

Accepted Manuscript

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PII: S0048-9697(18)34414-0
DOI: <https://doi.org/10.1016/j.scitotenv.2018.11.061>
Reference: STOTEN 29410

To appear in: *Science of the Total Environment*

Received date: 19 July 2018
Revised date: 3 November 2018
Accepted date: 5 November 2018

Please cite this article as: Meritxell Gros, Josep Mas-Pla, Mercè Boy-Roura, Irma Geli, Francesc Domingo, Mira Petrovic , Veterinary pharmaceuticals and antibiotics in manure and slurry and their fate in amended agricultural soils: Findings from an experimental field site (Baix Empordà, NE Catalonia). Stoten (2018), <https://doi.org/10.1016/j.scitotenv.2018.11.061>

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Veterinary pharmaceuticals and antibiotics in manure and slurry and their fate in amended agricultural soils: Findings from an experimental field site (Baix Empordà, NE Catalonia)

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Abstract

The fate and transport of 34 veterinary pharmaceuticals (PhACs) is investigated in swine slurry and dairy cattle manure-amended agricultural soils, from an experimental field site, by using both analytical and modelled data. Potential differences on PhACs fate, attributed to the application of distinct swine slurry fractions (total, solid, and liquid), are herein assessed for the first time. Surface and deep soil layers, up to a depth of 120 cm, were analyzed at different periods after an annual fertilization event. Using input data representing typical agricultural soil conditions and the PhACs concentration measured in organic fertilizers the transport of these pollutants was modelled for a period of 10 years, including the monitored annual fertilization event. Fluoroquinolone, tetracycline and pleuromutilin antibiotics, together with anti-helminthics and analgesic and anti-inflammatories, were detected in manure-amended soils, at average concentrations ranging from 0.078 to 150 $\mu\text{g}/\text{kg dw}$ in surface layers, with the highest levels found in the fields fertilized with the swine slurry solid fraction. Even though severe disagreements were observed between experimental and simulated PhACs concentrations along the soil column, both approaches pointed out that target compounds strongly adsorb onto surface layers, showing limited mobility along the soil profile. Thus, repeated manure and slurry fertilizations will contribute in building up persistent PhACs residues in the uppermost layers of the soil, while leaching will be a minor process governing their fate towards the subsurface. The ecotoxicological risks posed by the occurrence of PhACs in soils were estimated to be low for terrestrial organisms. Nevertheless the antibiotic enrofloxacin showed some potential to induce negative effects to crops.

Keywords: Pharmaceuticals and antibiotics; manure fertilization; soil pollution; groundwater pollution; transport modelling, ecotoxicological effect

1. Introduction

Animal manure and slurries have been extensively used as fertilizers in agriculture, because they contain nutrients that are essential for crop growth. Nevertheless, besides their valuable nutrient content, manure and slurries can also be an important reservoir of organic pollutants, specifically pharmaceuticals and antibiotics (PhACs) used in animal husbandry whose concentrations can be several-fold greater than the amounts used in human medicine (Nõlvak et al., 2016). Therefore, animal manure and slurry reuse as fertilizers stands as an important route of entry of these pollutants in the environment.

Following manure and slurry fertilization, veterinary pharmaceuticals and antibiotics (PhACs) accumulate in soils (Hamscher et al., 2002; Martínez-Carballo et al., 2007), they could enter the human food chain (Kumar et al., 2005; Dolliver et al., 2007; Thasho et al., 2016) or leach to surface and groundwater bodies (Frey et al., 2015; Sollicec et al., 2016; Boy-Roura et al., 2018). Furthermore, through ineffective removal during surface or groundwater treatment, they could reach potable water intended for human consumption. Besides the spread of veterinary PhACs, manure and slurry fertilization may also facilitate the dissemination of antibiotic resistance genes (ARGs) in the environment (Xu et al., 2015; Lin et al., 2016; Peng et al., 2017). Several studies have already reported that manure and slurry-amended agricultural soils are major reservoirs of ARGs, where both antibiotics produced by indigenous microorganisms and those applied externally via organic fertilizers, can serve as an effective stimuli for the development and dissemination of resistance (Allen et al., 2010; Marti et al., 2013; Tien et al., 2017). To date, European and national directives determine a control in the application rates of organic fertilizers (e.g. manure and slurry) so contamination of water resources can be limited and prevented. Nevertheless, none of the current policies addresses the pollution by PhACs yet. The EU Nitrates Directive (91/676/CEE) has set

170 kg manure-N/ha per year as a precautionary application threshold to prevent contamination in regions that are vulnerable to nitrate leaching to groundwater bodies. In Spain, the separation of solid and liquid swine slurry fractions is starting to be implemented as a suitable strategy to improve slurry surplus management in areas with intensive livestock, as well as to reduce groundwater nitrate contaminations in vulnerable areas. The main advantages offered by phase separation is that phosphorous and nitrogen are concentrated in the solid fraction, which can be transported at longer distances for crop-amendment, while liquid fractions, with a reduced nutrient content, are more efficiently applied in nearby agricultural areas. Moreover, this practice favours the application of fraction-specific post-treatments, considerably reducing environmental risks (Flotats et al., 2009; Lyngsø et al., 2011). Even though this recent agricultural practice has proved its suitability in reducing nutrients pollution, it is still not broadly applied as a routine fertilization approach. Until now, some studies have been conducted focusing on the fate and transport of PhACs in manure and slurry-amended agricultural fields (Karcı et al., 2009; García-Galán et al., 2010; Hou et al., 2015; Solliec et al., 2016; Topp et al., 2016; Bourdat-Deschamps et al., 2017; Boy-Roura et al., 2018). Most of the available investigations have been confined to the analysis and identification of a restricted number of target compounds or specific classes of PhACs in surface soil layers only (Hamscher et al., 2005; Vázquez-Roig et al., 2010; Xu et al., 2015; Topp et al., 2016) or to laboratory scale settings, using column experiments (Kay et al., 2005; Kreuzig et al., 2005; Blackwell et al., 2009), instead of investigating their occurrence and fate under realistic field conditions (Boy-Roura et al., 2018; Bourdat-Deschamps et al., 2017; Camotti Bastos et al., 2018). Moreover, to the authors' best knowledge, none of the available studies neither focused on the PhACs behaviour in manure and slurry-amended agricultural soils and their

transport along the soil unsaturated zone, nor evaluated the environmental consequences of single-fraction slurry fertilization. Hydrological dynamics and chemical processes (mainly sorption and degradation) that take place in the soil unsaturated zone control the infiltration rate and the impact of pollutants on groundwater resources. Sorption influences a compound's mobility and leaching potential, and it strongly depends on the compound's and soil's physico-chemical properties (e.g. K_{oc} , K_{ow} and pKa, pH, mineral concentrations, cation exchange capacity, ionic strength, dissolved organic matter (DOM), soil organic matter (OM), the presence of monovalent and multivalence metallic ions and structure; Thiele-Bruhn, 2003; Schafer et al., 2012; Wang and Wang, 2015; Wegst-Uhrich et al., 2014; Christou et al., 2017). Besides sorption, degradation also governs PhACs transport and fate in soils. Degradation depends on a number of factors (e.g. functional groups that make it labile to biodegradation, hydrolysis, etc.). Some studies have already reported biotic degradation of PhACs in agricultural soils (Wang et al., 2008; Topp et al., 2012; Sollicec et al., 2016) as well as abiotic reduction (Halling-Sørensen et al., 2003; Sturini et al., 2012). On the other hand, hydrological factors must forcedly be taken into account to explain the occurrence of PhACs in the subsurface. Percolation of PhACs from agricultural-amended soils depends on the soil water balance, controlled by the rainfall-irrigation regime and evapotranspiration losses. The resulting infiltration rate will determine the soil water flux towards the water table. Flow, and therefore solute transport, in the unsaturated zone depends on the relative proportion of air and water in the soil pores which vary in depth and time, controlling the hydraulic properties of the partially saturated porous media, and the geochemical processes. Thus, understanding reactive solute transport in impact assessment studies is of utmost importance for water quality management strategies and the mitigation of groundwater pollution.

In this study, we first characterize the PhACs content in distinct classes of manure and fractions of swine slurry, in particular 34 multiple-class veterinary pharmaceuticals and antibiotics. Our aim is to provide data on the pollutant load derived from the use of manure and slurry as fertilizers. From an analytical perspective, the paper also presents an improved methodology to analyze PhACs residues in dairy cattle manure, swine slurry and soil samples. Secondly, we investigate their occurrence, fate and transport in manure and slurry-amended agricultural soils, using analytical and modelled data from experimental field sites under different agricultural practices (e.g. fields fertilized with different manure types and distinct slurry fractions). This second goal has a two-fold objective: 1) to distinguish the effects of distinct manures (swine slurry and dairy cattle manure) on the soil PhACs concentration (field experiment 1), and 2) to investigate the impact of the different swine slurry fractions (liquid, solid, and whole slurry) when used as fertilizers (field experiment 2). By using modelled data, PhACs occurrence in depth is discussed at the light of the infiltration capacity of the site soils as well as of their reactive behaviour, so their presence, or absence, can be understood in the appropriate hydrological context. Finally, the potential ecotoxicological risks posed by long-term manure and slurry soil amendment were also evaluated.

2. Materials and methods

2.1. Chemicals and reagents

All analytical standards used for quantification were of high purity grade (>90%). PhACs and isotopically labelled standards (IS), used as surrogate standards, were purchased from Sigma-Aldrich (Madrid, Spain) and Toronto Research Chemicals (Ontario, Canada). More information about standards, chemicals and reagents used can be found in the supplementary material.

2.2. Field experiments, sample collection and pre-treatment

The experimental fields where trials have been carried out are located in the alluvial plain of the Ter River. Soils in those fields are very deep (>1.2 m), well drained, non-saline, silt-loam textured, calcareous and without pebbles. These fields have been used for experimental purposes for decades by the Institute of Agrifood Research and Technology (IRTA Mas Badia), located in La Tallada d'Empordà (NE Catalonia). Winter cereals (wheat and barley) have been the main crops implanted in the fields along those years. For this study, two experiments were conducted. Experiment 1 was performed on soils from two different trials placed in the same field. In those trials, some plots are fertilized each year with dairy cattle manure and others with pig slurry at a rate of 170 kg N/ha per year since 2001. Plots in the trials had a size of 3x8 m and 3x10 m and they have been cultivated with wheat and barley (Domingo Olivé et al., 2016). From those trials, plots selected for experiment 1 were: (i) two plots amended with swine slurry, (ii) two plots amended with dairy cattle manure, and (iii) four control plots (two for each trial), where no manure or slurry were applied.

In experiment 2, trials fertilized only with swine slurry were used. The plots had a size of 3x10 m and the ones selected consisted of: (i) two plots fertilized with whole swine slurry (including both liquid and solid fractions), (ii) two plots fertilized with swine slurry's solid fraction only, (iii) two plots amended with swine slurry's liquid fraction, and (iv) two non-fertilized control plots.

For both experiments 1 and 2, soil samples were taken before the yearly fertilization in December 2015, and after fertilization: two and seven months later (February and July 2016, respectively). Soil samples were taken at different soil depths, covering 0-30 cm, 30-60 cm, 60-90 cm, 90-120 cm, for experiment 1; and 0-30 cm, 30-60 cm and 60-90

cm for experiment 2. For each plot and depth, two sub-samples were taken using an Edelman auger, and pooled to achieve a more representative sample. For samples below 30 cm depth, the material of the plough layer was removed to avoid contamination with topsoil samples. Soil samples were placed in plastic bags for transport to the laboratory.

In addition to soil samples, dairy cattle manure and swine slurry used as soil fertilizer was also sampled and analyzed. Swine slurry used in experiment 1 was collected from well-mixed containers and stored in high-density polyethylene bottles. Conversely, for swine slurry used in experiment 2, liquid and solid fractions obtained after centrifugation in the field were collected in high-density polyethylene bottles, as well as the original swine slurry. All samples were immediately transported to the laboratory under cooling conditions. Upon arrival to the lab, soils and dairy cattle manure samples were transferred to polypropylene containers, wrapped in aluminium foil and immediately frozen at -20°C . In addition, swine slurry used in experiments 1 and 2, and the liquid fraction from experiment 2, were centrifuged. Swine slurry is a heterogeneous mixture formed by urine, faeces, feed residues, straw and wash water. The liquid fraction, even though it had already been separated from the solid part, still contained solid residues. Thus, centrifugation for these samples (swine slurry and liquid fraction) was performed to obtain more homogeneous and representative fractions. For centrifugation, approximately 1.5 L of swine slurry total and liquid fractions were distributed in six 250 mL containers and centrifuged, using a Beckman Coulter Avanti J-26 XPI centrifuge, at 10000 rpm for 15 min at 4°C . After centrifugation, the supernatant (liquid phase) was decanted to 1L amber polyethylene bottles whereas the solid residue was transferred to 50mL polypropylene containers. Both solid and liquid fractions were frozen at -20°C . Solid swine slurry fractions, dairy cattle manure and soil samples were freeze-dried (LioAlfa 6, Telstar, Spain) at 0.033 bar vacuum and at -82

°C for 96h, respectively, and were kept at -20°C until analysis. Prior to sample extraction, swine slurry's liquid fractions were filtered through 2.7 µm, followed by 1 µm glass fiber filters (Whatman, United Kingdom), while soil, swine slurry solid fractions and dairy cattle manure were sieved through a 2 mm (soil) and a 1 mm followed by a 0.5 mm (manure and solid swine slurry) sieve. Samples were further homogenized using mortar and pestle.

2.3. Soil, manure and swine slurry characterization

The percentage of soil organic carbon (%OC) and total nitrogen (%N) was determined in each sample using a LECO analyzer (Truspec Micro CHNS). Soil pH_(CaCl₂) was also measured using a Crison GLP-21 pH meter and results are presented in Tables 1 and 2. For soils collected within experiment 1 (Table 1), the %OC in surface soils (0-30 cm) ranged from 0.80 to 1.03, while lower levels were measured in deeper horizons, specifically from 0.44 to 0.79 in 30-60 cm, from 0.28 to 0.44 in 60-90 cm and from 0.13 to 0.38 in 90-120cm. For the %N, values measured ranged from 0.11 to 0.13 in 0-30 cm, from 0.059 to 0.12 in 30-60 cm, from 0.034 to 0.063 in 60-90 cm and from 0.013 to 0.055 in 90-120 cm. For soil pH, values ranged from 7.1 to 7.9. Similar values were achieved for the soils collected in experiment 2 (Table 2), with values somewhat higher for the %OC in 60-90 cm depth. Other soil characteristics, such as total Kjeldahl nitrogen, N-NO₃, N-NH₄, P (Olsen), Na, K, Ca, Mg, soil texture and humidity are included in Table S1 in the supplementary material.

In manure and swine slurry samples, used as organic fertilizers, dry matter, organic matter, pH, electrical conductivity, N-NH₄, total Kjeldahl nitrogen (TKN), phosphorous (P), potassium (K), copper (Cu) and Zinc (Zn) were measured following standard methods and results are summarized in Table 3. Manure and swine slurry showed basic

characteristics since pH values measured ranged from 8.4 to 9.0. More detailed information about the analytical methods can be found in the supplementary material.

2.4 Analysis of PhACs in soil, manure and swine slurry

Suitable analytical protocols were optimized for the analysis of PhACs in soils, dairy cattle manure and swine slurry samples (liquid and solid fractions), by testing different extraction techniques, solvents and polymeric materials. The analytical methods tested for solid samples (soil, swine slurry solid fraction and dairy cattle manure) included QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction, followed by dispersive solid phase extraction (dSPE) as extract purification, and ultrasonic assisted extraction (UAE) followed by solid phase extraction (SPE) as a clean-up step, while for the liquid manure fractions SPE was used. Detailed information about method optimization is provided in the supplementary material (section 2.4; Tables S2, S3 and S4; and Figures S1, S2 and S3). The analytical approaches that yielded higher recoveries for most target PhACs in all matrices were the ones used for sample analysis. Briefly, 10 mL of swine slurry's liquid fraction were measured and appropriate volumes of an isotopically labelled standard mixture and a 0.1M Na₂EDTA solution were added. Samples were diluted with Milli-Q water until 100 mL and they were extracted by Solid Phase Extraction (SPE), using Oasis AccellTM Plus QMA (500 mg, 6 mL) cartridges, connected in tandem with Oasis HLB (200mg, 6mL). For solid samples, 2 g of the <2mm soil fraction and 1g of the <0.5mm dairy cattle and swine slurry's solid fraction were spiked with an appropriate volume of the isotopically labelled standard mixture. Samples were extracted by ultrasonic assisted extraction (UAE), in combination with vortex, using a citrate buffer/acetonitrile mixture. Extracts were diluted in Milli-Q water, and after addition of a Na₂EDTA 0.1M solution, they were further purified using

Oasis Accell™ Plus QMA (500 mg, 6 mL) cartridges, in tandem with Oasis HLB (200mg, 6mL). After sample loading on SPE cartridges, for both solid and liquid samples, Oasis Accell™ Plus QMA cartridges were discarded and Oasis HLB were washed with HPLC water, dried under vacuum for 30 min and eluted with 4x2 mL of methanol. Extracts were evaporated under a gentle nitrogen stream and reconstituted with 1 mL methanol-water mixture (50:50, v/v), except for soil extracts, which were reconstituted in 0.5 mL methanol-water (50:50, v/v). Prior to instrumental analysis, extracts from solid manure and slurry samples were filtered through 0.22 µm PVDF syringe filters. PhACs were detected and quantified using an ultra-high-performance-liquid chromatography (UHPLC) Acquity system (Waters Corporation, MA, USA) coupled to a 5500 QTRAP hybrid quadrupole-linear ion trap tandem mass spectrometer (AB Sciex, Foster City, CA, USA). Detailed information about analytical methods, chromatographic conditions and performance parameters (e.g., extraction recoveries, instrumental detection limits, method detection and quantification limits and matrix effects) can be found in the supplementary material (Tables S5, S6, S7, S8 and S9).

2.5. Model description and parameters

Unsaturated flow and transport modelling was conducted using HYDRUS-1D software package (Simunek et al., 2013) through a 0-350 cm soil profile of a silt loam soil (USDA), as resulting from field soil analysis (sand 32%; coarse silt 28%; fine-medium silt 26%; clay 15%, BSI classification). Soil was considered homogeneous, and divided depth-wise into 3.5 cm elements with a total depth of 350 cm. Water table was initially placed at -300 cm from the surface. The total simulation ran for a 10-year period, with daily time steps, starting in January 1, 2006, until the end of the field experiments in

July 31, 2016. Such a long simulation is intended to overcome all potential errors set by guessed initial conditions.

Initial conditions were defined by constant soil moisture content all along the modelled column. As regards of boundary conditions, the top soil condition was set as a time dependent boundary atmospheric with surface runoff, which sets the potential water flux across the upper soil element. Daily precipitation and actual evapotranspiration were taken from the daily records of the Catalan Meteorological Service from La Tallada d'Empordà station, less than one kilometre away from the experimental fields. Evapotranspiration data were modified using local crop coefficients for wheat and barley (Bosch Serra, 2009). No irrigation was ever applied to the plots. Root water uptake is limited to the uppermost 35 cm of the soil column, consistent with the mean root length of these crops. The bottom boundary condition, at -350 cm, was defined as deep drainage.

Soil hydraulic properties were simulated using the van Genuchten equation (Leij et al., 2012), and the unsaturated soil hydraulic parameters were taken as those suggested by default in HYDRUS for a silt loam: residual wetness: 0.067, maximum wetness: 0.450; α : 0.02 cm⁻¹; n: 1.41, and saturated hydraulic conductivity: 10.8 cm/d.

Transport boundary conditions were set as a concentration flux boundary at both ends with zero initial concentration along the soil profile. Dispersivity was set at 5 cm (Vanderborght and Vereecken, 2007). Soil bulk density was uniform and equal to 1.5 g/cm³. The solid-water distribution coefficient K_d (cm³/g) was estimated for each PhACs using equation 1.

$$K_d = f_{oc} K_{oc} \quad \text{Eq. 1}$$

The distribution coefficient, K_d , for PhACs varies substantially depending on the soil type, its organic carbon content, f_{oc} , and the partition coefficient between water and soil organic carbon, K_{oc} . In this study, the K_{oc} values used were taken from the Chemspider database (www.chemspider.com), using the data predicted by the EPISuiteTM model (Table S10), which estimates K_{oc} based on predicted K_{ow} data. f_{oc} values were those obtained from the organic carbon measurements in surface soil layers (0-30 cm). Estimated K_d values for each of the detected PhACs are summarized in Table S10. Finally, a linear equilibrium relationship (isotherm) between the amount of solute sorbed onto the soil, $S = K_d C$, and the concentration of the solute on the liquid phase, C , was considered in the simulations to estimate the final mass of volume in the soil column. PhAC concentration in the top soil layers was estimated according to the amount of manure annually applied to each parcel, the measured concentration of each PhAC in manure, and the annual rainfall rate. This approach assumes that the annual applied mass of PhAC during a single fertilization event will be completely dissolved by annual precipitation and uniformly introduced to the soil column through the year. Actual data on the volume of swine slurry and dairy cattle manure application exist from 2011 to 2016. Volumes for the previous years were assumed equal to those of 2011 (see calculations on the supplementary material; Table S12). Modelling efforts have been only conducted to simulate results derived from experiment 1.

2.6. Evaluation of potential ecotoxicological risks

PhACs occurrence in soils might induce undesired toxic effects. We estimated the potential ecotoxicological risks posed by PhACs levels in surface soil layers, using the hazard quotient (HQ) ratios (EMEA, 2006). HQs were calculated by dividing PhACs measured concentrations with predicted no-effect concentrations in soil ($PNEC_{soil}$).

Regulatory guidelines allow the estimation of $PNEC_{soil}$ from $PNEC_{water}$, corrected with the compounds solid/water partition coefficient (K_d) and taking into account the soil bulk density and the field water content (Chen et al., 2016). However, some authors claim for the inaccuracy of this approach, given the fact that $PNEC_{water}$ values are derived from aquatic ecotoxicological endpoints, with different exposure pathways, metabolism and sensitivities than terrestrial organisms (Bourdat-Deschamps et al., 2017). Thus, to perform suitable risk assessment estimations, we instead used ecotoxicity data for terrestrial organisms (earthworms and invertebrates), when available, but we also took into account information on plants (seed germination or root elongation) and soil microbial respiration (see Table S13 in the supplementary material). PNEC values were derived from data gathered in the scientific literature, and NOEC (No-Observed Effect Concentrations) or EC_{50} data (effective concentration leading to 50% of the effect, compared to a non-exposed control) were used, divided by an appropriate assessment factor, as indicated in Table S13. Measured soil concentrations used were the highest detected for each compound in surface soil layers, including both experiments, while PNEC values considered were the lowest, in order to assess risks under the most critical situations. The criteria used for the interpretation of HQ was: a) low risk when $HQ < 0.1$; b) medium risk with $0.1 < HQ < 1$, and c) high risk for $HQ \geq 1$ (Martín et al., 2012; Verlicchi et al., 2015; Bourdat-Deschamps et al., 2017).

2.7. Statistical analysis

Kruskal-Wallis tests, at 95% confidence level, were performed to compare PhACs concentrations between fertilized and control plots (un-fertilized) over time, in both experiments. Conover post-hoc tests were used for pairwise comparisons when statistically significant differences were found. Prior to Kruskal-Wallis, a Shapiro-Wilk

test was employed to assess for normality in all data. Spearman correlations were performed to study correlations between PhACs concentrations in soils and their physico-chemical properties. For statistical calculations, the sample concentrations below the method limits of detection (MDL) and quantification (MQL) were substituted with values corresponding to MDL/2 and MQL/2. Statistical tests were performed using the RStudio software (RStudio, Inc).

3. Results

Given the scope of the study, results are described focusing first on the PhACs concentrations in the applied manure and slurry to the parcels. Then, PhACs concentrations in the soil surface and in depth, as a result of the transport processes, for both experiments are introduced and, those of experiment 1 are also compared to the outcome of the unsaturated flow and reactive transport model.

3.1. Occurrence of PhACs in manure and swine slurry

The concentrations of the PhACs detected in dairy cattle manure and swine slurry samples used as fertilizers in the agricultural plots are presented in Table 4. In general, only 5 out of the 34 compounds were found in dairy cattle manure, while 14 out of the 34 targeted substances were detected in swine slurry (Table 4). Identified compounds belong to fluoroquinolone (ciprofloxacin, enrofloxacin, marbofloxacin), macrolide (tilmicosin), tetracycline (tetracycline, oxytetracycline, chlortetracycline), lincosamide (lincomycin), sulfonamide (sulfamethazine), pleuromutilin (tiamulin) and ionophore (toltrazuril) antibiotics, analgesics and anti-inflammatories (salicylic acid and flunixin) and antihelmintic drugs (flubendazole).

For dairy cattle manure, concentrations ranged from 18 to 85 $\mu\text{g}/\text{kg}$ dw (dw: dry weight; Table 4), whereas PhACs levels for swine slurry were quite remarkable, notably varying between the liquid and the solid fractions. Indeed, levels in the later fraction were about one to two orders of magnitude higher than those detected in the liquid. Concentrations in the liquid fractions ranged from 0.77 to 51 $\mu\text{g}/\text{L}$ (except for lincomycin, which was detected at 20 mg/L in one of the samples), while levels in solid fractions ranged from 4.6 up to 7500 $\mu\text{g}/\text{kg}$ dw. The compounds found at the highest concentrations in the liquid fraction (>10 $\mu\text{g}/\text{L}$) were the antibiotics lincomycin, oxytetracycline and enrofloxacin, and the analgesics and anti-inflammatories salicylic acid and flunixin, respectively. Regarding solid fractions, the substances detected at the highest concentrations (>1000 $\mu\text{g}/\text{kg}$ dw in at least one of the samples) were the antibiotics oxytetracycline, tetracycline, chlortetracycline, lincomycin, ciprofloxacin, enrofloxacin, the analgesic and anti-inflammatory flunixin and the anti-helminthic drug flubendazole. Figure 1 shows the distribution of PhACs between swine slurry's solid and liquid fractions. As it is depicted in the figure, fluoroquinolone (ciprofloxacin, marbofloxacin and enrofloxacin), tetracycline (oxytetracycline and tetracycline), macrolide (tilmicosin) antibiotics and flubendazole mostly partition onto slurry's solid fraction (between 80-90% of total PhACs amounts are present in the solids), while lincomycin, sulfamethazine, tiamulin and salicylic acid are preferentially distributed in the liquid fraction. Compounds distribution between solid and liquid fractions could be attributed to both hydrophobic and electrostatic interactions. Even though hydrophobicity, as indicated by $\log K_{ow}$ values, has been used a model parameter to explain PhACs partitioning between solid and liquid phases, in this study, no clear relationship could be established between PhACs hydrophobicity and their sorption to the solid fractions. For instance, fluoroquinolone and tetracycline antibiotics, which showed some of the lowest

log K_{ow} values (-2.87 to 0.70), are the compounds with the higher distribution onto the solid fraction, while tiamulin, with higher log K_{ow} values (4.75), mostly partition onto the liquid fraction. These results point out that other factors (e.g. electrostatic interactions), may play an important role in compounds distribution (e.g. interaction between negatively charged molecules with positively charged metal ions present in solids). Finally, the concentrations detected in both dairy cattle manure and swine slurry are in good agreement with those previously reported in the scientific literature (Campagnolo et al., 2002; Schlüsener et al., 2003; Karcı and Balcioğlu, 2009; Martínez-Carballo et al., 2007; Zhao et al., 2010; Zhou et al., 2012).

3.2. PhACs accumulation at surface soil layer (0-30 cm depth)

In experiment 1, four substances were detected (enrofloxacin, oxytetracycline, flubendazole, and tiamulin) in swine slurry amended parcels; while in dairy cattle amended plots only flunixin was found. Conversely, up to six substances were identified in experiment 2 considering the distinct swine slurry fractions: the five mentioned ones plus tetracycline (Figures 2 and 3; Tables S14 and S15). For all detected substances and field experiments, concentrations in fertilized experimental plots were statistically significantly different than those in the control fields ($p < 0.05$), where most substances were not detected or, when found, they were present below or close to their limit of quantification.

In experiment 1, all fertilized plots already showed noticeable concentrations of target compounds before the yearly slurry application (Figure 2), and they were significantly different than those of the control parcels ($p < 0.05$). Enrofloxacin was the compound detected at the highest average concentrations, ($130 \pm 16 \mu\text{g/kg dw}$), followed by oxytetracycline ($29 \pm 3.4 \mu\text{g/kg dw}$). Flubendazole and tiamulin were detected at

concentrations approximately two orders of magnitude lower than these substances ($0.46 \pm 0.12 \mu\text{g/kg dw}$ and $0.078 \pm 0.024 \mu\text{g/kg dw}$, respectively). Concentrations of these PhACs remained in the upper soil layer and were also detected two and seven months after the yearly input at distinct proportions (Figure 2).

This high antibiotic content in the samples taken before the yearly fertilization could be explained by the fact that all experimental sites, except the controls plots, had been intensively fertilized with manure and slurry for several years before this experiment started. Subsequently, PhACs residues coming from previous applications had persisted over time. In experiment 1, all substances, except enrofloxacin, showed a slight increase in concentration in the soils taken two months after manure application, compared with the samples collected before the yearly fertilization. Nevertheless, this raise in concentrations was remarkable and only statistically significant ($p < 0.05$) for tiamulin and flunixin. In soil samples collected after seven months of soil amendment, a decrease in concentration was observed for most substances with respect to month two, and remaining concentrations were similar than those found before the yearly fertilization (except for enrofloxacin).

In dairy cattle manure amended plots, flunixin was neither detected in the controls nor in the samples taken before the yearly fertilization. However it was detected after manure additions.

Regarding the fertilization with separate swine slurry fractions as conducted in experiment 2 (Figure 3), all plots amended with the solid slurry fraction presented significantly higher levels ($p < 0.05$) in the upper soil layer than those fertilized with the liquid and total swine slurry fractions. Detected average concentrations in these soils two months after fertilization ranged from $3.2 \pm 0.08 \mu\text{g/kg dw}$ to $150 \pm 38 \mu\text{g/kg dw}$, being enrofloxacin the compound showing the highest concentration. In total and liquid

fraction slurry-amended soils, detected average concentrations two months after fertilization ranged from approximately 0.1 to 30 $\mu\text{g}/\text{kg dw}$. Such high concentrations in solid fraction-amended soils could be attributed to a slower release of PhACs from solid manure to the soil, compared with swine slurry and its liquid fraction, together with a higher concentration of PhACs present in the manure solid fraction (Table 4). Solid fractions, as a pollutant source, will be retained longer in the soil, whereas other fractions will quickly infiltrate and adsorb/degrade to the observed low concentrations. Furthermore, as suggested by Sabourin and co-workers (Sabourin et al., 2009), amendments using solid or liquid fractions would differently modify the pore space saturation resulting in a distinct exposure to soil microorganisms. In the case of liquids they will fill available pore spaces in soil, ensuring greater exposure to PhACs to soil microorganisms, thus enhancing biodegradation. In contrast, restricted diffusion of oxygen into solid fraction aggregates may result in a restricted diffusion of PhACs out of aggregates, which may enhance their persistence. Comparing the concentrations in samples taken at different times, concentrations increased or decreased, yet differences were mostly non-significant ($p>0.05$).

3.3. PhACs distribution along the soil profile

In soil samples analyzed within experiment 1, enrofloxacin and flubendazole were the only substances detected at soil layers below 30 cm in the plots fertilized with swine slurry (Figure 2), while for experiment 2 (Figure 3), these substances together with flunixin and tiamulin were also detected. Tetracycline antibiotics were not detected along the soil profile. In experiment 1 (Figure 2), enrofloxacin was found at high and at relatively constant concentration (~ 70 and $110 \mu\text{g}/\text{kg dw}$) at all depths before the yearly fertilization. Nevertheless, in the samples collected two and seven months after soil

amendment, it was not detected in deeper soil layers. In the second experiment (Figure 3), enrofloxacin also appears with reduced concentrations in depth for all sites (13-25% of those found in surface layers for solid fraction-amended plots after two months fertilization), and this decrease was more remarkable when liquid and total fractions had been applied. Conversely, flubendazole was detected at all depths in samples from both experiments, either before or after fertilization. Nevertheless, its concentrations at depths below 30 cm were approximately between 15-20% of those found in the surface horizons. Flunixin and tiamulin were only detected at all depths when solid slurry was applied, probably because concentrations at the surface layer were higher. Notably, for all experiments, concentrations after 7 months of fertilization were also lower than those found after 2 months. These results are in good agreement with laboratory experiments where PhACs leaching potential was studied in soil column experiments amended with manure and/or slurry. Several studies have shown the strong adsorption of tetracycline and fluoroquinolone antibiotics in soils and thus, their low mobility along the soil column (Rabolle et al., 2000; Kay et al., 2005; Ostermann et al., 2013; Domínguez et al., 2014). Even though fluoroquinolone antibiotics are mostly classified as substances with low mobility in soils, a recent study detected fluoroquinolone residues in leachates from fields fertilized with organic fertilizers (Bourdat-Deschamps et al., 2017). Finally, residues of anti-helminthic drugs (flubendazole) were detected in drainage waters from plots fertilized with swine slurry (Weiss et al., 2008), highlighting its potential mobility along the soil profile.

3.4. Unsaturated flow and reactive transport modelling results

HYDRUS-1D results for experiment 1 provide the concentration of dissolved compounds, C , in mass per unit of water volume, and these have been transformed to

mass of compound per unit mass of dry soil, considering the simulated soil moisture at each element and the soil bulk density in the unit conversion process. Adsorbed mass at each depth, S , is estimated using the linear isotherm and the distribution factor estimated for each PhAC, i.e., $S = K_d C$. The total mass of compound per unit of mass of dry soil is simply estimated by adding both concentrations in the adequate units ($\mu\text{g}/\text{kg}$; Figure 4).

However, transport simulations fail to reproduce the magnitude and distribution in depth of all simulated PhACs regardless of the samples (before soil amendment and at two and seven month later). Assuming that the simulated soil moisture, pressure head and water flux were adequately simulated based on the water balance between daily rainfall, actual evapotranspiration once corrected by the crop factor, and root water uptake, the total mass profile (expressed as total mass per unit of soil mass; right column in Figure 4) presents a range of values that do not compare with field measurements (Figure 2). In particular, enrofloxacin and oxytetracycline concentrations have been largely underestimated by the model, whereas flubendazole, tiamulin and flunixin are overestimated. It is worth mentioning that the ratios between the total PhACs mass in the input slurry and manure with the total mass estimated by the model also present large differences (Figure 4).

Simulations include the nine previous years before the experiment. Therefore, simulated curves before fertilization, showing significant compound occurrences below 30 cm depth, are also in disagreement with observed data for enrofloxacin and oxytetracycline, while they correctly simulate the absence of the other compounds below this depth. Indeed, as observed, models agree in the fact that, even after almost ten years of continuous input, most of the mass will be mostly retained in the upper soil layer (<30 cm) due to the retardation inherent to the transport of sorptive compounds.

Disagreements between observed and simulated results rely on the assumptions in which the model is based. First of all, a continuous input instead of a pulse input corresponding to the fertilization moment was applied based on the assumption that applied manure and slurry will not immediately infiltrate, yet it will be leached at a steady concentration along the year. However, the stronger assumptions relate to the considered adsorption models based on linear isotherms and the estimated K_d values. As it can be observed in Table S10, predicted K_d values used are considerably lower than those reported in the scientific literature which have been mainly estimated using experimental set-ups (Table S11). In fact, K_d spread over a large range of values, denoting the idiosyncrasy of each experimental layout and the type of soil. This fact indicates that the use of estimated K_{oc} data, instead of experimental values, could add some limitations in providing accurate K_d values, and this would directly impact the prediction of both PhACs concentrations, sorbed and in solution, in soils. On the other hand, literature references (e.g., Limousin et al., 2007; Al-Khazrajy and Boxall; 2016) show that linear isotherms are not always valid to describe PhACs adsorption, and that K_d values can vary over several orders of magnitude for each compound under distinct actual soil conditions.

Moreover, biodegradation has been recognized as a relevant attenuation process (Schlüsener et al., 2006; Wang; Yates 2008; Yang et al., 2009; Topp et al., 2012; Pan et al., 2016) as well as the formation of non-extractable soil residues after long term soil fertilization (Kreuzig; Höltge 2005; Heise et al., 2006; Bourdat-Deschamps et al., 2017). None of these processes have been considered in the numerical model, and these processes certainly have an influence on the estimated PhACs concentrations. In the case of biodegradation, no reliable decay factors were found in literature and, therefore, it was ruled out from this modelling exercise. Yet they will provide an even larger

decrease of observed contaminant mass, whether in solution or adsorbed in the soil particles. Consequently, there is a critical uncertainty on the conceptual approach to model the fate of these substances in the soil and, extensively, in the subsurface due to a lack of a precise knowledge of their geochemical behaviour.

3.5. Evaluation of potential ecotoxicological risks

The estimated hazard quotients (HQ), as well as the measured environmental concentrations (MEC) and predicted-no-effect-concentrations (PNEC) used for their calculation, are summarized in Table 5. Data about PhACs toxic effects to terrestrial organisms and crops is very scant in the scientific literature. Furthermore information available highlights that there are large differences in toxic potential, depending on the type of crops and endpoints investigated. For enrofloxacin and tetracycline, information about their toxicity to both earthworms and plants was found. However, for oxytetracycline and tiamulin, the only data available was related with the effects on the reproduction of soil invertebrates, while for flubendazole and flunixin, no information could be found. For this reason, HQ for flubendazole and flunixin are not included in Table 5. Estimated HQ, indicating toxicity to earthworms, were 0.0015 for enrofloxacin and 0.0075 for oxytetracycline, respectively, while HQ to endpoints representative for potential reproductive effects to earthworms were 0.015 for oxytetracycline and 0.0076 for tiamulin. All these values fall below the 0.1 threshold, indicating that the occurrence of these PhACs in soils poses low ecotoxicological risks for terrestrial organisms. Nevertheless, HQs estimated when assessing potential toxic effects to crops ranged from 0.0075 up to 12. The highest HQs were estimated for enrofloxacin, with values of 5.6 and 12 when evaluating the effects on root elongation in model crops, such as cucumber, and seedling growth in wheat, respectively. These HQ are higher than 1,

indicating that the concentrations to which enrofloxacin is found in soils can induce negative effects in crops. For tetracycline, a HQ of 0.16 was estimated when assessing effects on the seedling growth in tomato, showing that some risks might occur. However, for the other PhACs detected, estimated HQs were below 0.1, indicating low risk for potential ecotoxicological effects to crops. The estimated HQs are in good agreement with previous studies in the scientific literature. HQs of 0.01 and 0.013 were estimated for enrofloxacin and oxytetracycline, respectively, when evaluating potential toxic effects to earthworms (Slana et al., 2013). However, HQs were below one when estimating potential toxic effects to plants (Slana et al., 2013).

4. Discussion

Field experiments monitoring PhACs in amended soils using swine slurry and dairy cattle manure provide an insight of their occurrence in depth and time. Observed data suggest that PhACs will be mainly retained in the upper soil layers due to their sorptive behaviour, and that they will rarely be found deeper than 100 cm in a silt loam soil. Soil concentrations are thus highly governed by adsorption processes, as well as degradation. Both processes are responsible for a short travelling along the soil profile and for a decrease in the magnitude of their occurrence, as pointed out by the usual decrease between the second and seventh months after fertilization (Fig. 2). In this sense, large PhACs concentrations must be expected on the uppermost layers of low permeable soils, whereas they would seldom be found at lower depths. This deduction is in conflict with the occurrence of these compounds in groundwater as reported by several studies (Hu et al., 2010; Frey et al., 2015; Manamsa et al., 2016; Kivits et al., 2018; Boy-Roura et al., 2018), and it could not be surely applied to large permeable surfaces (e.g., sandy soils) and to those areas with a large irrigation return flow that enhance and generate an

additional leaching and infiltration of these compounds, despite their adsorption capacity, towards the subsurface. Irrigation excess, as in surface irrigation crops, will largely overcome the natural soil water budget, especially in Mediterranean areas and, therefore, enhance PhACs leaching. This point emphasizes the need about discussing PhACs pollution in the appropriate hydrological framework.

Results also point out the variability of PhACs concentrations found in slurries and manures. After the development of a sensitive analytical method for extracting and analyzing PhACs content in slurry and manure (Supplementary Material), the PhACs concentrations reported herein stand as an example of their occurrence in organic fertilizers. However, it is unlikely that these values can be taken as references or extrapolated to other slurries and manures, coming from different farms or storing conditions. Thus, regional studies should then rely on a larger dataset that establishes the more expected range of magnitude for PhACs concentrations in the used fertilizer.

Laboratory and field experiments referred in the literature report that adsorption and biodegradation are the most active processes that will retain and attenuate PhACs loads in soils and aquifers. They also describe the complexity of quantifying the magnitude of both processes because of the behaviour of these substances in natural environments is rather complex as it depends on their molecular structure and the environmental conditions (i.e. soil properties and biogeochemistry) in a particular spot. Indeed, several factors influence PhACs behaviour in soil, such as the electrostatic interactions between positively charged groups in the molecule (which are pH-dependent as well) and the negatively charged adsorption sites of soils, the interaction and complexation with soil multivalence metal ions, forming strong chelates, cation exchange processes and/or by the adsorption on the surface of metal oxides (Wang and Wang 2015; Gu et al., 2007). This is one reason why, to our understanding, modelling efforts have failed to reproduce

observed PhACs mass recovered from soil samples at distinct depths. Another reason is the use of estimated K_d values through predicted K_{oc} , instead of using experimental values for this particular soil type. Furthermore, several authors have indicated that K_{ow} are not sufficient for the assessment of PhACs sorption, because this process strongly depends on the compounds chemical structure and its speciation (charge state) at the soil pH (Schaffer et al., 2012; Wegst-Uhrich et al., 2014). Thus, the use of K_{oc} derived from the pH-dependant octanol-water partition coefficients, ($\log D$), are more recommended (Schaffer et al., 2012, Wegst-Uhrich et al., 2014). Nevertheless, in this study, we did not use the K_{oc} predicted based on $\log D$ data because K_d achieved were even lower than those obtained without taking into account the compounds ionization state, thus adding more inaccuracy in the prediction of soil PhACs concentrations.

The occurrence of target compounds (e.g., enrofloxacin, flubendazole) along the soil profile in those samples taken before the annual fertilization is attributed to the fact that the PhACs released from previous slurry applications have remained adsorbed along the soil profile. Assuming that periodic fertilizations have been taken place in these experimental plots over the past years, some kind of “resident concentration” has been reached at each depth, as illustrated by the transport models. However, the magnitude of the concentrations will depend on the sorption and biodegradation characteristics of each compound, on the hydrologic and fertilization regime, the application rate of the fertilizers and on the pollutant content in the organic fertilizers. Indeed, Spearman correlations between PhACs physico-chemical properties (e.g. solubility, $\log K_{oc}$, $\log K_{ow}$, $\log D$, pK_a , compounds half-lives in soil) and soil concentrations indicated that PhACs showing higher water solubility were those that were detected at higher concentrations in both surface and deep layered soils. Enrofloxacin is the compound with the highest solubility among all PhACs detected, which might explain its mobility

and ubiquity in deep soil layers. On the other hand, flunixin is negatively charged at soil pH while flubendazole is neutral. Thus, their retention to negatively charged soil components might be reduced, thus enhancing their mobility in soils. No relationship between compounds distribution along the soil column and their log K_{ow} and log K_{oc} values was observed, indicating that other processes other than just hydrophobic interactions (as degradation) dictate the fate and transport of PhACs in soils (Thiele-Bruhn, 2003; Christou et al., 2017).

A plausible hypothesis to explain the detection of remarkable enrofloxacin concentrations in the samples taken before the annual fertilization in the first field trial cannot be solely based on its lower estimated K_d value ($0.75 \text{ cm}^3/\text{g}$), yet on the hydrological regime and its influence on the adsorbed mass. We propose that rainfall recharge after previous fertilization events with a low antibiotic concentration had efficiently desorbed the mass attached to soil particles. This would assume that fertilizers will largely release their PhACs content just after amendment, and not as the continuous input defined in the numerical model. Another hypothesis may involve the role of distinct slurry fractions. While the solid part will mainly remain in the upper layer, the liquid one could reach further locations along the profile. Both hypotheses, however, are counteracted by the oxytetracycline behaviour which, having an estimated K_d value of $0.88 \text{ cm}^3/\text{g}$ had to show a similar pattern. These results highlight the difficulties and uncertainties in explaining observed PhACs behaviour on soil profiles and the outcomes of this study could be taken as a warning to managers about the difficulties and constraints about assessing the impact of such pollutants in the subsurface.

Conclusions

Dairy cattle manure and swine slurry are important reservoirs of PhACs residues. Thus, their reuse as fertilizers in agricultural fields is expected to be an important pathway for soil and groundwater pollution. This study confirms that a wide variety of PhACs is present in swine slurry, with concentrations up to 20 mg/L and 7500 $\mu\text{g}/\text{kg dw}$ in the liquid and solid fractions, respectively, while in dairy cattle manure, only a reduced number of PhACs has been detected, with concentrations between one and two orders of magnitude lower than those found in swine slurry. Among the PhAC detected classes, antibiotics (i.e. fluoroquinolones, tetracyclines, lincosamides, pleuromutilins and ionophores) are ubiquitous compounds. However, other veterinary drugs, such as anti-helminthics and analgesic and anti-inflammatories are also frequently detected. Results derived from the experimental field sites confirm that veterinary PhACs mostly reach agricultural soils via manure and slurry applications, as PhACs residues were detected in amended soils at levels up to 150 $\mu\text{g}/\text{kg dw}$, but rarely in control plots. Our data also indicates that fertilization with distinct manure classes (e.g. dairy cattle manure vs swine slurry) and slurry fractions has an influence on PhACs occurrence and fate in soils, since higher concentrations were found in fields fertilized with swine slurry solid fractions. The discrepancies encountered in PhACs concentrations between modelled and experimental data evidences the limitations of current modelling approaches. These differences, which are mostly attributed to the inaccuracies in predicting sorption coefficients (K_d) and to the influence of other biogeochemical factors, other than just the compounds physico-chemical properties in PhACs fate (e.g. soil properties, such as ion exchange interactions, complexation with metal ions and biodegradation), that are difficult to be accounted for in conventional modelling tools. Despite these discrepancies, both modelling and experimental data showed the same trend in terms of PhACs behaviour. Target compounds tend to accumulate in surface

soil layers, while they have limited mobility along the soil column. These results predict that repeated manure and slurry fertilizations over the years will certainly have an impact on building up persistent PhACs residues in soils, while their leaching potential to groundwater bodies will be limited, at least in silt loam soils. However, soils with higher permeability (i.e. sandy soils) as well as soils with higher irrigation rates will enhance PhACs leaching to groundwater bodies.

The ecotoxicological risk assessment approach used in this study also faced important limitations, mainly related with the lack of data regarding PhACs toxicity to soil organisms and crops. Results suggested that PhACs levels in soils do not exhibit toxicity to soil organisms, while particular substances, such as enrofloxacin, are able to induce adverse effects on crops ($HQ > 1$). This study evidences that, to improve risk assessment studies in soil ecosystems, it is necessary to expand current knowledge about the potential toxic effects of PhACs on soil organisms from various trophic levels. Furthermore, ecotoxicological studies should also focus on soil microorganisms, and on the spread of antibiotic resistance, to have a more comprehensive knowledge about the environmental risks associated with manure and slurry soil amendment.

Acknowledgements

This work has been supported by the Secretary of Universities and Research of the Counsel of Economy and Knowledge of the Catalan Government (AGAUR) and the COFUND Program of the Marie Curie Actions of the 7th R&D EU Program. It has also been supported by the projects PERSIST (EU Water Joint Programming Initiative, JPIW2013-118) and REMEDIATION (CGL2014-57215-C4-2-R), and the University of Girona funds MPCUdG2016/061. Authors also acknowledge the support from the

Economy and Knowledge Department of the Catalan Government through Consolidated Research Group (ICRA-ENV 2017 SGR 1124).

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Figure captions

Figure 1. Distribution of PhACs between the solid and liquid fractions in swine slurry.

Figure 2. Concentrations of PhACs detected along the soil profile in experiment 1.

Figure 3. Concentrations of PhACs along the soil profile in experiment 2, differentiating the distinct fractions of swine slurry used as fertilizers.

Figure 4. Fate and behaviour of PhACs according to the flow and transport 1D-model results. K_d refers to the considered distribution factor, and “ratio” expresses the quotient between the total PhACs mass in the input slurry and manure used in experiment 1 and the total mass estimated by the model.

Table captions

Table 1. Percentage of organic carbon (%OC), nitrogen (%N) and $\text{pH}_{(\text{CaCl}_2)}$ in soil samples from experiment 1 (soils fertilized with swine slurry and dairy cattle manure). Results presented in the table correspond to the average of the two plots sampled for each treatment.

Table 2. Percentage of organic carbon (%OC), nitrogen (%N) and $\text{pH}_{(\text{CaCl}_2)}$ in soil samples analyzed in experiment 2 (soils fertilized with swine slurry liquid, solid and total fractions). Results presented in the table correspond to the average of the two plots sampled for each treatment.

Table 3. Chemical parameters measured in animal manure

Table 4. Concentrations (mean and standard deviation, $n=3$) of the PhACs detected in dairy cattle manure and swine slurry used as fertilizer in the field experiments.

Table 5. Measured environmental concentrations in soil (MEC_{soil}), predicted-no-effect concentrations ($PNEC_{soil}$) and hazard quotients (HQ) calculated for several end-points.

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Table 1

| | | | BEFORE FERTILIZATION (BF) | | | TWO MONTHS AFTER FERTILIZATION | | | SEVEN MONTHS AFTER FERTILIZATION | | |
|-----------------------|--------------|-----------------|---------------------------|--------|--------|--------------------------------|---------|--------|----------------------------------|---------|--------|
| TYPE OF FERTILIZATION | TYPE OF PLOT | SOIL DEPTH (CM) | %O C | % N | P H | %O C | %N H | P H | %O C | %N H | P H |
| SWINE SLURRY | FERTILIZED | 0-30 CM | 0.89 | 0.11 | 7.1 | 0.83 | 0.07 | 7.5 | 0.99 | 0.11 | 7.5 |
| | | 30-60 CM | 0.44 | 0.05 | 7.9 | 0.41 | 0.05 | 7.2 | 0.48 | 0.06 | 7.9 |
| | | 60-90 CM | 0.28 | 0.03 | 7.4 | 0.25 | 0.02 | 7.6 | 0.25 | 0.04 | 7.0 |
| | | 90-120 CM | 0.13 | 0.01 | 7.3 | 0.13 | 0.01 | 7.5 | 0.11 | 0.01 | 7.5 |
| | CONTROL | 0-30 CM | 0.80 | 0.11 | 7.3 | 0.80 | 0.11 | 7.2 | 0.80 | 0.11 | 7.8 |
| | | 30-60 CM | 0.51 | 0.07 | 7.3 | 0.51 | 0.07 | 7.3 | 0.51 | 0.07 | 7.3 |
| | | 60-90 CM | 0.31 | 0.04 | 7.4 | 0.31 | 0.04 | 7.4 | 0.31 | 0.04 | 7.4 |
| | | 90-120 CM | 0.16 | 0.01 | 7.6 | 0.16 | 0.01 | 7.6 | 0.16 | 0.01 | 7.6 |
| DAIRY CATTLE | FERTILIZED | 0-30 CM | 1.03 | 0.13 | 7.2 | 1.1 | 0.12 | 7.1 | 0.91 | 0.12 | 7.6 |
| | | 30-60 CM | 0.59 | 0.08 | 7.2 | 0.59 | 0.07 | 7.3 | 0.58 | 0.08 | 7.7 |
| | | 60-90 CM | 0.37 | 0.05 | 7.7 | 0.33 | 0.04 | 7.9 | 0.41 | 0.05 | 7.1 |
| | | 90-120 CM | 0.29 | 0.04 | 7.9 | 0.26 | 0.03 | 7.6 | 0.30 | 0.04 | 7.1 |
| | CONTROL | 0-30 CM | 0.88 | 0.12 | 7.2 | 0.88 | 0.12 | 7.1 | 0.88 | 0.12 | 7.9 |
| | | 30-60 CM | 0.79 | 0.12 | 7.2 | 0.79 | 0.12 | 7.2 | 0.79 | 0.12 | 7.7 |
| | | 60-90 CM | 0.44 | 0.06 | 7.3 | 0.44 | 0.06 | 7.3 | 0.44 | 0.06 | 7.3 |
| | | 90-120 CM | 0.38 | 0.05 | 7.5 | 0.38 | 0.05 | 7.5 | 0.38 | 0.05 | 7.5 |

Table 2

| | | | TWO MONTHS AFTER FERTILIZATION | | | SEVEN MONTHS AFTER FERTILIZATION | | |
|------------------------------|--------------|-----------------|--------------------------------|-------|------|----------------------------------|-------|-----|
| TYPE OF FERTILIZATION | TYPE OF PLOT | SOIL DEPTH (CM) | %OC | %N | pH | %OC | %N | pH |
| SWINE SLURRY TOTAL FRACTION | FERTILIZED | 0-30 CM | 0.85 | 0.12 | 7.1 | 0.96 | 0.14 | 7.2 |
| | | 30-60 CM | 0.35 | 0.046 | 7.3 | 0.47 | 0.051 | 7.2 |
| | | 60-90 CM | 0.59 | 0.026 | 7.3 | 0.48 | 0.036 | 7.5 |
| SWINE SLURRY LIQUID FRACTION | FERTILIZED | 0-30 CM | 0.87 | 0.12 | 7.00 | 1.27 | 0.14 | 7.3 |
| | | 30-60 CM | 0.52 | 0.067 | 7.1 | 1.01 | 0.12 | 7.3 |
| | | 60-90 CM | 0.62 | 0.040 | 7.2 | 0.33 | 0.054 | 7.6 |
| SWINE SLURRY SOLID FRACTION | FERTILIZED | 0-30 CM | 0.87 | 0.13 | 7.2 | 0.95 | 0.13 | 7.1 |
| | | 30-60 CM | 0.36 | 0.050 | 7.3 | 0.49 | 0.063 | 7.3 |
| | | 60-90 CM | 0.70 | 0.052 | 7.2 | 0.66 | 0.024 | 7.4 |
| NON-FERTILIZED PLOTS | CONTROL | 0-30 CM | 0.80 | 0.13 | 7.1 | 0.80 | 0.13 | 6.9 |
| | | 30-60 CM | 0.29 | 0.010 | 7.1 | 0.29 | 0.01 | 7.2 |
| | | 60-90 CM | 0.93 | 0.046 | 7.3 | 0.93 | 0.046 | 7.2 |

Table 3

| Chemical parameters | Experiment I | | Experiment II | | |
|-----------------------------|--------------|---------------------|---------------|------------------------------|-----------------------------|
| | Swine slurry | Dairy cattle manure | Swine slurry | Swine slurry liquid fraction | Swine slurry solid fraction |
| Dry matter (105°C) % s.m.f. | 6.62 | 52.7 | 6.62 | <1.000 | 28.7 |
| Organic matter % s.m.s. | 73.4 | 31.7 | 73.4 | 48.4 | 70.7 |
| pH | 9.0 | 8.6 | 9.0 | 8.7 | 8.4 |
| Conductivity 25°C (dS/m) | 14.9 | 3.24 | 14.9 | 17.1 | 1.89 |
| N-NH ₄ % d.m. | 4.05 | 0.14 | 4.05 | 20.71 | 0.64 |
| N-Kjeldahl % d.m. | 1.79 | 1.58 | 1.79 | >10.0 | 1.28 |
| Phosphorous (P) % d.m. | 3.1 | 0.63 | 3.1 | 0.98 | 3.2 |
| Potassium (K) % d.m. | 2.54 | 1.77 | 2.54 | >10.0 | 0.71 |
| Copper (Cu) mg/kg d.m. | 172 | - | 172 | 53 | 58 |
| Zinc (Zn) mg/kg d.m. | 1610 | - | 1610 | 394 | 438 |

d.m.: dry matter

Table 4

| Compounds | Dairy cattle Experiment I | Swine slurry total fraction Experiment I* | | Swine slurry total fraction Experiment II* | | Swine slurry liquid fraction Experiment II* | | Swine slurry solid fraction Experiment II |
|------------------|----------------------------------|-------------------------------------------|----------------------------------|--------------------------------------------|----------------------------------|---------------------------------------------|----------------------------------|-------------------------------------------|
| | Solid $\mu\text{g}/\text{kg dw}$ | Liquid $\mu\text{g}/\text{L}$ | Solid $\mu\text{g}/\text{kg dw}$ | Liquid $\mu\text{g}/\text{L}$ | Solid $\mu\text{g}/\text{kg dw}$ | Liquid $\mu\text{g}/\text{L}$ | Solid $\mu\text{g}/\text{kg dw}$ | Solid $\mu\text{g}/\text{kg dw}$ |
| Ciprofloxacin | 54 (± 2.4) | 2.3 (± 0.051) | 880 (± 6.3) | 5.03 (± 0.49) | 3400 (± 530) | 5.4 (± 0.16) | 2900 (± 150) | 2900 (± 300) |
| Enrofloxacin | 18 (± 6.5) | 2.8 (± 0.12) | 640 (± 77) | 11 (± 0.59) | 6010 (± 540) | 13 (± 0.81) | 5300 (± 310) | 1900 (± 290) |
| Marbofloxacin | n.d. | 4.2 (± 0.090) | 660 (± 54) | 1.3 (± 0.015) | 390 (± 13) | 1.7 (± 0.039) | 390 (± 11) | 380 (± 46) |
| Tilmicosin | n.d. | 7.4 (± 0.96) | 750 (± 0.65) | blq | blq | blq | blq | 62 (± 15) |
| Tetracyclin | n.d. | 0.93 (± 0.024) | 201 (± 10) | 0.96 (± 0.080) | 1600 (± 50) | 1.01 (± 0.004) | 1200 (± 52) | 1300 (± 28) |
| Oxytetracyclin | 20 (± 2.1) | 23 (± 0.19) | 3400 (± 240) | 29 (± 0.87) | 7500 (± 610) | 27 (± 0.26) | 5800 (± 220) | 6700 (± 250) |
| Chlortetracyclin | n.d. | n.d. | n.d. | n.d. | 1700 (± 92) | n.d. | 1300 (± 13) | 1200 (± 4.7) |
| Lincomycin | n.d. | 20400 (± 4400) | 2700 (± 220) | 16 (± 1.1) | 570 (± 44) | 17 (± 0.26) | 209 (± 1.8) | 130 (± 14) |
| Sulfamethazine | n.d. | 2.8 (± 0.26) | 43 (± 8.3) | n.d. | n.d. | n.d. | n.d. | n.d. |
| Tiamulin | blq | 1.9 (± 0.014) | 24 (± 1.1) | blq | 4.9 (± 0.21) | blq | 4.6 (± 0.039) | 8.2 (± 0.56) |
| Flubendazole | n.d. | 0.94 (± 0.025) | 230 (± 18) | 0.77 (± 0.01) | 3400 (± 480) | 0.92 (± 0.013) | 2600 (± 180) | 630 (± 17) |
| Salicylic acid | 85 (± 19) | 16 (± 7.8) | 120 (± 16) | 51 (± 9.7) | 390 (± 14) | 66 (± 6.03) | 440 (± 48) | 180 (± 8.4) |
| Flunixin | 27 (± 0.60) | n.d. | n.d. | 37 (± 2.3) | 2300 (± 220) | 40 (± 0.69) | 1800 (± 140) | 800 (± 52) |
| Toltrazuril | n.d. | n.d. | n.d. | 5.9 (± 0.55) | 960 (± 190) | 9.9 (± 0.34) | 770 (± 11) | 720 (± 56) |

*Swine slurry total and liquid fractions were centrifuged before analysis and liquid and solid fractions were analyzed separately; blq: compounds detected below their limit of quantification; n.d.; non-detected compounds

Table 5

| Chemical groups | Antibiotics | MEC _{soil} (µg/kg) | PNEC _{soil} (µg/kg) | Ecotoxicological end-points | Hazard quotient (HQ) |
|---------------------------------|-----------------|--------------------------------|---------------------------------|------------------------------------------------------------------------------------------------------------------|----------------------|
| <i>Fluoroquinolones</i> | Enrofloxacin | 151 | 100000 | Earthworm <i>Lumbricus terrestris</i> | 0.0015 |
| | | 151 | 910 | Seed germ (soil) Cucumber | 0.17 |
| | | 151 | 27 | Root elongation Cucumber ^a | 5.6 |
| | | 151 | 13 | Seedling growth Wheat | 12 |
| | | 151 | 950 | Seedling growth (soil) Tomato | 0.16 |
| <i>Tetracyclines</i> | Tetracycline | 10 | 30000 | Seedling height Rice/Cucumber | 0.003 |
| | | 10 | 30000 | Root length Rice/Cucumber | 0.003 |
| | Oxytetracycline | 75 | 1000 | Earthworm <i>E. fetida</i> | 0.075 |
| | | 75 | 1000 | Soil microbial respiration | 0.075 |
| | | 75 | 10000 | Toxicity to plants | 0.0075 |
| | | 75 | 5000 | Reproduction effects to soil invertebrates (<i>Folsomia fimetaria</i> and <i>Enchytraeus cryptus</i>) | 0.015 |
| <i>Pleuromutilin antibiotic</i> | Tiamulin | 3.6 | 475 | reproduction effects to soil invertebrate (<i>Folsomia fimetaria</i>) | 0.0076 |

Highlights

- AN IMPROVED ANALYTICAL METHOD TO DETERMINE PHACs IN MANURE AND SLURRY IS DEVELOPED
- PHACs WERE QUANTIFIED IN THESE FERTILIZERS AT LEVELS UP TO 20 MG/L AND 7500 μ G/KG DW
- AFTER REPEATED MANURE APPLICATIONS, PHACs MOSTLY ACCUMULATE IN SURFACE SOILS
- MODELLING RESULTS ARE HAMPERED BY A LACK OF ACTUAL SORPTION PARAMETERS FOR PHACs
- ECOTOXICOLOGICAL EFFECTS TO TERRESTRIAL ORGANISMS WERE ESTIMATED TO BE LOW

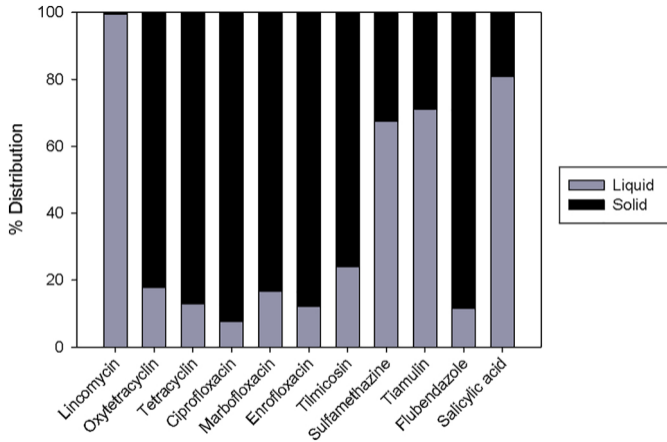
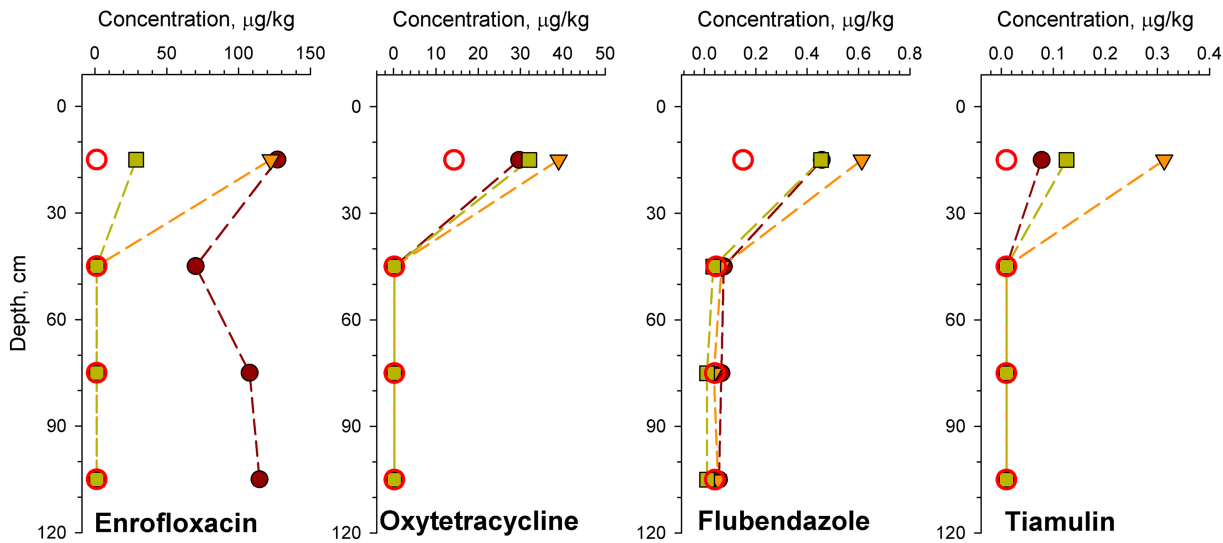


Figure 1

1) Crops amended with swine slurry



2) Crops amended with dairy cattle manure

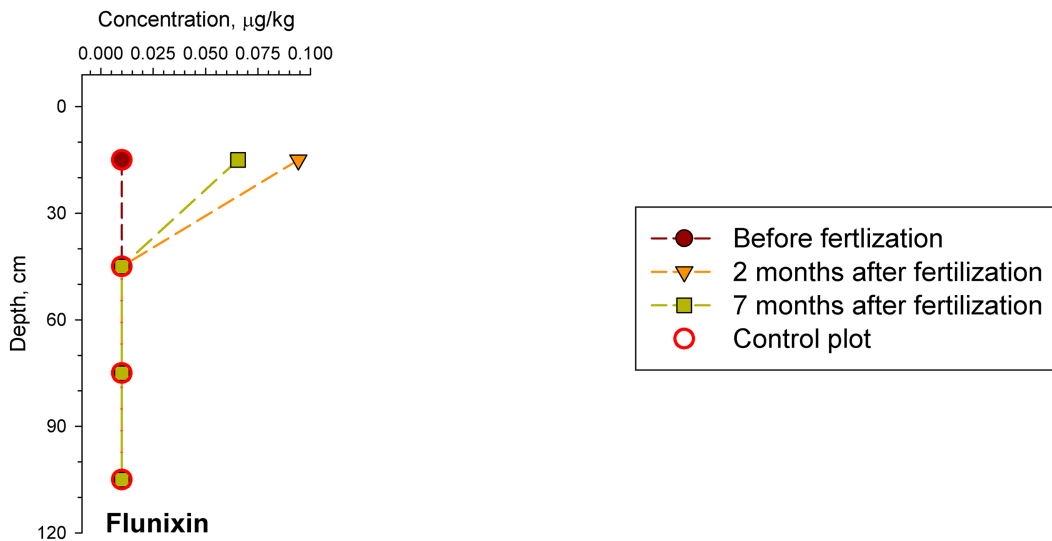


Figure 2

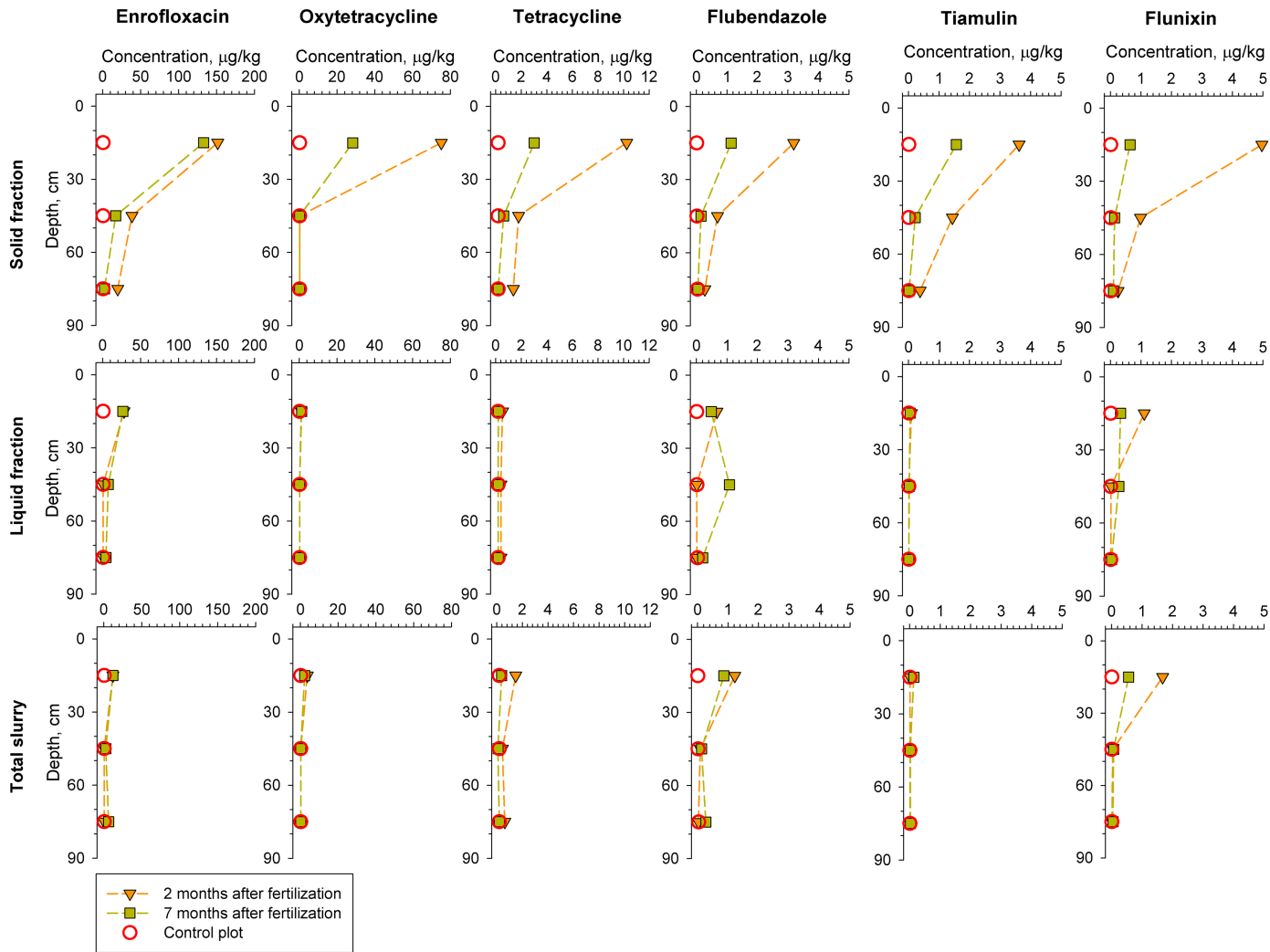


Figure 3

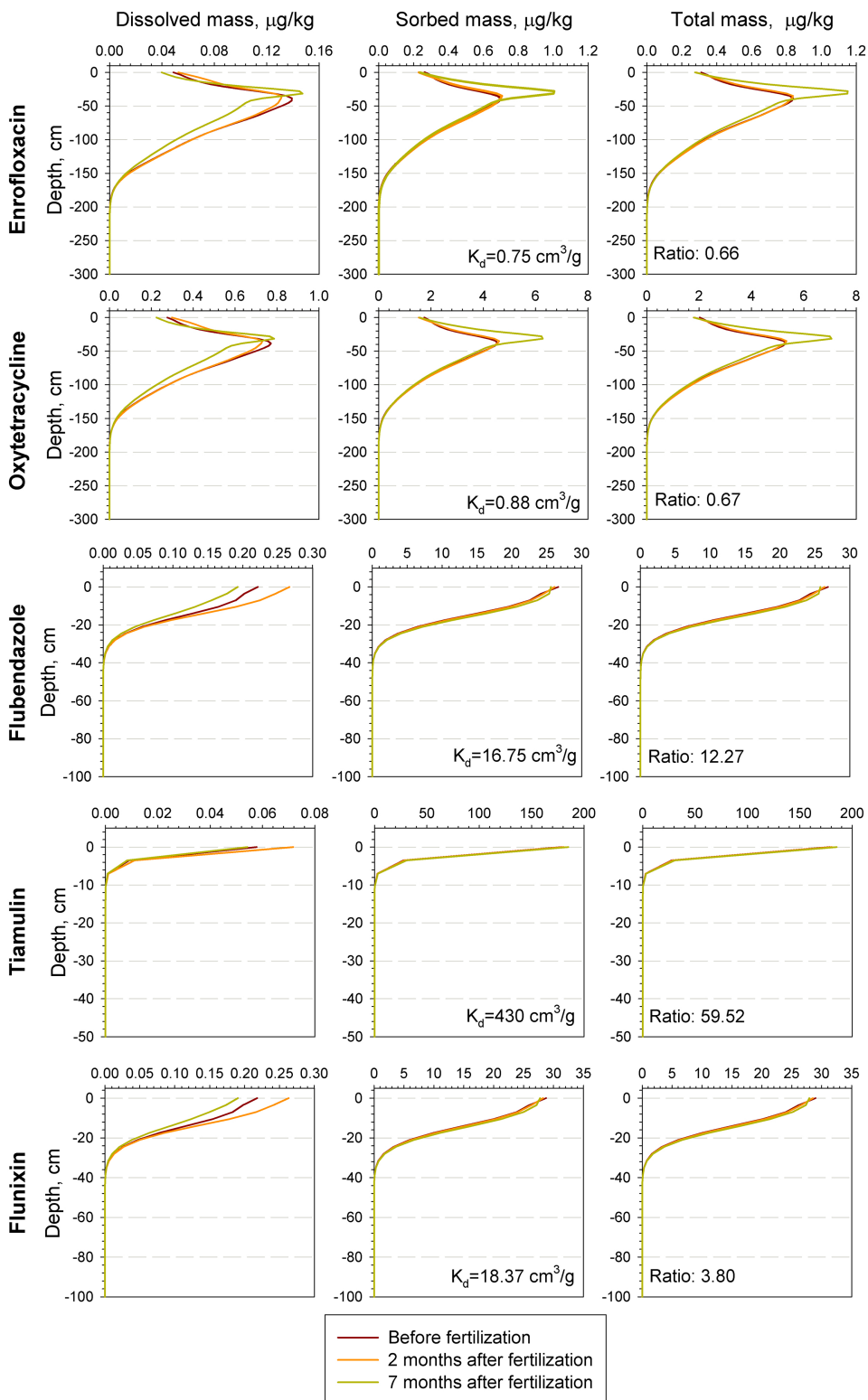


Figure 4