47
MnO	0,06	0,08	0,07	0,09	0,06	0,09
TiO_2	0,5	0,77	0,54	0,66	0,44	0,56
CaO	25,35	7,38	14,26	15,11	17,32	10,72
K_2O	1,71	2,4	2,11	2,9	2,33	2,15
P_2O_5	0,13	0,15	0,34	0,16	0,79	0,14
SiO_2	36,49	61,36	53,64	45,26	35,21	48,48
Al_2O_3	9,92	12,92	9,11	13,9	8,89	11,60
MgO	1,46	1,37	2,78	2,87	2,16	2,14
Na_2O	0,21<LL	0,51<LL	0,60 <LL	0,71<LL	0,48<LL	0,55<LL

* waiting for results; LL = lowest standard

The characterization of these soil samples was performed either by the *Applus Agroambiental S.A.* in Sidamon (Lleida) or the *Laboratori Polivalent de la Garrotxa*, and the x-ray fluorescence was executed by the *Serveis Cientificotècnics de la Universitat de Barcelona*.

2.3. PROCEDURES

- Isotherms study

Sorption experiments of ENR at multiple concentrations on soils were performed in the presence of $CaCl_2$ 0.01 M, as proposed by OECD [45] to simulate natural soil water and to minimize the suspension of soil particles. For the equilibration, 1 g of soil was weighed in each tube (after homogenized for 2h in the rollermix) and 10 mL of the correspondent antibiotic solution prepared in $CaCl_2$ 0.01 M was added. The soil:solution mixture was shown to be suitable for ENR sorption studies. This mixture was placed in the rotatory mixer for 24h and then centrifuged for 20 min at 3500 rpm. The samples were filtered through a nylon filter 0.45 µm and placed in vials for injection.

- HPLC conditions

After optimization, the conditions used for the chromatography were the following:

- Mobile phase: 80% Oxalic acid 0.01 M pH 2.2: 20% Methanol
- Flow rate: 1.5 mL/min
- Injection volume: 50 µL
- Detection: λ_{ex}= 280 nm and λ_{em}= 458 nm
- Cleaning: after each working session the column was cleaned with a mixture of water and methanol in a ratio of 50:50 for 120 minutes.

- X-ray sample preparation

The soil samples were ground with an agate mortar and pestle and sieved through a 64 µm sieve. Each bead was prepared with approximately 0.3 g of soil and lithium tetraborate was added in a proportion of 1:20 (soil: $Li_2B_4O_7$) and 120 µL of potassium iodide, 1 M. The samples were prepared in duplicate in a fully-automatic bead preparation system, and sent for analysis at the *Serveis Cientificotècnics de la Universitat de Barcelona*.

- Soil amendment

To investigate the effect of organic matter in soil sorption, three amended soil samples were prepared from the Papiol regadiu soil, using commercial humic acid. Each amended fraction contained 100 g of Papiol regadiu soil and different amounts of humic acids were added to each one: 1 g, 3 g, and 5 g. After adding a few milliliters of water, the samples were kept agitating in the rollermix for 72 hours, and left without agitation for 20 days. The excess of humic acid was removed with $CaCl_2$ 0.01 M by successive decantation, followed by vacuum filtration using cellulose filter paper (Whatman Grade No. 41) and, lastly, the samples were dried at room temperature.

3. RESULTS AND DISCUSSION

3.1. OPTIMIZING THE LIQUID CHROMATOGRAPHY CONDITIONS FOR ENROFLOXACIN DETERMINATION

Before starting the sorption experiments, the chromatographic conditions needed to be chosen in order to quantify ENR properly in the soil extracts obtained in the sorption studies (in a $CaCl_2$ 0.01 M medium). This study was performed with the Osca soil sample and considered as analogous for the other soil samples.

3.1.1. CHOOSING THE MOBILE PHASE

The type of column and the mobile phase used in LC are key factors in the separation of analytes. The quinolones are compounds that complex easily with metal ions and show typically asymmetric chromatographic peaks in silica-based columns. As to avoid this complexation, ultrapure silica columns are used, and particular reagents, such as oxalic acid, are added to the mobile phase to improve the symmetry of the peaks. Prior to this work, in the research group, the separation of quinolones was performed using a mobile phase based on acetonitrile (ACN) and water. Due to the problems of ACN price and supply, methanol (MeOH) was attempted as an alternative for ACN. After choosing the solvents – oxalic acid in water and MeOH – the best proportion for this study was still unclear.

Several proportions of mobile phase were investigated within the range of Oxalic acid 0.01M, pH 2.2: MeOH (70:30) and Oxalic acid 0.01M, pH 2.2: MeOH (90:10).

The best results were obtained for the proportion Oxalic acid 0.01 M, pH 2.2: MeOH (80:20) and the chromatogram obtained is shown in Figure 7.

Figure 8 shows a typical chromatogram for a soil extract containing a 4 ppm solution of ENR. The ENR peak shows a very good separation from the soil matrix peaks in a reasonable time frame – below 15 minutes.

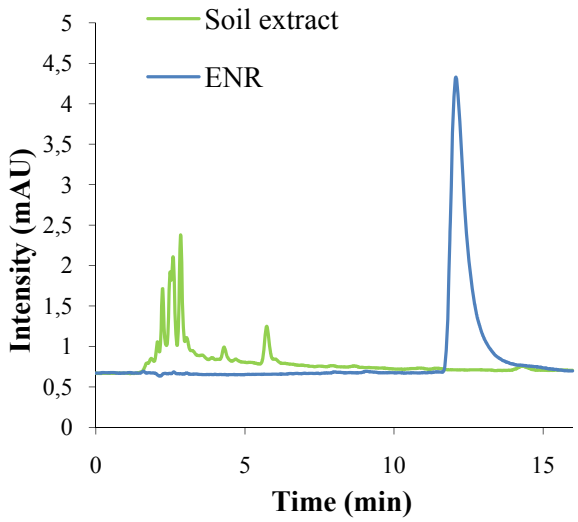

Figure 7 – HPLC-FLD chromatogram of a blank soil extract and of a standard solution of ENR using Oxalic acid 0.01M, pH 2.2: MeOH (80:20).

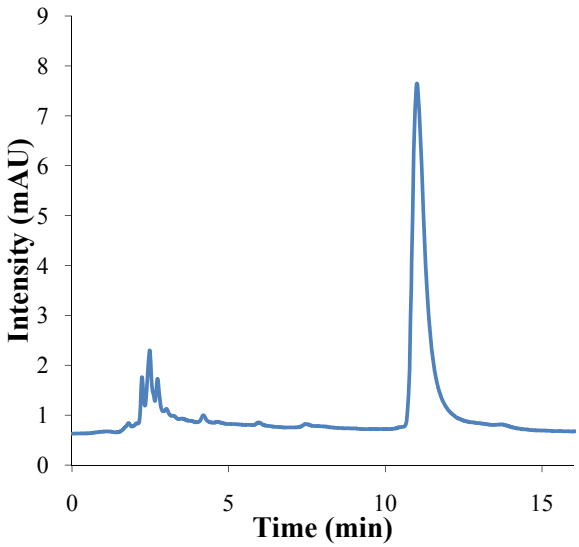

Figure 8 – HPLC-FLD chromatogram of an Osca soil sample after equilibration with a 4 ppm ENR solution using Oxalic acid 0.01M, pH 2.2: MeOH (80:20).

Using this analytical method, in order to quantify ENR in the equilibrated soil solutions, each day two calibration curves were plotted (one for low concentration levels and one for high concentration levels) using daily ENR standards. An example is shown in Figure 9. The method used is very sensitive to determine ENR for it allows the quantification of very low concentrations (starting at 0.5 µg/L).

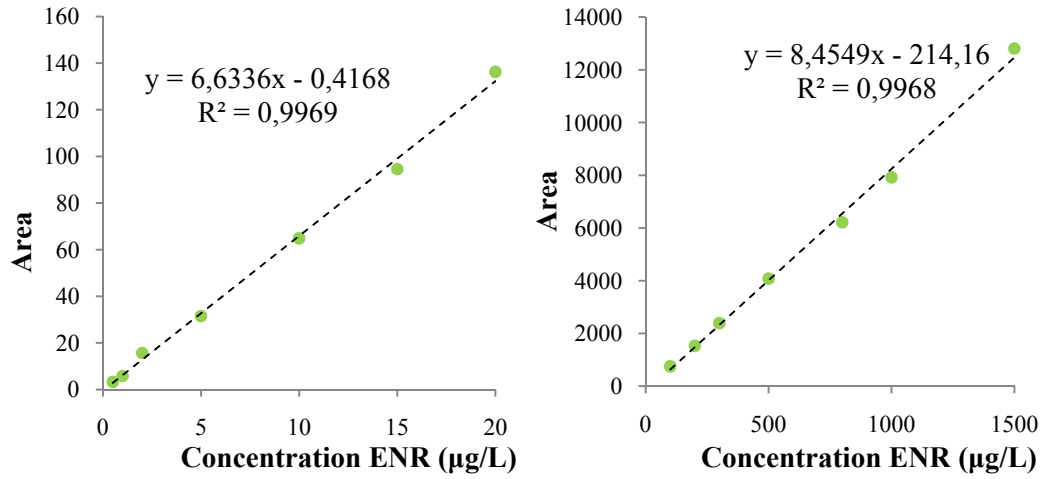

Figure 9 – Calibration curves from 0.5 to 20 ppb (left) and 100 to1500 ppb (right) used in this study.

3.2. SORPTION

Sorption to soils is an important process for determining the fate of contaminants in the environment. Not only are the analyte's properties important for the sorption behaviour, but the soil properties also play a significant part, being the most important organic matter (OM) content, clay minerals, cation exchange capacity (CEC) and pH.

3.2.1. PRELIMINARY STUDIES

3.2.1.1. DETERMINATION OF SORPTION EQUILIBRATION TIME

This study was performed to see if pre-equilibration of the soil with $CaCl_2$ 0.01 M was necessary before the sorption experiments, and to decide how long the soil would be equilibrating with the quinolone solution. Pre-equilibration is when the soil is left agitating for a certain period of time with a $CaCl_2$ 0.01 M solution prior to adding the quinolone solution.

Two experiments were performed, using M1 and M2 samples:

- M1 is an Osca soil sample left pre-equilibrating overnight with a $CaCl_2$ 0.01 M solution
- M2 is an Osca soil sample not pre-equilibrated.

Both tubes (M1 and M2) were left agitating for 95 hours with a 1 ppm ENR solution and 500 µL fractions were sampled at several times to monitor the ENR concentration. Standard solutions of ENR were prepared in $CaCl_2$ 0.01 M and the correspondent calibration curve was obtained. The areas given in the chromatogram were converted in concentrations using the equation of the calibration curve. These values are shown in Figure 10.

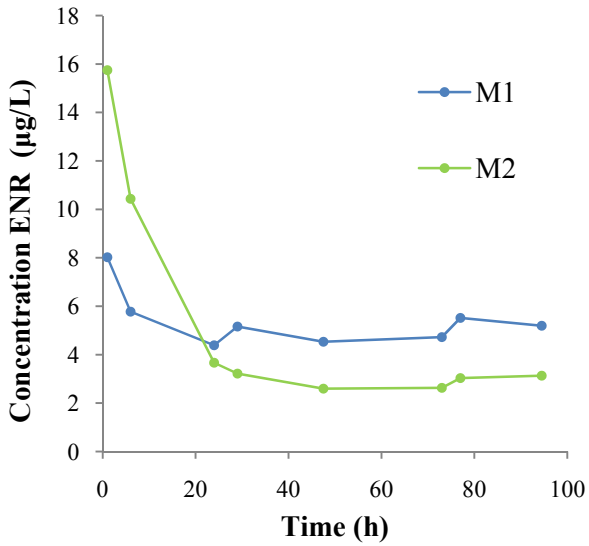

Figure 10 – ENR concentration in soil samples with an ENR solution, over time: M1 contained pre-equilibrated soil and M2 was not pre-equilibrated.

As can be seen in Figure 10, the ENR concentration decreases in the aqueous phase as it is adsorbed to the soil. Some significant differences are observed at early times, when the soil not pre-equilibrated decreases faster. However, both samples reach a steady-state at around 24 hours, when their concentration values are very similar. Therefore, the time chosen for the equilibration of soil with the ENR solution in $CaCl_2$ 0.01 M was 24 h, and it was decided to work with soil without pre-equilibration, as it facilitates the work.

3.2.1.2. STABILITY OF ENROFLOXACIN IN SOIL EXTRACT

In order to make sure the concentration of ENR in solution decreases because of its sorption to soil and not because it degrades, a stability study was carried out.

Two tubes (P1 and P2) were prepared with ENR standards of 5 ppb:

- P1 was prepared in $CaCl_2$ 0.01 M
- P2 was prepared in soil extract (in a $CaCl_2$ 0.01 M medium)

The tubes were shaken in a rotatory mixer for 24 h. Fractions of 500 µL were taken at different times to monitor the stability of the diluted ENR solutions in soil extract and in $CaCl_2$ 0.01M.

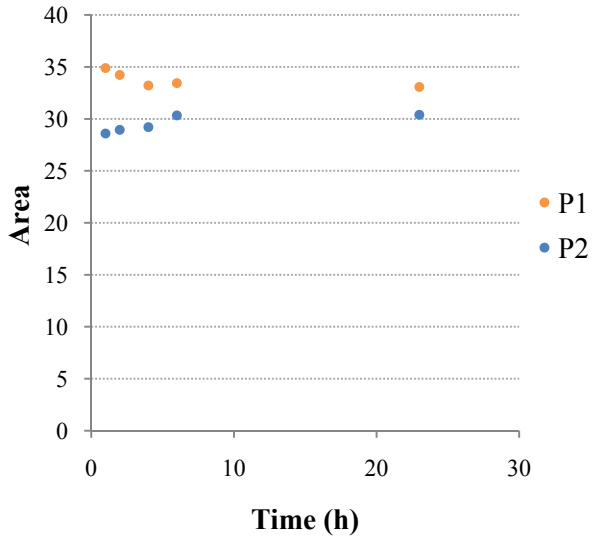

Figure 11 – Stability of 5ppb ENR solutions prepared in $CaCl_2$ 0.01 M (P1) and in soil extract (P2) over time.

Figure 11 shows that the concentration of ENR is constant in the time interval studied, confirming the stability of this quinolone in the conditions used for the sorption experiments.

3.2.2. SORPTION ISOTHERMS

An isotherm study was carried out in six soils of pH>7, typical from the Mediterranean area. The sorption usually decreases as soil pH increases, and thus mobility of contaminants also increases, meaning that the environmental risk is expected to be higher for soils if basic pH. Most of the studies found in the literature refer to the sorption of quinolones on acid soils. With this study we wanted to evaluate the effect of pH on the magnitude of ENR sorption, and to identify what parameters other than pH are important in the sorption process.

In order to plot the sorption isotherms the concentration of ENR in the solution and in the soil must be calculated. The concentration of ENR in the solution (C_w) was obtained directly converting the area taken from the chromatograms, using the equations from the calibration curves. On the other hand, the concentration of ENR in the soil (C_s) was calculated from the mass balance, by subtracting the C_w from the initial concentration of ENR added to the sample (C_i) and correcting it for the volume used (10 mL) and mass of soil (1 g). An example of these calculations is shown below for the Osca soil sample 1 (M1).

The results obtained experimentally were then compared with two sorption models: Freundlich and Langmuir (the latter only when applicable), using the *Solver* tool in *Microsoft Excel*. This tool optimizes parameters for the data submitted and was used to calculate the sorption parameters K_f, n, K_L and b.

- **OSCA SOIL**

$C_w = \frac{Area - b}{m}$, where b and m are the y-intercept and the slope of the calibration curve, respectively.

$$C_s = (C_i - C_w) \times \frac{V}{m_{soil}} = (250 - 1.90) \times \frac{0.01}{1} = 2.481 \mu g / g$$

The final results are gathered in Table 3.

Table 3 – ENR concentration values for the Osca soil obtained experimentally and estimated by the Freundlich model.

Samples	C_i (mg/L)	C_w (µg/L)	C_s (µg/g)	$C_{Freundlich}$ (µg/g)
M1	0.25	1.90	2.48	0.02
M2	0.50	4.54	4.95	0.13
M3	2	27.35	19.73	2.35
M4	2	15.63	19.84	8.64
M5	4	35.05	39.65	15.38
M6	8	63.12	79.37	60.47
M7	12	81.12	119.19	108.42
M8	16	110.32	158.90	221.74
M9	30	131.34	298.69	332.76
M10	50	146.48	498.54	428.91
M11	100	211.22	997.89	1005.32

In Figure 12 the adosption isotherm obtained for the Osca soil is represented.

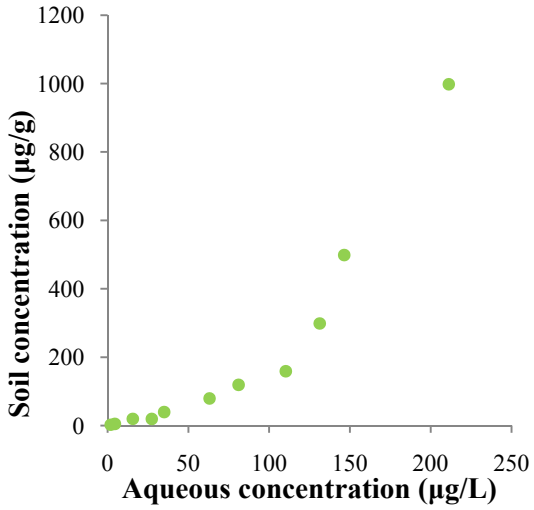

Figure 12 – Sorption isotherm for the Osca soil.

From the values obtained experimentally, the estimated concentration for ENR in soil can be calculated from the Freundlich equation. These values are shown in Table 3.

- Freundlich equation:

$$C_s = K_f C_w^n = 3.91 \times 10^{-3} \times C_w^{2.33}$$

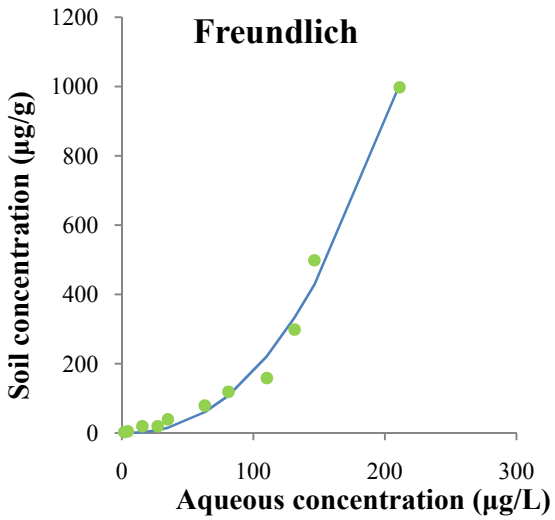

Figure 13 – Sorption isotherms obtained by HPLC-FLD (markers) and predicted by the Freundlich model (line) for the Osca soil.

All the calculations shown for the Osca soil are equivalent for the next soil samples.

- **PAPIOL SECÀ SOIL**

Table 4 – ENR concentration values for the Papiol secà soil obtained experimentally and estimated by the Freundlich and Langmuir models.

Samples	C_i (mg/L)	C_w (µg/L)	$C_{s,exp}$ (µg/g)	$C_{Freundlich}$ (µg/g)	$C_{Langmuir}$ (µg/g)
M1	0.25	1.14	2.49	6.53	1.75
M2	0.50	1.90	4.98	9.41	2.89
M3	1	4.51	9.95	17.63	6.87
M4	1.50	7.34	14.93	25.09	11.15
M5	2	10.74	19.89	33.06	16.28
M6	3	16.12	29.84	44.36	24.34
M7	5	29.91	49.70	69.42	44.71
M8	5	38.44	49.62	77.14	51.55
M9	10	34.61	99.65	83.24	57.10
M10	15	113.02	148.87	162.39	137.93
M11	20	96.69	199.03	181.82	159.45
M12	30	233.39	297.67	307.46	304.55
M13	40	329.89	396.70	395.05	406.03
M14	50	421.39	495.79	471.71	492.15
M15	60	578.07	594.22	593.10	620.82
M16	80	826.84	791.73	768.65	787.42
M17	100	1207.15	987.93	1011.06	979.91

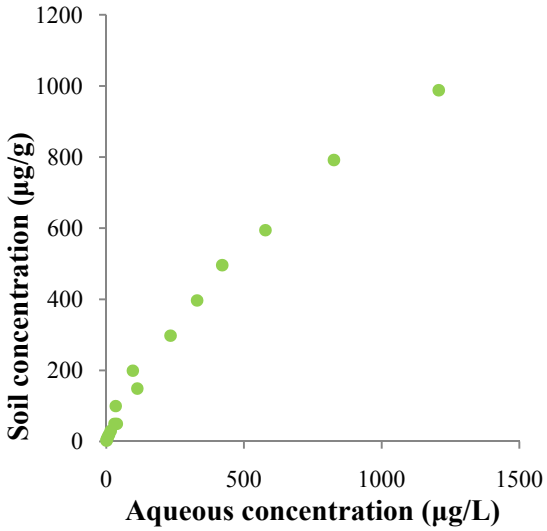

Figure 14 – Sorption isotherm for the Papiol secà soil.

- Freundlich equation:

$$C_s = K_f\, C_w^n = 5.92 \times C_w^{0.72}$$

- Langmuir equation:

$$C_s = b\frac{K_L\, C_w}{1 + K_L\, C_w} = 2092\ \frac{7.30 \times 10^{-4} C_w}{1 + 7.30 \times 10^{-4} C_w}$$

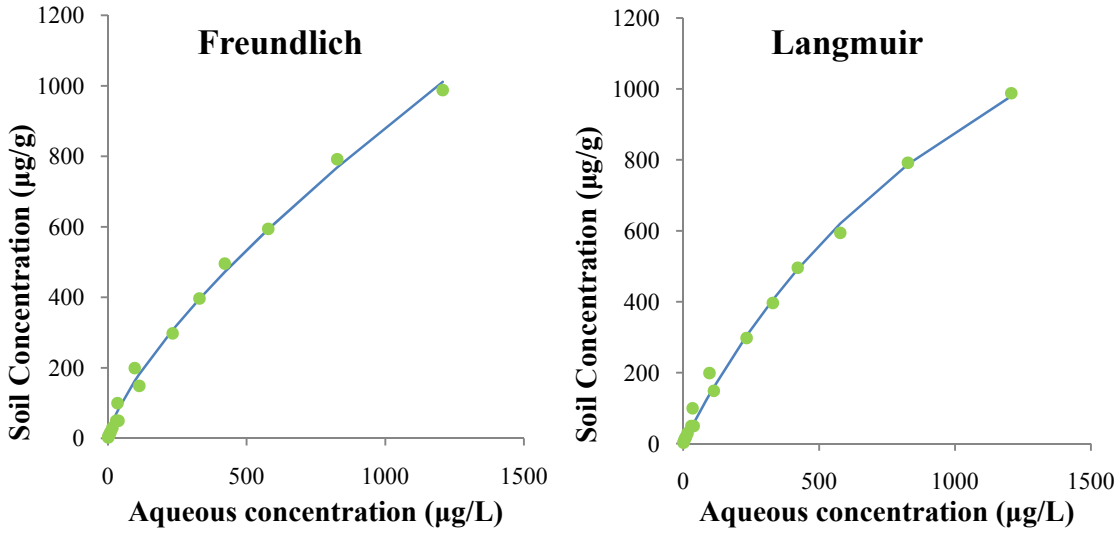

Figure 15 – Sorption isotherms obtained by HPLC-FLD (markers) and predicted (line) by the Freundlich and Langmuir models for the Papiol secà soil.

- **PAPIOL REGADIU SOIL**

Table 5 – ENR concentration values for the Papiol regadiu soil obtained experimentally and estimated by the Freundlich and Langmuir models.

Samples	C_i (mg/L)	C_w (µg/L)	$C_{s,exp}$ (µg/g)	$C_{Freundlich}$ (µg/g)	$C_{Langmuir}$ (µg/g)
M1	0.25	0.67	2.49	2.28	1.42
M2	0.50	1.39	4.99	4.36	2.92
M3	1	3.38	9.97	9.69	7.11
M4	2	8.50	19.91	22.18	17.84
M5	5	23.52	49.76	55.27	48.97
M6	10	47.65	99.52	104.14	97.98
M7	20	88.50	199.12	181.48	178.20
M8	20	112.91	198.87	225.83	224.60
M9	30	154.64	298.45	299.45	301.32
M10	40	216.27	397.84	404.61	409.09
M11	50	270.20	497.30	473.18	477.73
M12	50	257.50	497.42	494.07	498.34
M13	60	328.04	596.72	588.00	589.25
M14	70	406.03	695.94	712.01	704.57

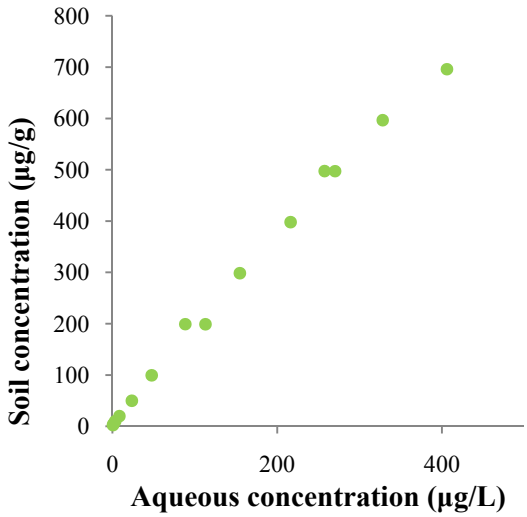

Figure 16 – Sorption isotherm for the Papiol regadiu soil.

- Freundlich equation:

$$C_{Freundlich} = K_f \, C_w^n = 3.25 \times C_w^{0.90}$$

- Langmuir equation:

$$C_{Langmuir} = b\frac{K_L C_w}{1+K_L C_w} = 3986\ \frac{5.29\times10^{-4} C_w}{1+5.29\times10^{-4} C_w}$$

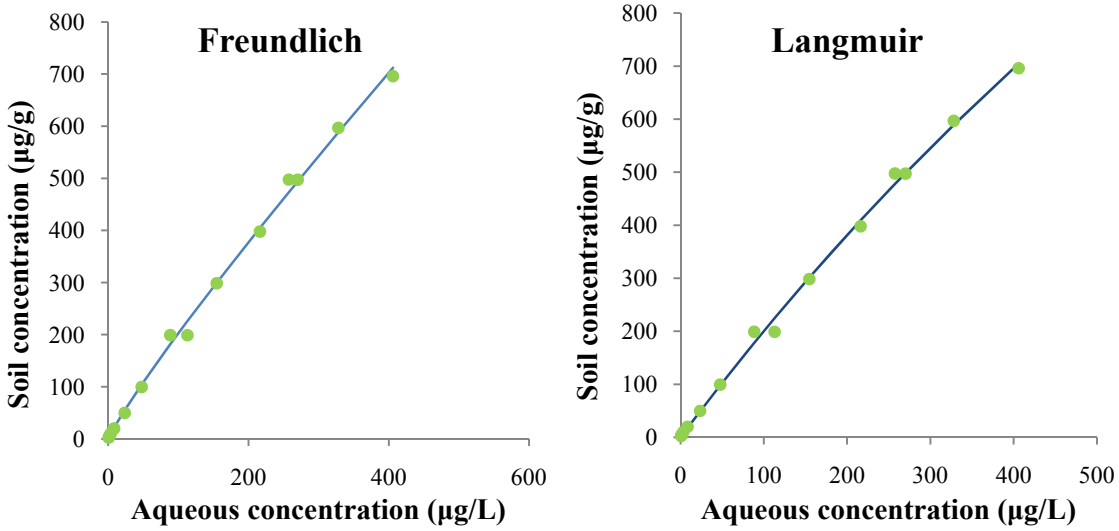

Figure 17 –Sorption isotherms obtained by HPLC-FLD (markers) and predicted (line) by the Freundlich and Langmuir models for the Papiol regadiu soil.

- **LLEIDA-2 SOIL**

Table 6 – ENR concentration values for the Lleida-2 soil obtained experimentally and estimated by the Freundlich model.

Samples	C_i (mg/L)	C_w (µg/L)	$C_{s,exp}$ (µg/g)	$C_{Freundlich}$ (µg/g)
M1	0.25	1.62	2.48	0.21
M2	0.50	3.43	4.97	0.57
M3	1	11.55	9.88	2.93
M4	2	46.30	19.54	19.07
M5	5	78.39	49.22	38.79
M6	10	137.44	98.63	82.75
M7	20	254.56	197.45	190.07
M8	20	258.96	197.41	194.52
M9	30	340.96	296.59	281.93
M10	40	464.34	395.36	427.66
M11	50	518.26	494.82	495.98
M12	50	543.04	494.57	528.24
M13	60	593.77	594.06	595.87
M14	70	636.78	693.63	654.83

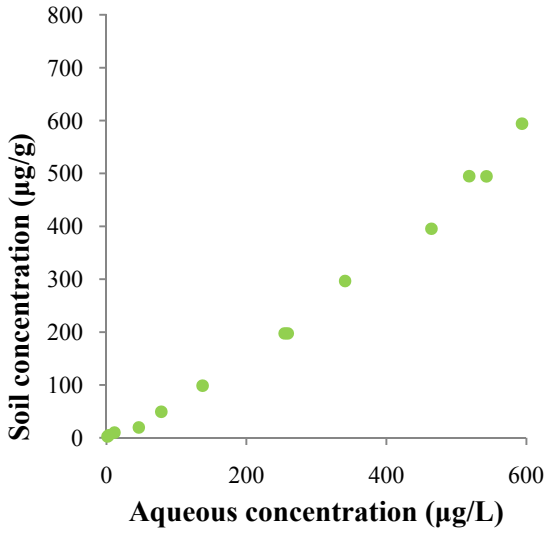

Figure 18 – Sorption isotherm for the Lleida-2 soil.

- Freundlich equation:

$$C_s = K_f\, C_w^n = 0.11 \times C_w^{1.35}$$

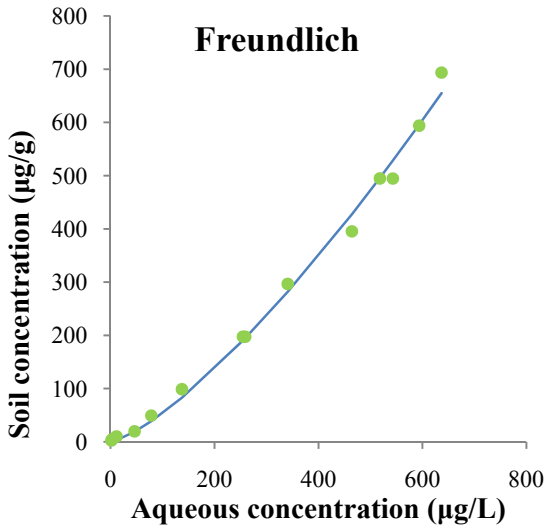

Figure 19 –Sorption isotherms obtained by HPLC-FLD (markers) and predicted by the Freundlich model (line) for the Lleida-2 soil.

- **LLEIDA-1 SOIL**

Table 7 – ENR concentration values for the Osca soil obtained experimentally and estimated by the Freundlich and Langmuir models.

Samples	C_i (mg/L)	C_w (µg/L)	$C_{s,exp}$ (µg/g)	$C_{Freundlich}$ (µg/g)	$C_{Langmuir}$ (µg/g)
M1	0.25	0.83	2.49	4.30	1.28
M2	0.50	1.32	4.99	6.08	2.03
M3	1	1.82	9.98	7.72	2.79
M4	2	7.30	19.93	21.84	11.13
M5	5	22.00	49.78	49.94	33.14
M6	10	59.59	99.40	105.36	86.84
M7	20	150.02	198.50	193.49	183.65
M8	20	134.13	198.66	210.42	202.79
M9	30	238.90	297.61	298.18	301.49
M10	40	334.24	396.66	383.48	393.75
M11	50	498.90	495.01	480.05	491.35
M12	50	451.09	495.49	517.68	527.17
M13	60	587.67	594.12	585.26	588.29
M14	70	743.00	692.57	697.68	681.07

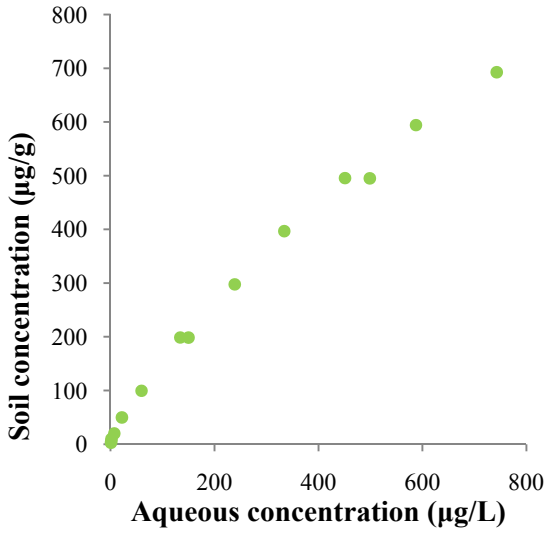

Figure 20 – Sorption isotherm for the Lleida-1 soil.

- Freundlich equation:

$$C_s = K_f C_w^n = 4.93 \times C_w^{0.75}$$

- Langmuir equation:

$$C_s = b\frac{K_L C_w}{1+K_L C_w} = 1689\ \frac{9.10\times10^{-4} C_w}{1+9.10\times10^{-4} C_w}$$

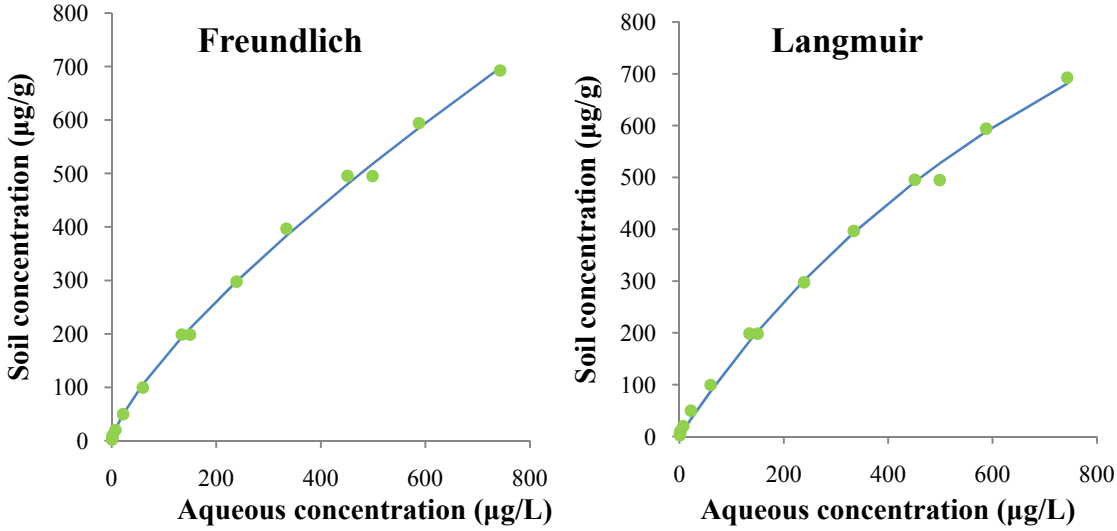

Figure 21 –Sorption isotherms obtained by HPLC-FLD (markers) and predicted (line) by the Freundlich and Langmuir models for the Lleida-1 soil.

- **ST. JOAN SOIL**

Table 8 – ENR concentration values for the St. Joan soil obtained experimentally and estimated by the Freundlich model.

Samples	C_i (mg/L)	C_w (µg/L)	$C_{s,exp}$ (µg/g)	$C_{Freundlich}$ (µg/g)
M1	0.250	1.31	2.49	1.22
M2	0.50	2.49	4.98	2.37
M3	1	5.81	9.94	5.73
M4	2	14.10	19.86	14.43
M5	5	42.33	49.58	45.33
M6	10	75.52	99.24	82.83
M7	15	129.78	148.70	145.56
M8	20	172.17	198.28	195.35
M9	20	176.75	198.23	200.77
M10	30	272.90	297.27	315.58
M11	40	346.32	396.54	404.44
M12	50	443.22	495.57	477.37
M13	50	406.10	495.94	522.89
M14	60	484.63	595.15	573.85
M15	70	529.09	694.71	628.77

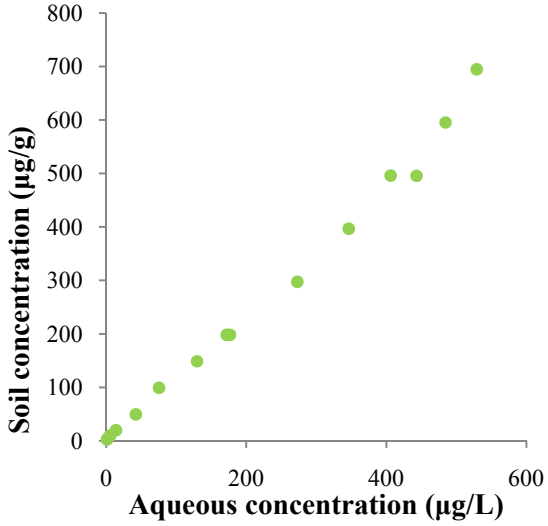

Figure 22 – Sorption isotherm for the St. Joan soil.

- Freundlich equation:

$$C_s = K_f C_w^n = 0.92 \times C_w^{1.04}$$

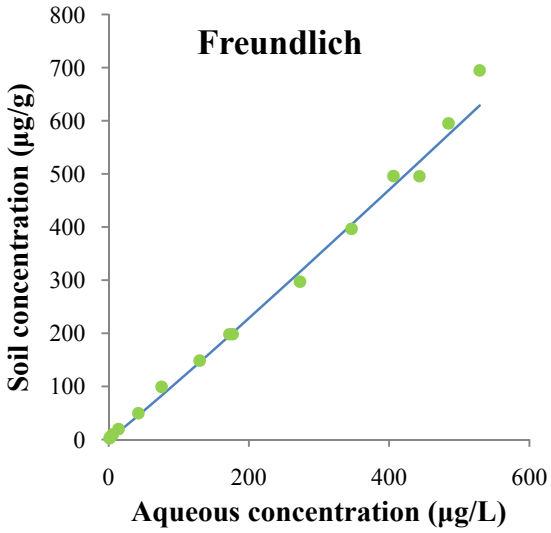

Figure 23 – Sorption isotherms obtained by HPLC-FLD (markers) and predicted by the Freundlich model (line) for the St. Joan soil.

Along with the Freundlich and Langmuir coefficients, a distribution coefficient (K_d) was estimated for all soils, using the initial points of the isotherm curve, and are represented in Table 9.

Table 9 – Sorption parameters for the soils studied.

SOIL	Linear fit	Freundlich fit		Langmuir fit	
	K_d	K_f	n	K_L	b
Osca	1.47	3.91×10^{-3}	2.33	–	–
Papiol secà	1.62	5.92	0.72	7.30×10^{-4}	2092
Papiol regadiu	2.04	3.25	0.90	5.29×10^{-4}	3986
Lleida-2	1.45	4.93	0.75	–	–
Lleida-1	0.69	0.11	1.35	4.08×10^{-5}	1689
St. Joan	1.16	0.92	1.04	–	–

Analyzing the shape of the six isotherm curves, they can be separated in two groups.

- On one side the Papiol secà, Papiol regadiu and Lleida-1 soils
- On the other the Osca, St. Joan and Lleida-2 soils

The first group shows L-shaped sorption isotherms. This indicates that the affinity between ENR and the soil is high at an early stage and decreases progressively, as sorption sites are being occupied.

Regarding the other group, the Langmuir equation was not fitted to estimate the ENR concentration because this model cannot be applied to the type of isotherms obtained for these soils. The Osca soil shows a clear S-shaped curve, while the other two represent only the beginning of an S-shape. The S-type isotherm, contrary to the L-type, begins with a small slope, which increases as more ENR molecules are present in solution, and start binding to the soil.

Analyzing the values of K_d for the sorption of ENR obtained for the set of soils, they are similar to the values given in the literature for acidic soils (Table 10). These findings do not agree with other authors that in a basic medium the compounds are not so easily sorbed to the soil.

Table 10 – Values of K_d for the sorption of ENR in soils with different properties as seen in the literature and obtained experimentally.

SOIL	pH	OC (%)	Clay	K_d (L/g)
Brazil*	4.9	1.63	41.7	3.04
Philippines*	5.3	0.73	17.2	5.61
Germany*	5.3	0.70	2.5	0.50
Sweden*	6.0	1.23	7.2	1.23
Sandy**	6.0	0.59	2.5	0.27
Loamy sand**	6.1	2.27	6.6	0.97
Sandy loam**	6.6	1.24	9.5	0.46
France*	7.5	1.58	23.4	0.26
St. Joan	7.9	2.61[a]	19.6	1.16
Papiol regadiu	8.0	1.50[a]	18.9	2.04
Osca	8.2	1.14[a]	30.9	1.47
Papiol secà	8.2	1.45[a]	23.7	1.62
Lleida-2	8.3	0.99[a]	34.0	1.45
Lleida-1	8.4	7.42[a]	23.4	0.69

References: * [28] and ** [29];

[a] the conversion of organic matter to organic carbon (OC) of the studied soils was done based on the assumption that organic matter contains 58% OC, using the conversion factor 1.724.

By just comparing the values of the sorption parameters obtained experimentally and using the Freundlich and Langmuir models for the studied soils, it was not possible to decide which properties were more important in the sorption of ENR. Therefore, a multivariate data treatment was performed by principal component analysis (PCA) to clarify this decision, using the *PLS Toolbox* of the *MATLAB* software (version 6.5).

In the PCA, the sorption parameter used was the experimental K_d and the included soil properties were pH, OM, sand, clay, silt, and oxides of Al, Fe, Ca and Mg. The CEC was not included because not enough values were available, and at this pH range the predominant species is the zwitterion, decreasing the importance of this parameter. The more significant factors for the sorption of ENR were found to be the content in Fe, Al and Mg oxides. These results may indicate that the binding mechanism of ENR to soils occurs mainly via cation binding, and can be supported by previous studies on FQ [28, 46].

This study was conducted to give an idea of the most influent soil characteristics in the sorption process. More experiments must be done, using a larger set of soils, to come to a more confident conclusion.

3.2.2.1. EFFECT OF ORGANIC MATTER ON ENROFLOXACIN IN SOILS

Although it is usually assumed that OM plays an important role in the sorption process, the PCA of the data obtained in this study didn't identify it as a significant factor. Nevertheless, OM includes a huge amount of organic compounds, with a wide range of molecular range, functional groups, etc. In this study, we investigated the sorption of ENR to soils amended with different amounts of humic acids.

As previously mentioned, the studies with amended soils (see section 2.3.) were done by adding different amounts of humic acids to the Papiol Regadiu soil. The organic matter content for these amended soils is shown in Table 11.

Table 11 – Organic matter content for the Papiol regadiu soil and for the correspondent amendments of 1 g, 3 g, and 5 g of HA.

SOIL	Papiol regadiu	Papiol regadiu + 1g HA	Papiol regadiu + 3g HA	Papiol regadiu + 5g HA
OM (%)	2.58	3.14	4.08	4.60

The amount of HA added is not directly proportional to the organic matter content observed in the samples. However, it can be seen that, as expected, the OM content increases with the increase of added HA.

The isotherm studies were carried out for the amended soils as for the other soil samples, and the results are shown and discussed below.

- **PAPIOL REGADIU SOIL + 1 g HUMIC ACIDS**

Table 12 – ENR concentration values for the Papiol regadiu + 1 g HA soil obtained experimentally and estimated by the Freundlich and Langmuir models.

Samples	C_i (mg/L)	C_w (µg/L)	$C_{s,exp}$ (µg/g)	$C_{Freundlich}$ (µg/g)	$C_{Langmuir}$ (µg/g)
M1	1	3.34	9.97	23.76	4.52
M2	2	8.21	19.92	44.71	11.11
M3	5	23.50	49.77	93.60	31.74
M4	10	48.97	99.51	156.83	66.00
M5	20	74.09	199.26	209.83	99.63
M6	75	441.77	745.58	736.18	575.45
M7	100	661.19	993.39	977.46	845.43
M8	150	1138.42	1488.62	1432.16	1399.69
M9	200	1862.25	1981.38	2024.17	2163.51

- Freundlich equation:

$$C_s = K_f\, C_w^n = 10.17 \times C_w^{0.70}$$

- Langmuir equation:

$$C_s = b\frac{K_L\, C_w}{1 + K_L\, C_w} = 15265\ \frac{8.87 \times 10^{-5}\, C_w}{1 + 8.87 \times 10^{-5}\, C_w}$$

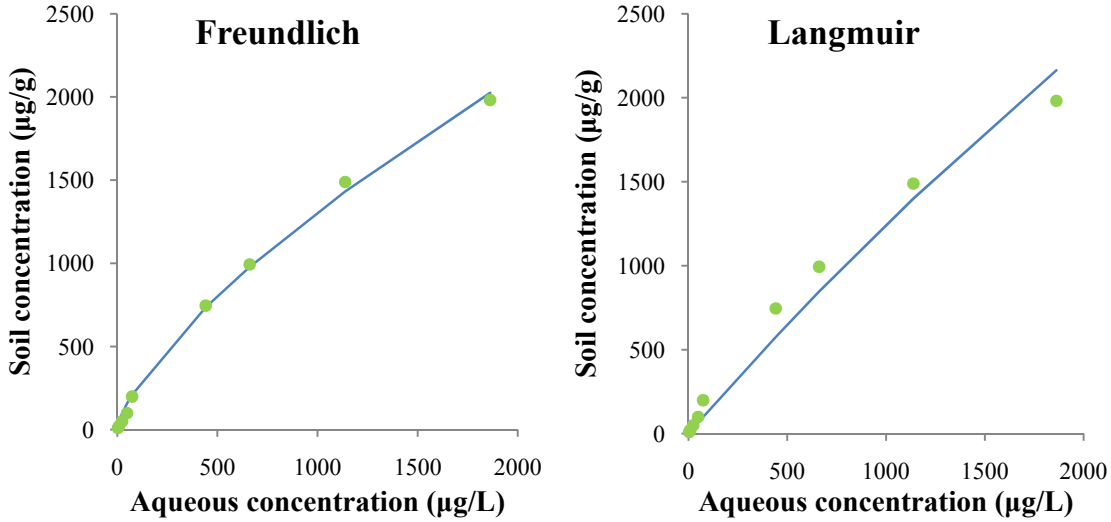

Figure 24 –Sorption isotherms obtained by HPLC-FLD (markers) and predicted (line) by the Freundlich and Langmuir models for the Papiol regadiu soil + 1 g HA.

- **PAPIOL REGADIU + 3 g HUMIC ACIDS**

Table 13 – ENR concentration values for the Papiol regadiu + 3 g HA soil obtained experimentally and estimated by the Freundlich and Langmuir models

Samples	C_i (mg/L)	C_w (µg/L)	$C_{s.\,exp}$ (µg/g)	$C_{Freundlich}$ (µg/g)	$C_{Langmuir}$ (µg/g)
M1	0.25	3.02	2.47	35.12	9.31
M2	0.5	2.50	4.97	31.04	7.73
M3	1	5.11	9.95	49.76	15.76
M4	2	7.01	19.93	61.33	21.59
M5	5	10.58	49.89	80.44	32.47
M6	10	20.10	99.80	122.86	61.17
M7	20	32.03	199.68	167.15	96.53
M8	50	165.94	498.34	495.16	449.63
M9	100	466.18	995.34	979.29	1030.16
M10	150	856.77	1491.43	1463.56	1526.91
M11	200	1387.30	1986.13	2011.86	1957.83

- Freundlich equation:

$$C_s = K_f\, C_w^n = 19.95 \times C_w^{0.66}$$

- Langmuir equation:

$$C_s = b\frac{K_L\, C_w}{1 + K_L\, C_w} = 3597\ \frac{8.61\times10^{-4} C_w}{1 + 8.61\times10^{-4} C_w}$$

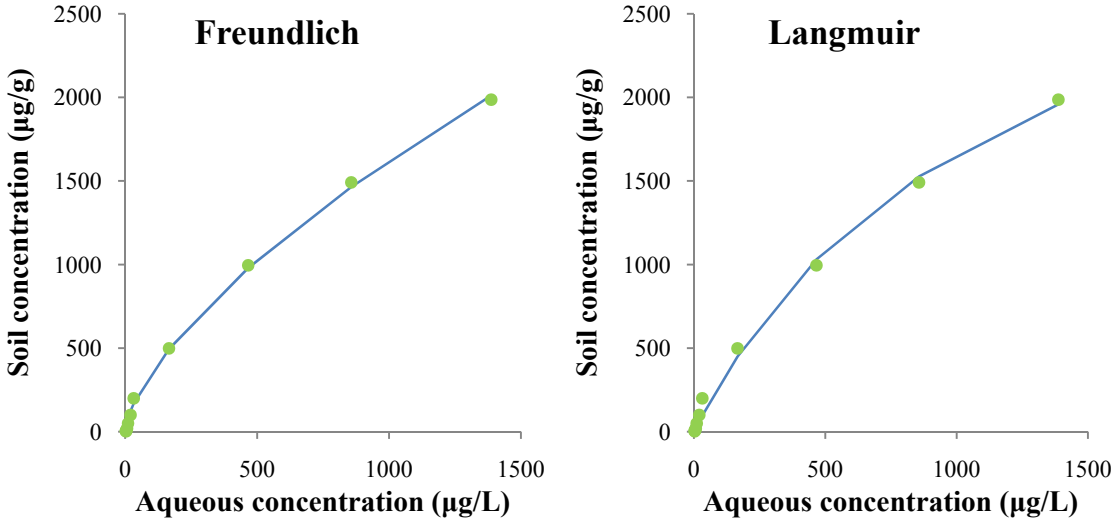

Figure 25 –Sorption isotherms obtained by HPLC-FLD (markers) and predicted (line) by the Freundlich and Langmuir models for the Papiol regadiu soil + 3 g HA.

- **PAPIOL REGADIU + 5 g HUMIC ACIDS**

Table 14 – ENR concentration values for the Papiol regadiu + 5 g HA soil obtained experimentally and estimated by the Freundlich and Langmuir models

Samples	C_i (mg/L)	C_w (µg/L)	$C_{s.\,exp}$ (µg/g)	$C_{Freundlich}$ (µg/g)	$C_{Langmuir}$ (µg/g)
M1	1	0.14	10.00	2.86	0.44
M2	2	1.80	19.98	18.48	5.65
M3	5	8.08	49.92	55.25	25.18
M4	10	21.62	99.78	113.42	66.77
M5	20	45.15	199.55	194.28	137.27
M6	50	167.17	498.33	505.62	470.06
M7	75	278.11	747.22	733.40	732.02
M8	100	410.25	995.90	974.33	1003.44
M9	150	765.63	1492.34	1537.10	1573.32
M10	200	1073.64	1989.26	1967.87	1937.81

- Freundlich equation:

$$C_s = K_f\, C_w^n = 12.01 \times C_w^{0.73}$$

- Langmuir equation:

$$C_s = b\frac{K_L\, C_w}{1 + K_L\, C_w} = 4569\ \frac{6.86 \times 10^{-4} C_w}{1 + 6.86 \times 10^{-4} C_w}$$

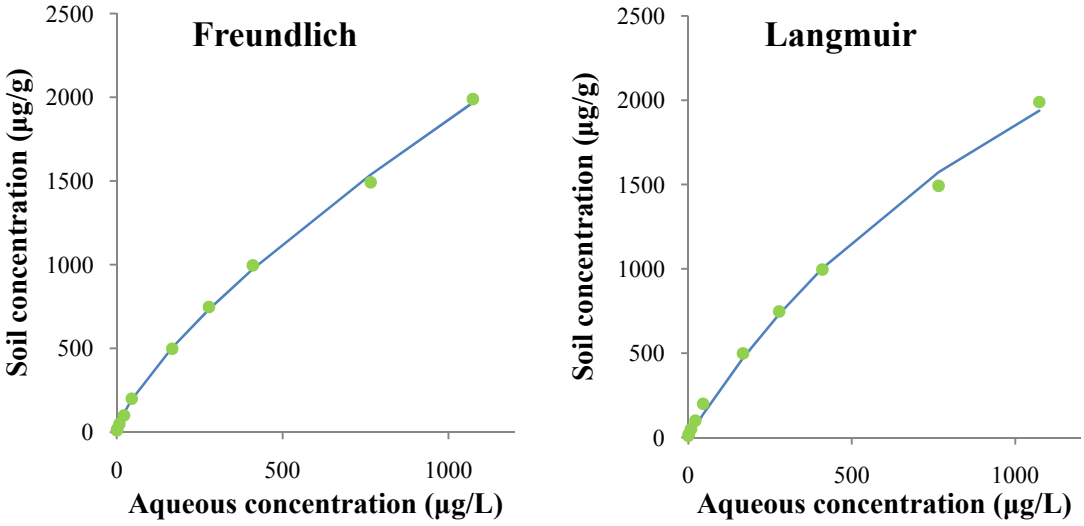

Figure 26 – Sorption isotherms obtained by HPLC-FLD (markers) and predicted (line) by the Freundlich and Langmuir models for the Papiol regadiu soil + 5 g HA.

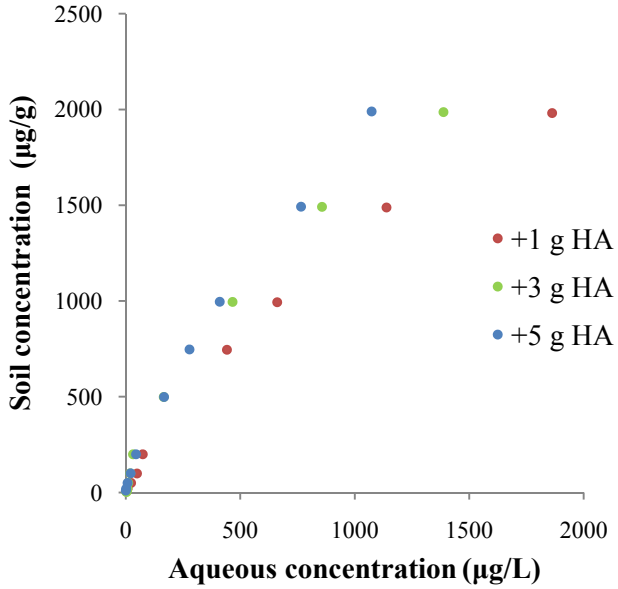

Figure 27 – Sorption isotherms for the three amended soils.

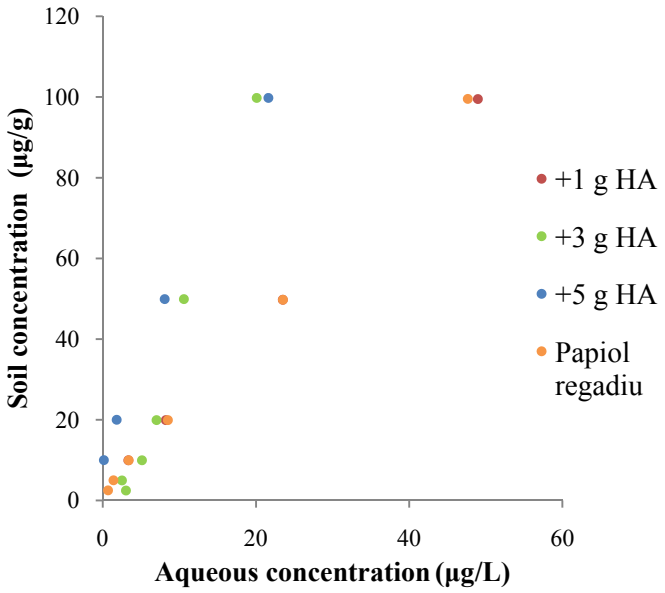

Figure 28 – Sorption isotherms at the lower concentrations for the Papiol regadiu soil and the three amended soils.

Table 15 – Sorption parameters for the Papiol regadiu soil and correspondent amendments.

SOIL	Linear fit	Freundlich fit		Langmuir fit	
	K_d	K_f	n	K_L	b
Papiol regadiu	2.04	3.25	0.90	5.29×10^{-4}	3986
Papiol regadiu + 1g HA	1.96	10.17	0.70	4.93×10^{-4}	4133
Papiol regadiu + 3g HA	4.35	16.95	0.66	8.61×10^{-4}	3597
Papiol regadiu + 5g HA	4.14	12.01	0.73	6.86×10^{-4}	4569

As seen in Figure 27, as the amount of HA increases, the sorption increases, since for the same aqueous concentration the concentration of ENR in the soil is greater. More particularly, as detailed in Figure 28, the soil with + 1 g of HA shows a similar behavior to the original Papiol regadiu soil and no differences can be found among them. The soils with added 3 g and 5 g of HA also have similar sorption isotherms in the studied range. This behavior is supported by the values of the sorption parameters obtained experimentally and estimated by the Freundlich and Langmuir models (collected in Table 15) that are alike between each pair of samples (Papiol regadiu/Papiol regadiu + 1 g HA, and Papiol regadiu + 3 g HA/Papiol regadiu + 5 g HA).

The results obtained in this study suggest that the higher the OM content, the higher the sorption, i.e. the greater value of K_d. On the contrary, other authors observed that by removing OM from the soil the sorption of quinolones was higher [47].

As stated before, the sorption process is not dependent only on the amount of OM; its composition also plays an important role. In this case humic acids were used, which are known to be particularly influent in the sorption process [48]. It is clear that these are just preliminary experiments and more studies have to be executed in order to gain deeper insight on the role of organic matter in the sorption of ENR to soils.

4. CONCLUSIONS

In this Master project, the sorption of ENR to six different basic soils was studied based on the OECD Guideline for the Testing of Chemicals No. 106. From the work developed, the following conclusions can be made:

- The experimental data fitted the Freundlich model adequately for all soils, and the Langmuir model was only suitable for the three soils exhibiting L-type isotherms

- Basic soils showed distribution coefficient values for ENR from 0.69 to 2.04 L/g, which are similar to the ones in acid soils determined by other authors

- The properties that appear to have a stronger influence on the sorption of ENR to basic soils are the content in Fe, Al and Mg oxides

- The amendment of soils with humic acids seems to indicate an increase in the sorption of ENR with the increase in OM content, but further studies are necessary.

5. REFERENCES

1. http://toxics.usgs.gov, *The U.S. Geological Survey's website - Toxic substances.*
2. wellcare®, *wellcare® information for you about Emerging Water Contaminants*. 2005, Water Systems Council.
3. Snow, D.D., et al., *Detection, Occurrence, and Fate of Emerging Contaminants in Agricultural Environments.* Water Environment Research, 2009. **81**(10): p. 941-958.
4. Kümmerer, K., *Pharmaceuticals in the Environment - Sources, fate, effects and risks*. 1st ed. 2001: Springer.
5. Halling-Sørensen, B., et al., *Occurrence, fate and effects of pharmaceutical substances in the environment- A review.* Chemosphere, 1998. **36**(2): p. 357-393.
6. Kümmerer, K., *Sustainable from the very beginning: rational design of molecules by life cycle engineering as an important approach for green pharmacy and green chemistry.* Green Chemistry, 2007. **9**: p. 899-907.
7. McBride, M.B., *Environmental Chemistry of Soils*. 1994: Oxford University Press.
8. Zhou, X., et al., *Excretion of enrofloxacin in pigs and its effect on ecological environment.* Environmental Toxicology and Pharmacology, 2008. **26**(3): p. 272-277.
9. Madden, J.C., et al., *Pharmaceuticals in the environment: Good practice in predicting acute ecotoxicological effects.* Toxicology Letters, 2009. **185**(2): p. 85-101.
10. Drillia, P., K. Stamatelatou, and G. Lyberatos, *Fate and mobility of pharmaceuticals in solid matrices.* Chemosphere, 2005. **60**(8): p. 1034-1044.
11. Kümmerer, K., *The presence of pharmaceuticals in the environment due to human use - present knowledge and future challenges.* Journal of Environmental Management, 2009. **90**(8): p. 2354-2366.
12. Lubick, N., *Opening the "green pharmacy".* Environmental Science & Technology, 2008. **42**(23): p. 8620-8621.
13. Richman, C. and S. Castensson, *Impact of waste pharmaceuticals: an environmental hazard or "greenwash".* Pharmaceutical Journal, 2008. **208**: p. 335-342.

14. Kümmerer, K., *Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources - a review.* Chemosphere, 2001. **45**(6-7): p. 957-969.
15. Hirsch, R., et al., *Occurrence of antibiotics in the aquatic environment.* The Science of The Total Environment, 1999. **225**(1-2): p. 109-118.
16. Kümmerer, K., *Resistance in the environment.* J. Antimicrob. Chemother., 2004. **54**(2): p. 311-320.
17. http://www.merckvetmanual.com/. *The Merck Veterinary Manual online*.
18. Laurence, D.R., P.N. Bennett, and M.J. Brown, *Clinical Pharmacology*. 8th ed. 1997: Churchill Livingstone.
19. http://www.merck.com/mmpe. *The Merck Manual of Diagnosis and Therapy online*.
20. *WHO Global Strategy for Containment of Antimicrobial Resistance*. 2001, World Health Organization.
21. Park, H.-R., T.H. Kim, and K.-M. Bark, *Physicochemical properties of quinolone antibiotics in various environments.* European Journal of Medicinal Chemistry, 2002. **37**(6): p. 443-460.
22. Tillotson, G.S., *Quinolones: structure-activity relationships and future predictions.* J Med Microbiol, 1996. **44**(5): p. 320-324.
23. Knapp, C.W., et al., *Fate and Effects of Enrofloxacin in Aquatic Systems under Different Light Conditions.* Environmental Science & Technology, 2005. **39**(23): p. 9140-9146.
24. Golet, E.M., et al., *Trace Determination of Fluoroquinolone Antibacterial Agents in Urban Wastewater by Solid-Phase Extraction and Liquid Chromatography with Fluorescence Detection.* Analytical Chemistry, 2001. **73**(15): p. 3632-3638.
25. Thiele-Bruhn, S., *Pharmaceutical antibiotic compounds in soils - a review.* Journal of Plant Nutrition and Soil Science, 2003. **166**(2): p. 145-167.
26. Pouliquen, H. and H. Le Bris, *Sorption of oxolinic acid and oxytetracycline to marine sediments.* Chemosphere, 1996. **33**(5): p. 801-815.
27. Tolls, J., *Sorption of Veterinary Pharmaceuticals in Soils: A Review.* Environmental Science & Technology, 2001. **35**(17): p. 3397-3406.

28. Nowara, A., J. Burhenne, and M. Spiteller, *Binding of Fluoroquinolone Carboxylic Acid Derivatives to Clay Minerals.* Journal of Agricultural and Food Chemistry, 1997. **45**(4): p. 1459-1463.
29. Uslu, M., et al., *Analysis and Sorption Behavior of Fluoroquinolones in Solid Matrices.* Water, Air, & Soil Pollution, 2008. **190**(1): p. 55-63.
30. Parshikov, I.A., et al., *Microbiological Transformation of Enrofloxacin by the Fungus Mucor ramannianus.* Applied and Environmental Microbiology, 2000. **66**(6): p. 2664-2667.
31. Evangelou, V.P., *Environmental Soil and Water Chemistry - principles and applications*. 1998: John Wiley & Sons, inc.
32. Sposito, G., *The Chemistry of Soils*. 1989: Oxford University Press.
33. Essingtion, M.E., *Soil and Water Chemistry - an integrative approach*. 2004: CRC Press.
34. Essington, M.E., *Soil and Water Chemistry - an integrative approach*. 2004: CRC Press.
35. Sparks, D.L., *Environmental Soil Chemistry*. 2nd ed. 2003: Academic Press.
36. Lizondo, M., et al., *Physicochemical properties of enrofloxacin.* Journal of Pharmaceutical and Biomedical Analysis, 1997. **15**: p. 1845-1849.
37. Jiménez-Lozano, E., et al., *Determination of pKa values of quinolones from mobility and spectroscopic data obtained by capillary electrophoresis and a diode array detector.* Analytica Chimica Acta, 2002. **464**(1): p. 37-45.
38. Vasudevan, D., et al., *pH-dependent ciprofloxacin sorption to soils: Interaction mechanisms and soil factors influencing sorption.* Geoderma, 2009. **151**(3-4): p. 68-76.
39. Golet, E.M., et al., *Determination of Fluoroquinolone Antibacterial Agents in Sewage Sludge and Sludge-Treated Soil Using Accelerated Solvent Extraction Followed by Solid-Phase Extraction.* Analytical Chemistry, 2002. **74**(21): p. 5455-5462.
40. Morales-Muñoz, S., J.L. Luque-García, and M.D. Luque de Castro, *Continuous microwave-assisted extraction coupled with derivatization and fluorimetric monitoring for the determination of fluoroquinolone antibacterial agents from soil samples.* Journal of Chromatography A, 2004. **1059**(1-2): p. 25-31.

41. Turiel, E., A. Martín-Esteban, and J.L. Tadeo, *Multiresidue analysis of quinolones and fluoroquinolones in soil by ultrasonic-assisted extraction in small columns and HPLC-UV*. Analytica Chimica Acta, 2006. **562**(1): p. 30-35.
42. Golet, E.M., et al., *Environmental Exposure Assessment of Fluoroquinolone Antibacterial Agents from Sewage to Soil.* Environmental Science & Technology, 2003. **37**(15): p. 3243-3249.
43. KarcI, A. and I.A. BalcIoglu, *Investigation of the tetracycline, sulfonamide, and fluoroquinolone antimicrobial compounds in animal manure and agricultural soils in Turkey.* Science of The Total Environment, 2009. **407**(16): p. 4652-4664.
44. Martínez-Carballo, E., et al., *Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria.* Environmental Pollution, 2007. **148**(2): p. 570-579.
45. *OECD Guideline for the Testing of Chemicals No. 106: Adsorption - Desorption Using a Batch Equilibrium Method*. 2000, Organisation for Economic Co-operation and Development.
46. Carrasquillo, A.J., et al., *Sorption of Ciprofloxacin and Oxytetracycline Zwitterions to Soils and Soil Minerals: Influence of Compound Structure.* Environmental Science & Technology, 2008. **42**(20): p. 7634-7642.
47. Zhang, J., et al., *Impacts of soil organic matter, pH and exogenous copper on sorption behavior of norfloxacin in three soils.* Journal of Environmental Sciences, 2009. **21**(5): p. 632-640.
48. Dercová, K., et al., *Bioremediation of soil contaminated with pentachlorophenol (PCP) using humic acids bound on zeolite.* Chemosphere, 2007. **66**(5): p. 783-790.