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RESEARCH ARTICLE

Fate of pharmaceutical compounds and steroid hormones in soil: study of transfer and degradation in soil columns

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Abstract Numerous chemical products are dispersed into the environment, and the consequences can be sometimes harmful to humans and ecosystems. Pharmaceutical compounds and hormone steroids are among these substances that concern the scientific community. Currently, little data are available on the presence and fate of these compounds in the environment and, in particular, for solid matrices. Therefore, the aim of this work was to perform soil column experiments to evaluate the accumulation, transfer and degradation of these substances in soil. The analyses were based on efficient sample preparation followed by sensitive and selective liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). For this purpose, 23 compounds were chosen including both pharmaceutical compounds as well as steroid hormones. In addition, this experiment was performed on two soils with different properties (% clay, pH, etc.). To the best of our knowledge, no soil column experiments have been performed previously on a large number of pharmaceutical compounds and steroid hormones. Significant transfer was observed only for sulphonamides that can be justified by their polarity (log K_{ow}<3). Furthermore, some compounds have a cationic characteristic and are likely to be not much mobiles in soil due to cation exchange process. However, it was observed that the migration of the substances depends on the soil characteristics, such as the amount of clay and the pH values. Regarding the degradation, it was noticed that substances degraded rapidly in the two soils. Indeed, for most substances, their half-lives were lower than 20 days. Furthermore, it was observed that the degradation rate depended on the soil.

Keywords Soil columns · Transfer · Degradation · Emerging contaminants · LC-MS/MS · Leachate

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Introduction

Chemical products are increasingly used for agriculture and domestic activities and are responsible for the spread of several substances in the environment that can be harmful to humans and animals, including pharmaceutical compounds and steroid hormones. These compounds can cause adverse effects. For example, the presence of antibiotics in the environment can lead to pathogen resistance (Chee-Sanford et al. 2009; Finley et al. 2013). Such compounds can also be toxic to ecosystems. Steroid hormones are endocrine disruptors and can affect growth, reproduction and other finely tuned hormonally regulated processes of species (Porte et al. 2006). In addition, more breast and testicular cancers in humans have been noted in recent years (Balabanic et al. 2011).

Large proportions of the antibiotics or animal hormones are not used by animals and are excreted unchanged. Consequently, these compounds can enter the environment through land application of manure. The presence of human drugs and

hormones is resulting from the spread of sewage sludge on agricultural soils. Thus, the monitoring of these substances in the environment is required. However, the presence, distribution and fate of veterinary substances or human drugs and hormones regularly introduced into the soil via land application are far from known and characterised at the present time.

As soon as contaminants are introduced into soil, they are submitted to both biotic (biodegradation and metabolisation) and abiotic (chemical reactions hydrolysis and oxydoreduction) transformations. These processes lead to the reduction of the native substance from the ecosystem or, in the long run, to its total removal. However, during this transformation step, some breakdown products are produced that can be more toxic and/or more persistent in soil (Barriuso et al. 1996; Bavcon et al. 2003; Laganà et al. 2002; Nawab and Aleem 2003; Andreu and Pico 2004; Chaabane et al. 2008). Some works have already reported that sulphonamides, macrolides and β-lactams are sensitive to hydrolysis (Huang et al. 2001; Bialk-Bielinska et al. 2012; Yang et al. 2009). Others have noted that steroid hormones are subject to microbial degradation (Ying et al. 2002). On the other hand, organic compounds can disperse and/or accumulate in the different compartments of the environment (water, soil). Understanding the retention/transfer phenomena into soils is important to predict the risks of contamination. These processes, both degradation and retention/transfer, can be studied either in real conditions or at the laboratory with reconstituted soil columns. Only a few research teams have studied the retention/transfer of antibiotics and steroid hormones with soil columns. Furthermore, their studies addressed a limited number of compounds (Das et al. 2004; Kay et al. 2005). In addition, emerging contaminants have not been studied much compared with "classical" pollutants such as pesticides, which have been examined for a longer time and for which more data are available.

In this context, the goal of this work was to develop methodologies based on soil column experiments to study the fate of numerous pharmaceutical compounds as well as steroid hormones in soil. The objective was to examine the accumulation, transfer and degradation in this matrix. Experiments were performed with two types of soil with distinct amounts of clay, organic matter and different pH values. This work is innovative because, as mentioned previously, no such experiment was performed previously on a large number of compounds belonging to emerging contaminants. Moreover, both solid and liquid phases were analysed, using efficient and sensitive methods allowing to achieve quantization thresholds rarely achieved before.

To perform this experiment, 14 veterinary or human antibiotics were chosen belonging to antiparasitics (dicyclanil), sulphonamides (sulphanilamide, sulphadiazine, sulphathiazole, sulphameter, sulphadimidine, sulphabenzamide, sulphadimethoxine, sulphamethoxazole), diaminopyrimidines (trimethoprim), macrolides (erythromycin, tylosin, roxithromycin) and β-lactams (penicillin G). Six steroid hormones were chosen, most of which naturally occur in all vertebrates, whereas others are synthetic. The natural androgens androstenedione and testosterone; the natural progestagen progesterone and the synthetic progestogens norethindrone, levonorgestrel and gestodene were selected. Finally, three human drugs that we considered as markers of human contamination were also chosen: carbamazepine, fluvoxamine and paracetamol.

The fate of the compounds in soils and leachates were followed using innovative analytical methods based on efficient sample preparation followed by selective and sensitive analyses by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

Experimental

Chemicals and materials

Vetranal (V) analytical standards were used in addition to others that meet United States Pharmacopeia (USP) testing specifications. Dicyclanil (V), sulphanilamide (V), sulphadiazine (V), sulphathiazole (99 %), sulphameter (V), sulphadimidine (V), sulphabenzamide, sulphadimethoxine, sulphamethoxazole, trimethoprim (V), erythromycin (USP), tylosin tartrate (V), roxithromycin (>90 %), penicillin G potassium salt (V), carbamazepine (USP), fluvoxamine, paracetamol (99 %), androstenedione (V), testosterone (V), progesterone (>99 %), norethindrone (V) and levonorgestrel (99 %) were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). The steroid gestodene (98 %) was purchased from AK Scientific (CA, USA).

Deuterated compounds were also chosen as internal standards. These substances are commercially available for only few compounds. However, a minimum of one deuterated compound per family was obtained (except for dicyclanil). Sulphamethoxazole-d_4 (>98 %) was chosen for sulphonamides, and trimethoprim-d_3 (99.6 %) was chosen for trimethoprim (diaminopyrimidine). As for macrolides, erythromycin ^{13}C-d_3 (>98 %) was chosen, and penicillin G-d_7 (>98 %) was used for the β-lactam family. Concerning human contaminants, carbamazepine-d_{10} (99 %), fluvoxamine-d_4 (98.2 %) and paracetamol-d_3 (99.1 %) were employed. Finally, deuterated substances were also used for steroid hormones: androstenedione-d_7 (98.8 %), testosterone-d_2 (99.7 %), progesterone-d_9 (99.1 %), norethindrone-d_6 (99.6 %), levonorgestrel-d_6 (99.4 %) and gestodene-d_6 (98.2 %). These deuterated compounds were purchased from C/D/N isotopes (Cluzeau, Sainte Foy La Grande, France) except sulphamethoxazole-d_4, erythromycin ^{13}C-d_3 and

penicillin G-d_7, which were acquired from Toronto Research Chemicals Inc. (TRC, Canada).

Individual stock solutions were prepared at concentrations of 200 mg/L in methanol and stored at −23 °C. Working solutions were prepared by the appropriate mixture and dilution of the stock solutions.

Methanol (MeOH), acetonitrile (ACN) (LC-MS grade), acetone and ethanol (grade HPLC) were purchased from Sigma-Aldrich. Pure water was obtained from a MilliQ device from Millipore (Saint-Quentin-en-Yvelines, France). Formic acid (98 %, LC-MS grade), ammonium hydroxide solution (NH_3, aq 25 % in water), citric acid monohydrate and calcium chloride were purchased from Sigma-Aldrich. Acetic acid and sodium salt anhydrous was acquired from Arcos (Geel, Belgium).

QuEChERS extract tubes (AOAC method) were obtained from Agilent Technologies (Massy, France). They contained 1.5 g of NaOAc and 6 g of $MgSO_4$.

Solid-phase extractions were performed using OASIS HLB (60 mg, 3 mL) cartridges from Waters (Saint-Quentin-en-Yvelines, France), SAX (500 mg, 3 mL) cartridges from Agilent Technologies (Courtaboeuf, France) and Strata-X cartridges (200 mg, 3 mL) from Phenomenex (Le Pecq, France).

Soil samples

Both transfer and degradation were studied for two soils, A and B. Their properties are displayed in Table 1. The cation exchange capacity (CEC) was evaluated by using a solution of chloride cobalt hexamine trichloride ($Co(NH_3)_6Cl_3$) 50 mmol + /L (millimoles of positive charges per litre), according to the norm NF X 31-130. The pH value was measured after suspending the soil sample air-dried in water with a 1:5 (*v*/*v*) ratio, according to the standard NF ISO 10390. The soil samples were dried and passed through a 3-mm sieve to remove coarse particles. They were subsequently introduced into the soil columns.

Soil column protocol

Soil columns in PVC with a diameter of 10 cm and a length of 30 cm were created in the laboratory (Fig. 1). The inferior end of the column had a funnel shape to recover the leachate into flasks. The bottom of the column was made of a grid covered with glass beads that allowed recovery of the leachate in limiting the loss of solid particles. A watering can was also created that permitted the introduction of water dropwise, allowing a homogeneous and reproducible watering over the whole surface.

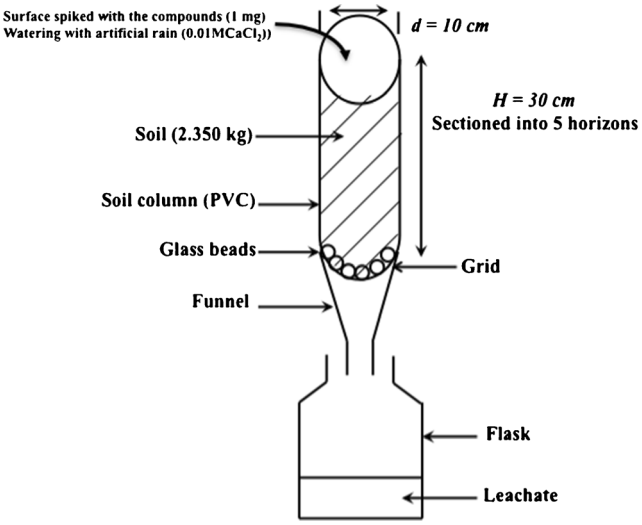

Fig. 1 Soil column design

Each soil column was filled with 2.350 kg of soil. Two series of columns were prepared, one with soil A and the other with soil B. The soil properties are shown in Table 1.

As the substances were not soluble in the same solvent, the following protocol was performed to ensure that each substance was introduced identically at the top of the column. First, 22 mg of each compound was weighed. Dicyclanil, sulphadimethoxine, sulphameter, erythromycin, roxithromycin, carbamazepine, fluvoxamine, paracetamol, androstenedione, testosterone, progesterone, norethindrone, levonorgestrel and gestodene were dissolved in 550 mL of ethanol. Sulphanilamide, sulphadimidine, sulphadiazine, sulphabenzamide, sulphamethoxazole and trimethoprim were dissolved in 220 mL of acetone. Finally, sulphathiazole, tylosin and penicillin G were dissolved in 220 mL of MilliQ water. Then, for each soil A and B, 275 g of soil, 275 mL of spiked ethanol and 110 mL of spiked acetone were placed in a 2-L round flask. This mixture was subsequently homogenised. The solvents were evaporated under vacuum at a temperature of 37 °C with a rotary evaporator. The temperature was chosen to be lower than 40 °C, because we had previously verified that a temperature of 40 °C does not degrade the target

Table 1 Properties of soils A and B

Soils	Soil texture	Clay (%)	Silt (%)	Sand (%)	OC (%)	N_{tot} (%)	OM (%)	pH	CEC (cmol/kg)	Bulk density (g/cm^3)
A	Silty clay loam	32.4	45.1	22.5	1.73	0.173	2.99	6.25	15.6	1.52
B	Sandy loam	9.5	21.5	69	3.76	0.207	6.5	8.07	8.72	1.29

OC organic carbon, N_{tot} total nitrogen, *OM* organic matter, *CEC* cation exchange capacity

substances (Salvia et al. 2012). Thus, homogeneous spiked soil was obtained. At the top of the column, 25 g of spiked soil and 10 mL of spiked water were placed on the soil surface. Finally, each column was contaminated with 1 mg of each substance. For each soil type, ten columns were prepared: five were spiked and five were conserved as controls.

Before the soil spiking, at the beginning of the experiment, all of the columns were moistened by introducing 150 mL of 0.01 M $CaCl_2$ two times per day for 4 days. After the spiking, they were regularly watered with 40 mL of 0.01 M $CaCl_2$ every 2 days for 30 days. The columns were surmounted by a cap with small holes which prevents drying of the soil surface. At predefined intervals (0, 5, 10, 15 and 30 days), one spiked and one control entire column were placed in a freezer prior to analysis. The 40 mL of leachates were recovered every 2 days in amber flasks. They were also conserved in a freezer prior to analysis. The volume of watering was selected based on the precipitation recorded in the Rhône-Alpes region. According to the literature, annual pluviometry is between 750 and 900 mm (Blanchet 1993). Therefore, the columns were watered with 40 mL of "artificial" rain every 2 days, which corresponds to an annual pluviometry of 930 mm.

Before the analysis, the frozen soil columns were cut into five equal slices. They were subsequently defrosted to homogenise each horizon. Then, approximately 50 g of each slice was lyophilised for 24 h to dry the matrix. Then, the soil was ground in a mortar and passed through a 0.63-mm sieve to obtain a homogeneous sample. Finally, 5 g of soil sample was placed in a QuEChERS tube and spiked with internal standards at 100 ng/g. The extraction step was then performed followed by the LC-MS/MS analysis.

Soil sample preparation

For the analysis of the soil samples, a modified-QuEChERS extraction followed by a tandem solid-phase extraction (SPE) purification was performed. The extracts were the analysed by liquid chromatography coupled to tandem mass spectrometry.

For the modified-QuEChERS extraction step, 10 mL of MilliQ water and 15 mL of ACN were added to the tube that contained 5 g of soil. The tube was shaken with a vortex device (Vortex Fisher Scientific FB15013 TopMix). Acetate buffer was then added, and the tube was immediately manually shaken for 30 s and swirled on a vortex mixer for 30 s. The tube was then shaken for 3 min at 750 rpm in a sample homogeniser (SPEX Sample Prep, 2010 GenoGrinder, Delta Labo, Avignon, France). After centrifugation at 5,000 rpm for 2 min (Sigma Laboratory Centrifuges 3K30H, Fisher Bioblock Scientific), 10 mL of the ACN layer was transferred into a 12-mL glass tube. The extract was then evaporated to dryness under a gentle stream of nitrogen at a temperature of 40 °C.

The extract obtained was cleaned using SPE (RapidTrace SPE Workstation, Caliper) with two cartridges in tandem. First, after a preconditioning of the SAX cartridge with MeOH and a conditioning buffer (0.04 M citric acid), the extract (dissolved in 97/3 citric acid (0.04 M)/MeOH, pH=2.5) was passed through the cartridge at a flow rate of 1 mL/min and recovered in a tube. Second, a Strata-X cartridge was also conditioned with both MeOH and 0.04 M citric acid, and then, the previously recovered extract was passed through the cartridge at 1 mL/min. The Strata-X cartridge was subsequently washed with 2 mL of 0.04 M citric acid and 2 mL of 0.1 M NaOAc. The cartridge was then dried for 30 min under a stream of nitrogen and eluted with 10 mL of MeOH at a flow rate of 1 mL/min. Finally, the recovered extract was evaporated to dryness under a gentle stream of nitrogen at a temperature of 40 °C.

The dry residue was dissolved in 200 μL of 95/5 % H_2O/MeOH and mixed for 20 s prior to LC-MS/MS analysis. All of the details of the development and performance of the methods have been described previously (Salvia et al. 2012).

Leachate sample preparation

The sample preparation of the leachate was performed using SPE with an AutoTrace SPE Workstation Lifesciences system from Dionex (Courtaboeuf, France). For the procedure, 20 mL of leachate were first diluted into 180 mL of MilliQ water to obtain a total volume of 200 mL. Then, this solution was adjusted to pH=7 with NH_4OH. An OASIS HLB cartridge was subsequently used to extract target analytes. After a preconditioning of the cartridge with 5 mL of MeOH and then 5 mL of MilliQ water, the diluted leachate was passed through the cartridge at a flow rate of 5 mL/min. The cartridge was then washed with 5 mL of MilliQ water adjusted to pH=7, dried for 30 min under a stream of nitrogen and eluted with 10 mL of MeOH at a flow rate of 2 mL/min. Finally, the extract was evaporated to dryness under a gentle stream of nitrogen at a temperature of 40 °C. The dry residue was dissolved in 200 μL of 95/5 % H_2O/MeOH before the LC-MS/MS analysis.

The performances of the method were evaluated from the leachate collected at the outlet of a control soil column (soil A). The recoveries were calculated from the surfaces of each compound by dividing the average of the surfaces "spiked before SPE extraction" on the average surface "spiked after SPE extraction". The "spiked after SPE extraction" corresponds to the extracted samples; the obtained extract was spiked just before analysis. This calculation was performed three times at 200 ng/L of each of the compound. From these experiments, it was possible to deduce the repeatability that measures the minimal variability of the results on the same day under the same conditions. It was expressed as the relative standard deviation (RSD, %). The linearity was evaluated on

12 levels (from leachate spiked from 0.1 to 500 ng/L). Ranges with a regression coefficient (r^2) above 0.9900 were considered linear. To determine the limits of detection (LOD) and limits of quantification (LOQ), leachate samples spiked with various concentrations were extracted and analysed. The LOD and LOQ corresponded to the lowest concentration that gave a signal-to-noise ratio of 3 and 10, respectively. The performances of the method are summarised in Table 2.

Analysis by liquid chromatography-tandem mass spectrometry

A HP 1100 chromatographic system (Agilent Technologies) equipped with a degasser, a binary pump, an autosampler and a column oven was used to perform the liquid chromatography. The LC system was coupled to a triple-stage quadrupole mass spectrometer 3200 QTrap from ABSciex (Les Ulis, France) with an electrospray ion (ESI) source (TurboV, ABSciex). The analytes were identified by both their chromatographic characteristics and their specific multiple reaction monitoring (MRM) fragmentation patterns. The chromatographic conditions as well as the parameters used for the detection were described by Salvia et al. (2012). Data processing was performed with Analyst software (version 1.5.1).

Data analysis

For leachates, the quantification of the compounds was performed by matrix matching. This method is typically

Table 2 Performances of the method

Compounds	Linear range (ng/L)	r^2	Recoveries (%)	RSD (%)	LOD (ng/L)	LOQ (ng/L)	pKa
Veterinary antibiotics							
Antiparasitic							
Dicyclanil	2–500	0.995	101	2.5	2	6.27	4.58
Sulphonamides / trimethoprim							
Sulphathiazole	0.1–500	0.998	99	1.6	0.03	0.11	pKa_1=2.40—pKa_2=7.10
Sulphadimethoxine	0.5–500	0.999	97	3.3	0.27	1.23	pKa_1=2.00—pKa_2=6.70
Sulphadimidine	0.1–500	0.999	99	1.0	0.03	0.11	pKa_1=2.40—pKa_2=7.40
Sulphanilamide	50–500	0.981	15	20.5	32	117	pKa_1=2.40—pKa_2=10.40
Sulphadiazine	0.1–500	0.993	97	1.4	0.03	0.09	pKa_1=2.00—pKa_2=6.50
Sulphabenzamide	0.5–500	0.997	98	1.5	0.30	1.01	pKa_1=1.20—pKa_2=4.60
Sulphameter	0.1–500	0.999	95	0.8	0.04	0.30	pKa_1=1.48—pKa_2=6.69
Sulphamethoxazole	0.5–500	0.999	101	2.8	0.34	0.79	pKa_1=1.90—pKa_2=6.00
Trimethoprim	0.1–500	0.992	95	1.5	0.05	0.50	pKa_1=1.32—pKa_2=7.45
Macrolides							
Erythromycin	1–500	0.999	89	8.1	0.71	1.54	8.80
Roxithromycin	0.1–500	0.998	84	2.9	0.03	0.11	9.17
Tylosin	0.5–500	0.997	82	3.0	0.21	0.69	7.73
B-lactam							
Penicillin G	2–500	0.992	98	2.6	1.18	4.61	2.80
Human drugs							
Carbamazepine	0.25–500	0.997	100	4.4	0.15	0.93	13.90
Fluvoxamine	5–500	0.990	87	7.2	2.39	9.33	9.40
Paracetamol	10–500	0.991	43	3.2	6.40	9.21	9.90
Steroid hormones							
Androgens							
Androstenedione	1–500	0.999	98	3.0	0.50	1.92	
Testosterone	5–500	0.998	99	4.3	4.96	13.73	
Progestagens							
Levonorgestrel	10–500	0.995	103	10.0	10.18	18.04	
Norethindrone	5–500	0.996	100	6.7	2.39	9.91	
Progesterone	2–500	0.994	92	9.9	1.02	3.71	
Gestodene	10–500	0.993	94	8.1	10	34	

employed to quantify compounds in complex matrices. Indeed, this methodology compensates for matrix effects due to interfering substances that exhibit influence on the analytical response. To employ this procedure, a non-contaminated matrix must be available. In our case, leachates collected from soil A or B that had not been spiked were used. These samples were previously checked to ensure that they did not contain any target analytes. A calibration curve was created from 0.1 to 500 ng/L.

For the analysis of soil horizons, an internal standard calibration was employed. This technique allows elimination of errors due to both experimenter and equipment. It also permits correction for matrix effects. At least one internal standard was found for each family, except for dicyclanil. Trimethoprim-d_3 was used as the antiparasitic reference.

Results and discussion

Study of the transfer of the target compounds in the soil column

The transfer of the compounds corresponds to their displacement through the soil due to the water infiltration through soil layers. It was evaluated by both the presence of the substances into the leachate and their distribution in the different soil horizons. The transfer was studied for soils A and B. Data represent the average of two series of measurements.

The analyses of the leachates of the two soils indicate that very few compounds were present. Indeed, only five compounds were present in few leachates of the soil A with levels comprised between 0.04 and 2 ng/g, as compiled in the Table 3. These values are negligible compared with the amount of substance put at the top of the column at the beginning of the experiment (1 mg of each compound). The results indicate that the number of compounds detected or their levels exhibit no tendency to increase or decrease with time. The target substances were retained in the solid matrix or present in their degraded form in the leachate.

First, we compared the transfer between the different substances for the same soil type. Thus, for the five sampling times (0, 5, 10, 15 and 30 days), the distribution of the native compounds present throughout the column was evaluated. Horizon 1 corresponds to the top of the column, and horizon 5 is the lowest part of the column.

Globally, only low transfer through the columns was observed for all of the target substances (one example of each category of compound is shown in Fig. 2; all the results are presented in the Fig. S1). The compounds were, for most of them, retained in the first horizon of the column. Only a significant portion of sulphonamides were present in the second layer. For example in soil B, 24 % of sulphamethoxazole and 10 % of sulphadimethoxine were found in the second horizon after 30 days of the experiment, whereas the other substances stayed, for most of them, in the first layer. In addition, only a very low quantity of compounds (less than 1 %) was found in layers 3, 4 and 5. Some of these results are displayed in Fig. 2, which shows the transfer of sulphamethoxazole, progesterone and fluvoxamine through soils A and B.

These results could firstly be explained by the polarity of sulphonamides. Indeed, log K_{ow} values of target sulphonamides are between −0.62 and 1.63. Most of the other compounds exhibit log K_{ow} values between 2.45 and 3.87. Therefore, these other compounds did not transfer through the column after their adsorption in the top layer. Furthermore, sulphonamides have low values of K_d (some L/kg) that could also justify their strongest mobility in soil. Besides, the low mobility of some substances could also be explained by their cationic characteristics. It is the case of macrolides that are basic compounds, their amine function being protonated at pH<pKa. In the two studied soils (pH 6.25 and 8.07 for the soil A and B, respectively), these substances are cationic as erythromycin, roxithromycin and tylosin exhibit pKa of 8.80, 9.17 and 7.73, respectively. Fluvoxamine is also protonated in the two soils as it has a pKa of 9.90. These substances can exchange with cations present in soil (in the clay layers for instance) and are therefore trapped in this medium. The influence of cation exchange processes on the transport behaviour of cationic compounds was already mentioned in some works (Ter Laak et al. 2006; Franco and Trapp 2008; Shaffer et al. 2012). Most of the other compounds are neutral such as steroids that exhibit a cyclopentanophenanthrene ring that is hydrophobic and could explain their low mobility.

Table 3 Levels (ng) of compounds detected in the leachate of the soil A

	Days												
Compound	2	5	7	9	12	14	16	19	21	23	26	28	30
Dicyclanile		3.6											
Sulphadimethoxine				0.1									
Sulphadimerazine										0.04			0.04
Tylosine	0.4	1	0.5		0.2		0.1						
Paracetamol				2									

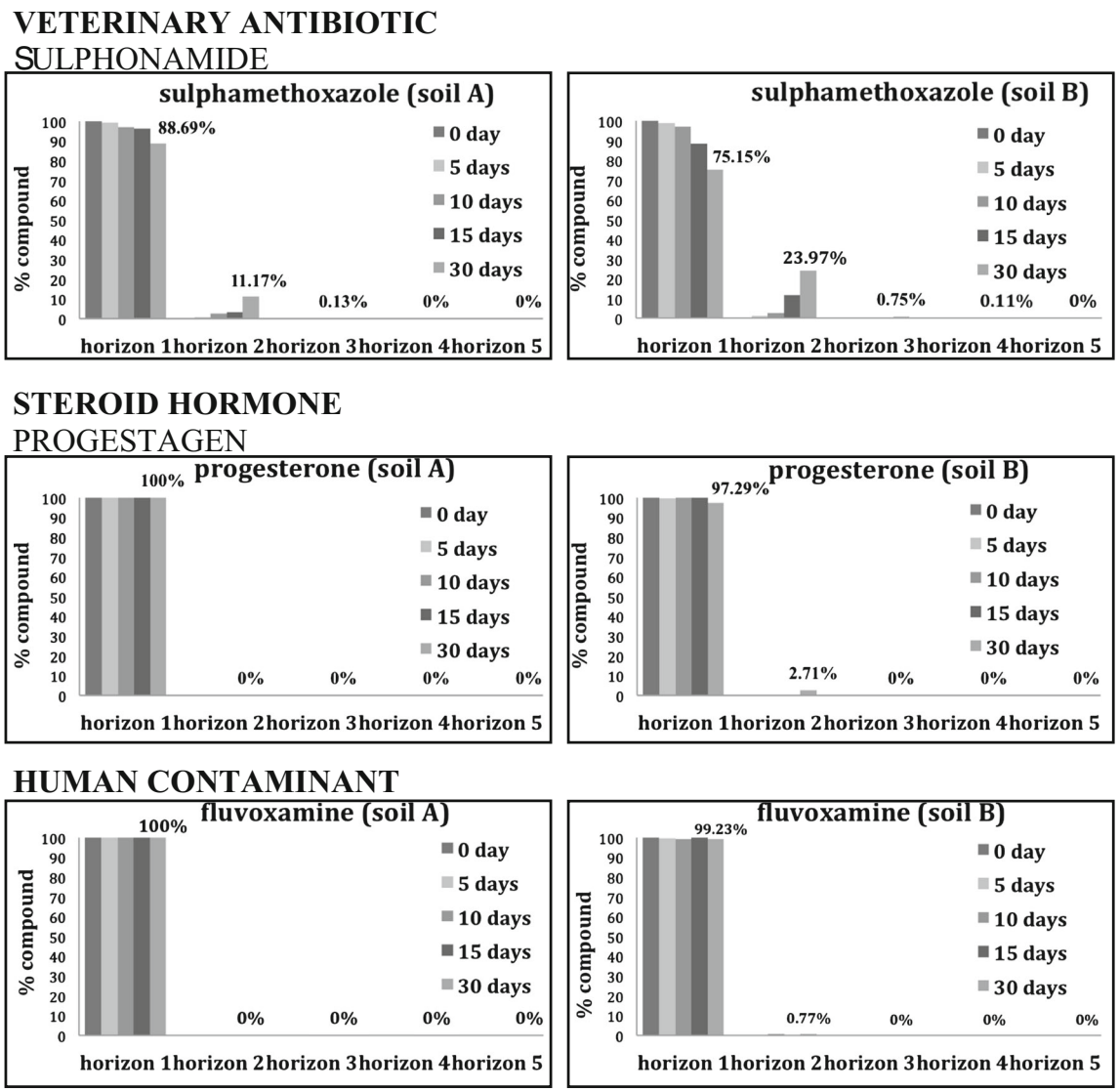

Fig. 2 Transfer of sulphamethoxazole, progesterone and fluvoxamine through soils A and B (*y* axis corresponding to the percentage of compound present in each horizon of soil column)

Then, the difference of transfer between the two soils was investigated.

It was observed that the compounds were more mobile in soil B than in soil A (Fig. 2, all the results are presented in the Fig. S1). This was true for most of the sulphonamides. For instance, after 30 experimental days, 24 % of sulphamethoxazole and 10 % of sulphadimethoxine were found in the second horizon of the column containing soil B, whereas only 11 and 1 % of sulphamethoxazole and sulphadimethoxine, respectively, were present in the second layer in the case of soil A. The same phenomenon was observed for sulphadimidine, sulphadiazine and sulphabenzamide. As for the steroid hormones, although their distribution through the soil column was very low, most likely due to their low polarity and their low solubility in water, a tiny quantity of progesterone (3 %) was found after 30 days in the second horizon of the soil column filled with soil B. This substance was only found in the first layer in the case of soil A (Fig. 2). The same phenomenon was noted for androstenedione.

These observations could be explained by the clay content of each soil type. Indeed, there was 32.4 and 9.5 % of clay in soils A and B, respectively. Previous works demonstrated that the higher the clay quantity is, the higher the adsorption of sulphonamides (Jacobsen et al. 2004; Kumar et al. 2005), macrolides (Jacobsen et al. 2004; Kumar et al. 2005) and steroid hormones (Dekissa 2008) into the soil matrix. A statistical study that we performed previously (Salvia et al. 2013) also indicated that the clay percentage had an impact on both sulphonamides and macrolide recoveries (variables negatively correlated). This result confirms an interaction between clay and the examined substances.

The difference of migration between the two soils could also be explained by their different pH values (6.25 and 8.07 in soils A and B, respectively). At a pH higher or equal to 7.5, sulphonamides (pKa_2 between 6 and 7.5) exist mainly under their anionic form and are therefore more soluble in aqueous solutions and more easily pulled down by water. Moreover, in these conditions, these substances lose their cationic characteristic, and therefore, they are less adsorbed on the soil (Kurdwadkar et al. 2007). This could explain why sulphonamides are more mobile in soil B than in soil A.

Moreover, sulphonamides are more mobile than the other compounds because they are the most polar and are anionic in some conditions (soil B). Futhermore, they exhibit low values of K_d that justify their strongest mobility in soil. Besides, some compounds (macrolides, fluvoxamine) have a cationic characteristic and therefore are likely to be not much mobile in soil. Others are neutrals and hydrophobic as steroid hormones that could explain why there are not pulled down by water.

Degradation study

The degradation of the compounds was evaluated in the two soil types. Target compound concentrations measured after different time intervals were plotted (examples are shown Fig. 3 and Fig. S2), and the disappearance curves of each chemical were fitted to the exponential decay model to estimate the degradation rate constant k and half-life $t_{1/2}$ (which corresponds to the time required to observe 50 % of disappearance of the initial content of the chemical substance). The

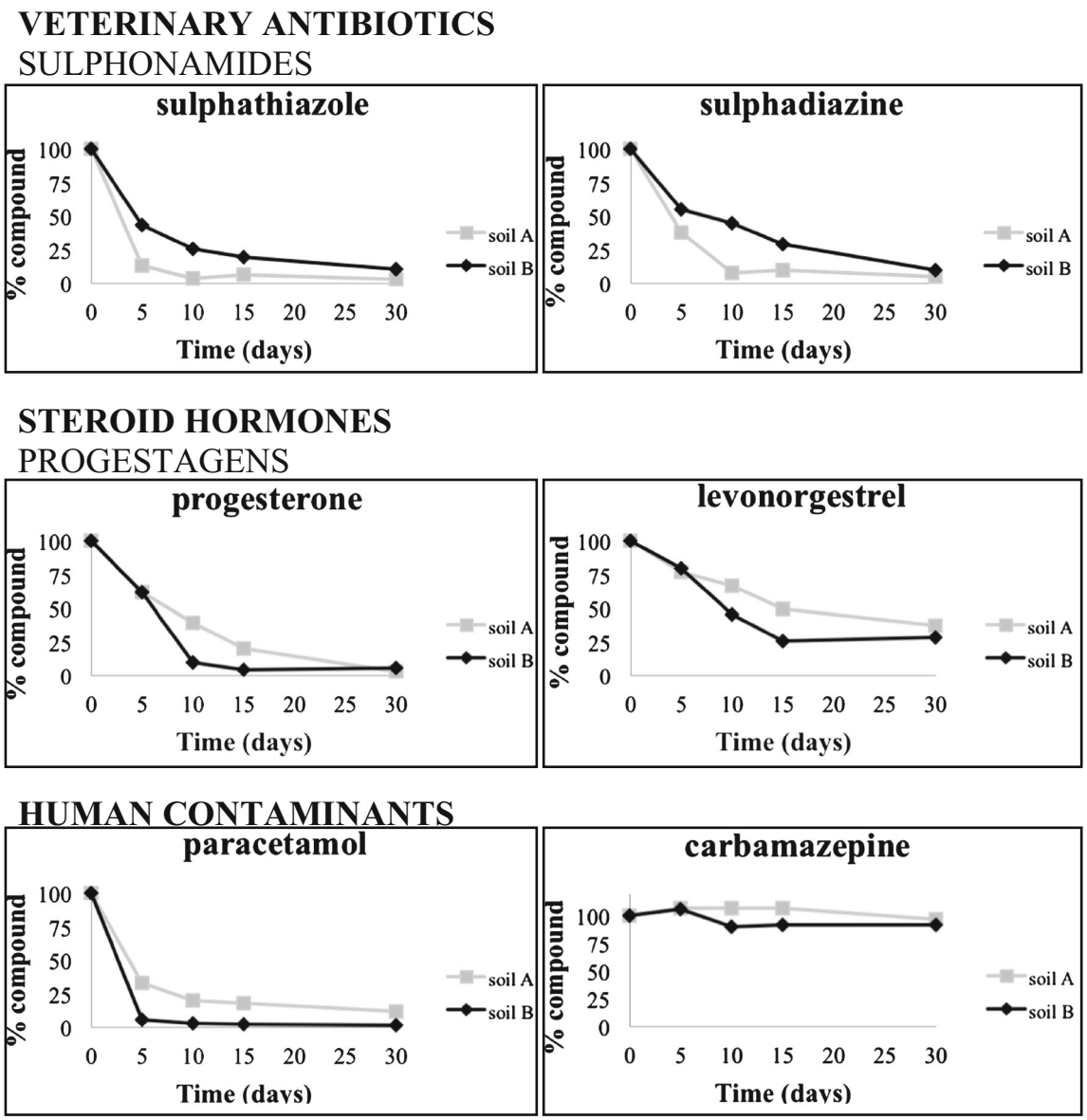

Fig. 3 Examples of degradation curves in soils A and B. The rate takes into account the overall content of the column

concentration considered is the total content of the compound in the column. All of the fits were excellent with correlation coefficient superior to 0.95, suggesting that the degradation rate of selected compounds in soil could be well described with first-order exponential decay model % degradation=e^{-kt}.

It was observed that the substances were degraded relatively rapidly. Indeed, for most of the compounds, $t_{1/2}$ values were lower than 20 days (Table 4). However, two compounds appeared persistent in the soil matrix: roxithromycin and carbamazepine. Indeed, they were only degraded approximately 15–20 and 4–9 %, respectively, after 30 experimental days.

These results are in agreement with those mentioned in previous works. Indeed, for sulphonamides, Accinelli (2007) mentioned average $t_{1/2}$ values between 18.6 and 21.3 days for sulphadimidine and sulphachloropyridine, respectively, in silt loam and sandy soils. Feng et al. (2010) reported half-lives between 4 and 11 days for trimethoprim, under aerobic and anoxic conditions, respectively. In regards to macrolides, Schlüsener and Bester (2006) reported half-lives of 20 and 8 days for erythromycin and tylosin, respectively. They also noticed that roxithromycin is relatively persistent in the soil as they observed that this compound remained nearly unchanged during their whole experimental period (120 days). Penicillin G is known to be relatively unstable and degraded quickly in soil, transforming into penicilloic acid (Beausse 2004; Chee-Sanford et al. 2009). Concerning the human contaminants, Walters et al. (2010) mentioned half-lives between 462 and 533 days for carbamazepine. The persistence of carbamazepine in soil was also confirmed by Monteiro and Boxall (2009). Paracetamol is known to be rapidly degraded as a $t_{1/2}$ of 2.1 days was found by Lin et al. (2010). Finally, the degradation of steroid hormones was also studied in previous works. A $t_{1/2}$ between 0.7 and 3.2 days was mentioned for androstenedione and half-lives between 0.3 and 14 days for testosterone have already been found (Lee et al. 2007; Yang et al. 2010). Tang et al. (2012) mentioned $t_{1/2}$ values between 4.32 and 11.55 days for levonorgestrel from experiments performed using five different soil types.

In addition, it was observed that the degradation was more rapid in soil B than in soil A for some substances (Table 4). It was the case for dicyclanil, trimethoprim, macrolides (erythromycin and tylosin), fluvoxamine, paracetamol and most of the steroid hormones (androstenedione, levonorgestrel, norethindrone and progesterone). On the contrary, sulphonamides seemed degraded more rapidly in soil A compared with soil B. Figure 3 shows some examples of degradation in both soils A and B (sulphathiazole, sulphadiazine, progesterone, levonorgestrel, paracetamol and carbamazepine). These differences in degradation rate could be explained by the pH difference of the two soils as biotic and abiotic degradations are influenced by the soil properties, mainly pH, temperature, clay and organic matter contents. However, no conclusion could be drawn and more experiments are required to understand this phenomenon.

Table 4 $t_{1/2}$ values for soils A and B and for the 23 studied substances

Compounds	Soil A		Soil B	
	k (/day)	$t_{1/2}$ (day)	k (/day)	$t_{1/2}$ (day)
Veterinary antibiotics				
Antiparasitic				
Dicyclanil	0.057	12.2	0.079	8.8
Sulphonamides/trimethoprim				
Sulphathiazole	0.329	2.1	0.113	6.1
Sulphadimethoxine	0.061	11.2	0.034	20.4
Sulphadimidine	0.171	4.0	0.045	15.4
Sulphanilamide	0.180	3.8	0.050	13.9
Sulphadiazine	0.187	3.7	0.078	8.9
Sulphabenzamide	0.240	2.9	0.183	3.8
Sulphameter	0.106	6.5	0.052	13.3
Sulphamethoxazole	0.046	14.9	0.059	11.8
Trimethoprim	0.013	51.3	0.051	13.6
Macrolides				
Erythromycin	0.034	20.2	0.039	17.4
Roxithromycin	0.007	87.7	0.012	57.3
Tylosin	0.026	25.9	0.040	17.3
B-lactam				
Penicillin G	Nd	<2	Nd	<2
Human drugs				
Carbamazepine	0.002	330.0	0.004	170.0
Fluvoxamine	0.043	16.0	0.108	6.4
Paracetamol	0.115	6.0	0.150	4.6
Steroid hormones				
Androgens				
Androstenedione	0.090	7.7	0.229	2.3
Testosterone	0.143	4.8	0.257	2.7
Progestagens				
Levonorgestrel	0.033	20.8	0.097	7.2
Norethindrone	0.034	20.1	0.052	13.3
Progesterone	0.10	6.4	0.233	3.0
Gestodene	0.086	8.0	0.061	11.4

Nd not determined

Conclusion

This study is innovative as, to our knowledge, no soil column experiments have been conducted previously on a large number veterinary and human drugs as well as steroid hormone compounds.

The results revealed a high and rapid degradation as in both soils, most of the substances exhibit $t_{1/2}$ lower than 20 days

except roxithromycin and carbamazepine that are relatively persistent in soil. Furthermore, a low migration of the target molecules was observed. Only sulphonamides migrate significantly in the second layer of the soil column (which contains five horizons) that could be explained by their polarity and low values of K_d. On the other hand, the low mobility of some substances could be explained by their cationic characteristics. It is the case for instance of macrolides and fluvoxamine. As rapid degradation and low transfer occurred, it can be assumed that only a low quantity of these parent compounds is present in the inferior layers.

Furthermore, different behaviour for both transfer and degradation was observed in the two soils. Indeed, some compounds (sulphonamides, steroid hormones) seem to migrate more in soil B than in soil A that could be explained by both the clay percentage and the soil pH. Finally, different degradation rates were observed for the two soils and the different substances that might be due to the soil pH, but more experiments must be performed to explain this result.

Some breakdown products are formed which can be more toxic and/or more persistent in soil. Additional studies must be conducted to identify them and evaluate their fate in the soil matrix.

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