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Occurrence of tetracyclines and sulfonamides in manures, agricultural soils and crops from different areas in Galicia (NW Spain)

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Abstract

Antibiotics released to the environment are causing public health and sustainability concerns. Taking that into account, we studied the presence of tetracyclines (Tetracycline, Oxytetracycline, Chlortetracycline and Doxycycline) and sulfonamides (Sulfadiazine, Sulfamethazine, Sulfachlorpyridazine and Sulfamethoxypyridazine) in manures, soils and crops from Galicia (Spain), where a high number of cattle, pig and poultry farms exist. We used the HPLC-MS/MS technique to analyze 40 samples of cattle, pig and poultry manure, as well as 65 soil samples, and 27 vegetation samples. The presence of antibiotics was detected in 42% of the manures, 17% of the soils and 44% of crop samples, with maximum concentrations of 106.0 mg kg^{-1} for individual antibiotics in manures and 0.6 mg kg^{-1} in soils and plants. The simultaneous presence of several antibiotics was infrequent in soils (only three soils presented two or three antibiotics), and more common in manures and plants, some of them with up to five antibiotics. Pig slurries showed the highest antibiotic concentrations, as well as the highest number of different antibiotics. Crops fertilized with these slurries also showed the highest number of different antibiotics. Antibiotics were detected in 71% of grass and corn samples, and in 33% of wheat grain samples, while they were not detected in potato samples. These results can be very relevant taking into account potential environmental and public health repercussions of antibiotics in soil and water, as well as antibiotics uptake and accumulation in plants, and subsequent incorporation to the food chain.

Keywords: Antibiotics; Crops; Slurries; Soils; Sustainability

1 Introduction

Antibiotics spread in the environment have become a global concern, mainly due to the increase in the development of resistant bacterial strains, which can infect humans and be extremely difficult to fight (Fisher and Scott, 2008; World Health Organization, 2015). One of the causes of the emergence and increase of resistances to antibiotics is the extensive use of these compounds in animal production, to control infections in animals, and in particular their sub-therapeutic use as animal growth promoters (Pikkemaat et al., 2016). In this sense, the European Commission has banned the marketing and use of antibiotics as growth promoters in animal feed since January 2006 (EC, 2003), although it is still a common practice in many parts of the world (Pikkemaat et al., 2016). Sustainability of the involved industries and productive activities, with consequent social and economic implications, is also very relevant (Boholm and Arvidsson, 2014; Xie et al., 2017; Bottoni and Caroli, 2018).

Veterinary antibiotics are excreted in animal feces and urines, reaching the environment mainly through manure and slurries, but also through food products and direct animal contact. Once they reach the soil, these compounds may enter surface water bodies and the food chain. This transfer is affected by adsorption/desorption and transport processes (Fernández-Calviño et al., 2015; Zhou et al., 2017), as well as by degradation processes acting on these antibiotics (Liu et al., 2017a). In areas where a high density of animal farms exists, there can be a high risk associated to the use of liquid and solid manures as fertilizers in agricultural soils (Kumar et al., 2005a). Indeed, abundant studies have reported the presence of a number of antibiotics of different groups in soils fertilized with animal manures and in water bodies close to animal farming areas (Thiele-Bruhn, 2003; Kemper, 2008; Du and Liu, 2012; Pan et al., 2014; Carballo et al., 2016). Other authors have reported antibiotics uptake and accumulation in different crops, such as corn, wheat, lettuce, and rice (Kumar et al., 2005b; Bassil et al., 2013; Pan et al., 2014), alerting about possible development of allergies and antibiotics resistance in human consumers and animals eating these vegetables.

In Galicia (NW Spain), both animal farming and agricultural activities are of economic and social relevance. As shown in Supplementary Material, livestock density is high in Galicia (>1.4 livestock units per ha, Fig. S1), clearly higher than in the rest of Spain (between 0.56 and 0.79 livestock units per ha, Fig. S2). In fact, a high density of dairy farms exists in Galicia associated to the milk industry (mainly cow), and agriculture is in a clear relation to the production and use of forage crops at the farm scale, needed to maintain dairy cattle production systems. The manure obtained in liquid or pasty form in the farms (slurry) is commonly applied to grasslands and forage crops to avoid or diminish the cost of mineral fertilizers (Louro et al., 2015). A comparable situation occurs in areas with pig farms or poultry farms, which are also abundant in the region: in these areas, where forage crops are not necessary for animal feed, animal manures are employed as amendments in agricultural soils. Under these circumstances, there is a potential risk of transference of veterinary antibiotics to the environment through the agricultural use of solid, pasty and liquid manures and slurries from farms, as previously shown for various chemical substances and microorganisms (Núñez-Delgado et al., 1997, 2002; López-Periago et al., 2000, 2002). This risk could be aggravated by the climatic conditions of the region, with most geographic areas characterized by high rainfall distributed all along the year (average annual rainfall over 1000 mm).

Although some previous studies have reported results corresponding to concentrations of several antibiotics in waters, wastewaters and sewage sludge in Galicia (Carballa et al., 2004, 2007, 2008; Iglesias et al., 2014; Álvarez et al., 2016; Alvarino et al., 2018), no data is available regarding antibiotics concentrations in soils and crops in this geographic area. In view of that, the main objective of this work is to carry out an evaluation of the environmental risk associated to veterinary antibiotics spread on soils in Galicia. For this, we have studied the presence of two groups of antibiotics (tetracyclines and sulfonamides) in manures, agricultural soils and crops from two areas with relevant cattle, pig and poultry farming activities.

2 Materials and methods

2.1 Study area

Two areas in Galicia (NW Spain) were selected for the study: Sarria (Lugo province), characterized by a high density of dairy, poultry and pig farms, and A Limia (Ourense province), with abundance of poultry farms.

The area of Sarria is part of a small tectonic depression filled with Tertiary and Quaternary sediments, located in a zone of contact between two-mica granites and schists, at an average above sea level of 450 m. Average yearly temperature and rainfall are 11.6 °C and 824.8 mm, respectively. This area presents abundance of poultry farms, pig farms and dairy farms aimed to milk production, with a very intensive use and high generation of pasty and liquid manures (slurries). Given the high density of intensive dairy farms, an important percentage of agricultural land is dedicated to pasture growth in artificial grasslands.

These pasture lands are usually spread with cattle, pig or poultry slurries or manures, without systematic patterns of application. Usual average yearly doses for slurries (with average dry matter~20%) are around 100 m^3 ha^{-1}. Combinations of inorganic nitrogen, phosphorus and potassium (NPK) fertilizers are also added, in average yearly doses of around 1000 kg ha^{-1} for NPK formulations 8:24:16.

A Limia is one of the best-defined territorial units of Galicia, constituted by a depression originally occupied by Lake Antela and filled with Quaternary sediments reaching a thickness up to 200 m. The sediments that filled the depression were eroded from its borders where several types of granite and schists were the most abundant rocks. A Limia has an average altitude of 640 m above sea level. The mean annual temperature is 11 °C, with total annual precipitation 881 mm on average, irregularly distributed through the year. The lake was drained in the mid-twentieth century and the region is now intensively cultivated. In the study area, potato, grown in rotation with wheat, extends through 3804 ha, producing 90,000 Mg of potatoes annually. Due to the abundance of poultry farms in the area, poultry manure (with average dry matter ~50%) is routinely used as fertilizer and also for its liming effect, with usual yearly doses averaging around 40 m^3 ha^{-1}. NPK fertilizers are also added, in average yearly doses equivalent to those used in Sarria (around 1000 kg ha^{-1} for NPK formulations 8:24:16). The water table is located very close to the soil surface (0-3 m, with large seasonal fluctuations, waterlogging easily taking place in winter and spring medium intensity rainfall events), causing that the area has a significant risk of environmental pollution from agricultural soils.

2.2 Sampling and general analysis

Forty composite samples (each of them resulting from 10 subsamples mixed and homogenized) of different animal slurries were taken in Sarria and A Limia exploitations, transported to the laboratory, stored, and then analyzed. Specifically, 5 composite samples of cattle slurry were taken at five farms in Sarria; 5 composite samples of pig slurry were taken at five farms in Sarria; and, finally, composite samples of poultry manure were taken at 25 farms in A Limia, and at 5 farms in Sarria. Slurries (from cow and pig) were sampled from the pits, whereas poultry manures were sampled directly at heaps. All manure samples were transported to the laboratory, freeze-dried shortly after sampling and stored at -4 ºC until analysis. The analysis of the manures included pH and electrical conductivity in 1:5 distilled water suspensions (Crison pHmeter, model 2001, and Crison conductivity-meter model 524, Crison, L'Hospitalet de Llobregat, Barcelona, Spain); total (organic) C and total N contents were determined by auto-analyzer (LECO CHN-1000, LECO Corporation, St. Joseph, MI, USA).

A total of 65 composite soil samples were collected, with the following distribution: 15 grassland soils were sampled at Sarria, and 50 cropland soils were sampled at A Limia (15 of these soils cultivated with wheat and 35 with potato). Sampling took place during July-August 2016. To perform composite soil samples, 10 soil sub-samples (0–20 cm depth) were collected with a soil auger along each plot, and subsequently mixed into a single composite soil sample (~3 kg). A fraction of each fresh sample (~100 g) was stored at 4 ºC for the determination of total antibiotics, and the remaining was air-dried and sieved by a 2-mm mesh. Soils were characterized using standard methods as described by Tan (1996). Particle size analysis was performed after organic matter destruction with H_2O_2, elimination of Fe and Al oxihydroxides with HCl and dispersion by hexametaphosphate + sodium carbonate. Particles > 50 μm were separated by wet sieving, and particles < 50 μm were obtained by the pipette method. Soil pH was measured in water (soil:solution 1:2.5) by means of potentiometric determinations (pHmeter, model 2001, Crison, L'Hospitalet de Llobregat, Barcelona, Spain). Total organic carbon (TOC) and N were determined on an elemental analyzer (LECO CHN-1000, LECO Corporation, St. Joseph, MI, USA). Exchangeable Ca^{+2}, $Mg^{+2,}$ Na^{+}, K^{+}, Al^{+3} and cation exchange capacity were determined following extraction with 1 M ammonium chloride, using atomic absorption and emission spectroscopy (AAnalyst 200, Perkin Elmer, Boston, MA, USA). Available P was extracted in 0.5 M $NaHCO_3$, with colorimetric determination of the phosphomolybdic complex, using UV-visible spectroscopy (UV-1201, Shimadzu, Kioto, Japan). Acid ammonium oxalate-extractable Fe and Al (Fe_o and Al_o, respectively) were determined after extraction of 0.5 g of soil with 50 mL of 0.2 M oxalic acid–ammonium oxalate at pH 3 during 4 h in the dark (corresponding to non-crystalline Fe and Al oxides). Pyrophosphate-extractable Fe and Al (Fe_p and Al_p, respectively) were determined after a 16-hour extraction of 0.5 g of soil with 50 mL of 0.1 M sodium pyrophosphate at pH 10 (corresponding to Fe and Al bound to soil organic matter). All chemicals used were high-purity analytical grade, from Sigma-Aldrich (Barcelona, Spain).

Crop samples were taken as follows. In Sarria, 5 composite crop samples were taken for each of the 3 kinds of slurry spread on soils. Specifically, 4 crop samples corresponded to grass, and 1 to corn, within plots treated with cattle slurry and within plots treated with pig slurry. However, all 5 crop samples were corn in plots treated with poultry manure. In A Limia (where all plots received poultry manure), 6 composite crop samples were wheat grain, and other 6 were potato tubercle. To perform composite crop samples, the following procedures were carried out. Grass and wheat grain samples were taken using a 50 x 50 cm^2 square. It was randomly thrown 5 times in each plot, then cutting and collecting the vegetable material from each square, to finally perform a composite sample for each one in each plot. Corn samples were taken randomly, choosing 10 plants in each plot and cutting their aerial part, then mixing to perform composite samples. Potato tubercles were taken after a random selection of 20 plants in each plot, then preserving for analysis the two largest and the two smallest tubercles (mixed to perform composite samples). All plant samples were crushed, ground, and shaken for 24 h to homogenize, then frozen at -4 ºC until analysis.

2.3 Extraction, analysis for antibiotics

Four tetracyclines (tetracycline -TC-, oxytetracycline -OTC-, chlortetracycline -CTC-, doxycycline -DC-) and four sulfonamides (sulfadiazine -SDZ-, sulfamethazine -SMZ-, sulfachlorpyridazine -SCP- and sulfamethoxypyridazine -SMP-) were analyzed in this study (see general details of these chemicals in Table 1). Tetracyclines are poliprotic acids with three pK_a values, they are characterized by high water solubility, and relatively stable in acid but not in alkaline conditions. Sulfonamides are more polar but less water-soluble; they present two pK_a values and behave as weak acids at soil pH.

Previously to analyses, all samples were lyophilized on a Scanvac lyophilizer model Scanlaf 230 VAC (Labogene, Denmark). Blank lyophilized plant, manure and soil samples were used for the preparation of the matrix-matched calibration, by spiking them with the mixture of antibiotics (sulfonamides and tetracyclines) to a level corresponding to 0, 25, 50, 75, 100, 150, 200 µg kg^{-1}. Standard solutions containing mixtures of antibiotics, which were employed to build the matrix-calibration-curves, were prepared by mixing stock solutions of each individual antibiotic in methanol. All antibiotics were high-purity analytical grade and obtained from Sigma-Aldrich (Barcelona, Spain).

Analyses of tetracyclines and sulfonamides were always conducted in batches of samples containing matrix-calibration-curves and samples. Extraction and analyses of antibiotics were conducted as described by Martínez-Carballo et al. (2007) and Iglesias et al. (2012, 2014). Briefly, tetracyclines and sulfonamides were extracted form lyophilized plant, manure and soil samples with a mixture of acetonitrile and EDTA-McIlvaine buffer at pH 4 and 6, respectively, followed by purification with SPE cartridges. As indicated by Martínez-Carballo et al. (2007), the extraction procedure here used gives exceptionally good recoveries (77-105%) with standard deviations between 5 and 23%. The final extracts were injected in a HPLC-ESI-MS/MS system, comprised of a HPLC model 1100 from Agilent Technologies (Waldbronn, Germany), equipped with a P680 quaternary pump, a degasser and an auto-sampler, and coupled to a mass spectrometer (MS) model API 4000™ from Applied Biosystems/MDS Sciex (Toronto, Canada), with an integrated TurboIonSpray® for molecule ionization. The software Analyst 1.4.1, also from Applied Biosystems/MDS Sciex (Toronto, Canada), was employed to acquire the data and control the system. The chromatographic analyses were performed by injecting 10 µL of extract into a BEH C18 1.7 um, 100×2.1 mm column (for Tetracyclines) or Synergi 2.5-µm Polar-RP 100A column (50×2.0 mm) (for Sulfonamides). Samples spiked with the selected antibiotics were employed to establish the limits of detection (LOD), and of quantification (LOQ), for the method. These limits are defined as concentration at which the signal-to-noise ratio was above 3 (LOD) and 10 (LOQ), respectively. As indicated in Iglesias et al. (2012) regarding the method used for quantification of the antibiotics under study, "The analytical method was validated in terms of selectivity, linearity, instrument detection limit (IDL), instrument quantification limit (IQL), LOD, limit of quantification (LOQ), method detection limit (MDL), repeatability, precision and accuracy, following the Environmental Protection Agency (EPA), Method 136, Appendix B criteria (EPA 1984)". All quantifications of the antibiotics here studied were carried out in the laboratories of the researchers who developed these analytical procedures.

Triplicate samples were used in all analytical steps. All chemicals used in HPLC determinations were high-purity analytical grade, from Sigma-Aldrich (Spain), and ultrapure water from Millipore equipment (Millipore, Spain) was used to prepare all dissolutions needed.

2.4 Data treatment and statistics

Descriptive statistics, specifically mean, maximum, and minimum values corresponding to general characteristics of all soil and manures/slurries samples, were calculated. Furthermore, analyses of correlation and multiple regressions between total antibiotics concentration and soil and manures/slurries characteristics, as well as between antibiotic concentrations in soils and in vegetation, were performed (significance was considered at $p < 0.05$). The statistical package SPSS 19.0 (IBM, USA) was used.

3 Results and discussion

3.1 General characteristics of manures and soils

As shown in Table 2, the average pH values were 7.9, 7.1, 7.6 and 7.5 for cattle, pig, and poultry manures/slurries sampled at "Sarria" locations, and poultry manures at "A Limia" locations, respectively. At "Sarria" locations, these pH values ranged from 6.6 to 9.1 in cattle manure, from 6.7 to 7.9 in pig manure, and from 7.5 to 7.7 in poultry manure. At "A Limia" locations pH ranged from 5.8 to 8.3 in poultry manure. Cattle slurry presented the highest variability, whereas the lowest corresponded to poultry manure from Sarria (Table 2). All these values are similar to those reported for animal slurries in other studies (Søgaard et al., 2002; Sánchez and González, 2005; Álvarez et al., 2010; Fernández-Sanjurjo et al., 2010). Electrical conductivity values were also dependent on the origin of the manure, with cattle slurry (Sarria) showing the highest average level (8.78 dS m^{-1}), oscillating between 5.33 and 13.96 dS m^{-1}, and the lowest average level corresponding to poultry manure from A Limia (5.32 dS m^{-1}), oscillating between 0.59 and 13.46 dS m^{-1}, all of them being lower than those previously reported for pig slurries in Galicia (Álvarez et al., 2010). Average C and N contents were higher in poultry manure from "A Limia", being 306.5 g kg^{-1} and 28.7 g kg^{-1}, respectively, with the lowest values detected in pig slurry (Sarria) for C (265.3 g kg^{-1}), and in cattle slurry (Sarria) for N (21.1 g kg^{-1}) (Table 2). The ranges for C and N contents were similar to those previously found in other studies in Galicia (Sánchez and González, 2005; Fernández-Sanjurjo et al., 2010). Regarding C/N ratio, the highest average value was 14, and corresponded to cattle slurry (Sarria), and the lowest was 10, corresponding to pig slurry (Sarria) (Table 2).

The soils studied presented textures that were predominantly loam in Sarria, and sandy-clay loam in "A Limia" (Table 3). In fact, sand percentage values were higher in "A Limia" soils, ranging from 39 to 64% (average 55%), whereas percentage sand for "Sarria" soils (n=15) ranged from 27 to 61% (average 39%). Clay percentage values were similar for most soils, ranging from 19 to 31% (average 24%) in "A Limia", and from 15 to 31% (average 21%) for "Sarria" (Table 3). As usual in Galicia, due to the combination of climatic conditions (high rainfall) and parent material (mostly siliceous acid rocks), most cropland soils in A Limia presented acid pH (average pH_{H2O} = 5.0), and grassland soils in Sarria had pH_{H2O} around 6.0. These higher pH values found in soils from Sarria were in accordance with their higher concentrations for Ca, Mg and eCEC (and lower levels for Al) in the exchange complex, all of them indicative of the generalized practice of liming artificial grasslands in Galicia. All the studied soils presented C and N contents that are common values in natural soils of the region (Calvo-de-Anta et al., 2015). Available P contents were very high in A Limia and high in Sarria. Iron and Al compounds were much more abundant in Sarria than in A Limia, which is mainly due to their different parent materials (Table 3).

3.2 Presence of antibiotics in manures and soils

Most samples analyzed in this work did not present detectable concentrations of the antibiotics studied, neither in manures nor in soils, being below LOD and LOQ (Fig. 1). The presence of antibiotics was detected in 17 manure samples (42% of the manures) (Fig. 1a), and in 11 soil samples (17% of the soils) (Fig. 1b).

We carried out an analysis of correlations between total concentration of antibiotics in soils and different soil parameters, using only those soil samples where antibiotics were detected ($n = 10$). The results of the test showed a significant relation between clay content and the presence of antibiotics in soils ($r = 0.635$, $p \leq 0.05$). Furthermore, a multiple regression analysis was performed, using total concentration of antibiotics as dependent variable, and soil characteristics as independent variables. In this case, results showed that clay percentage explained 40% of the variation of the total content of antibiotics, by means of the following equation:

$$[\text{Total Antibiotic}] = -0.7 \pm 0.4 + 0.04 \pm 0.02\text{Clay}$$

$$F = 5.4;\ p < 0.05$$

The influence of the clay content on the concentration of antibiotics detected in soils may be related to adsorption phenomena. In fact, previous works found significant relations between clay contents and adsorption parameters for Oxytetracycline (Jones et al., 2005), Tetracycline and Chlortetracycline (Sassman and Lee, 2005; Teixidó et al., 2012). Similar results were found for sulfonamides, showing that clay fraction increases adsorption (Doretto and Rath, 2013; Pereira-Leal et al., 2013; Doretto et al., 2014).

In the present study, tetracyclines were in general present in a higher number of samples and at higher concentrations than sulfonamides (Tables 4 and 5), in agreement with previous works (Li et al., 2011; Pan et al., 2011). This higher occurrence of tetracyclines with respect to sulfonamides may be related to the higher adsorption of tetracyclines in soils, facilitating their persistence in the environment (Sarmah et al., 2006; Carvalho and Santos, 2016). In fact, Laak et al. (2006a) studied the adsorption of oxytetracycline, tylosin and sulfachloropyridazine in 11 soils of different characteristics, obtaining for SCP adsorption coefficients (K_d) that varied between 0.4 and 34.8 L kg^{-1}, while they were much higher (between 946 and 5257 L kg^{-1}) for OTC.

In the present study, the frequency of apparition of antibiotics was lower than the values reported by other authors, which can reach over 90% in manures (Pan et al., 2011) and manure-amended soils (Zhang et al., 2008). In this study, the simultaneous presence of antibiotics was infrequent in soil samples (only 3 of the 65 soils studied presented two or three antibiotics), and more common in the manures, some of them with up to 5 antibiotics (Tables 3 and 4). A negative and significant correlation was found between total concentration of antibiotics in manures and the pH of these manures ($r = 0.328$, $p \leq 0.05$, $n = 17$). This may be due to the following. Firstly, an increase in pH leads to an increase in the degradation rate of both tettacyclines (López-Peñalver et al., 2010; Conde-Cid et al., 2018) and sulfonamides (Periša et al., 2013), decreasing their concentrations in the medium. Secondly, adsorption of both tetracyclines and sulfonamides decreases as pH increases (Teixidó et al., 2012; Park and Huwe, 2016).

As they are ionizable compounds, a change in pH can lead to protonation or deprotonation of the molecules, changing their physicochemical properties and, therefore, highly affecting their adsorption. Thus, an increase in pH leads to an increase in the proportion of negatively charged species for both the studied sulfonamides and tetracyclines, and at the same time causes an increase in the negative variable-charges of organic matter, generating an electrostatic repulsion between the species of the negatively charged antibiotics and the negative charges of the organic matter present in manures. In addition, the anionic species of these compounds are more soluble in water, thus decreasing their adsorption and increasing their mobility. This effect of pH has been observed for sulfonamides (Boxall et al., 2002; Wegst-Uhrich et al., 2014; Liu et al., 2017b) and for tetracyclines (Sassman and Lee, 2005; Zhao et al., 2011), as well as for other antibiotics (Hari et al., 2005). In this sense, Laak et al. (2006b) studied the adsorption of different species of sulfachloropyridazine (SCP^0 and SCP^-) and oxytetracycline (OTC^+, OTC^0, OTC^- and OTC^{2-}) and found K_d values approximately one order of magnitude lower for negatively charged species, as compared to neutral or positively charged species. Specifically, these authors found K_d values of between 8.1 and 16.6 L kg^{-1} for SCP^0, and much lower for SCP^- (between 0.0 and 1.1 L kg^{-1}). Likewise, K_d values ranged between 4200 and 4740 L kg^{-1} for OTC^+ and OTC^0, and between 310 and 630 L kg^{-1} e for OTC^- and OTC^{2-}.

Tetracycline (TC) and OTC were the most common antibiotics of their group (tetracyclines) in all samples. Tetracycline (TC) was found in 5 manures and 5 soils, in many cases together with other tetracyclines or with SCP. Oxytetracycline (OTC) was found in 7 manures and 2 soils, in almost all cases with other tetracyclines and at concentrations higher than the rest. Chlortetracycline (CTC) was only found in 3 pig slurries, always with other tetracyclines, and DC was found in 6 manures, but not in soils. Regarding the sulfonamides group, SCP appeared in 11 samples (5 manures and 6 soils), showing concentrations <0.2 mg kg^{-1}, whereas SMZ was present in 2 soils and 2 manures. Finally, SMP and SDZ were detected in 1 sample of manure (not both sulfonamides simultaneously), but not in soils.

The maximum concentration observed for individual antibiotics in manures reached 106.0 mg kg^{-1} for DC, 35.0 mg kg^{-1} for OTC, 4.0 mg kg^{-1} for CTC, 0.9 mg kg^{-1} for TC, 0.6 mg kg^{-1} for SDZ and 0.1 mg kg^{-1} for SCP (Table 3). The concentrations detected for tetracyclines are in the common range for manures, except for doxycycline, far from the maximum values observed in other studies, which reported levels that regularly exceed 100 mg kg^{-1} (Karcı and Balcıoğlu, 2009; Hu 2010; Pan et al., 2011; Widyasari-Mehta et al., 2016). In the present study, sulfonamide concentrations were also lower than the extreme values reported by other authors, which can reach up to 35 mg kg^{-1} for SCP (Karcı and Balcıoğlu, 2009), and up to 91 mg kg^{-1} for SDZ (Martínez-Carballo et al., 2007). Nevertheless, antibiotic concentrations in manures usually show large variations and are highly dependent on factors such as the moment of sampling, the region studied or the source of manure. In the present study, total concentrations of antibiotics found in manures followed a decreasing sequence, as follows: pig manure > poultry manure > cattle manure, in agreement with results by Zhang et al. (2008). This sequence might be related to differences in the treatments received by livestock, which could be larger for pigs, although reliable data on the exact quantity, dose and frequency of application are limited (Pikkemaatt et al., 2016). Despite the scarcity of data, evidences exist in the sense that pig and poultry manures present higher antibiotic concentrations than cattle manure due to a lower use of antibiotics in cattle (Kemper et al., 2008). This is supported by recent estimations that suggest that the global average use of antimicrobials per kilogram of animal produced was 45 mg kg^{-1} for cattle, 148 mg kg^{-1} for chicken and 172 mg kg^{-1} for pigs (Van Boeckel et al., 2015).

Guo et al. (2016) also found a lower presence of antibiotics in cattle than in pig and poultry manure, which they attributed to variations in the prescriptions and doses for cattle, and to the different metabolic characteristics of the animals. In general, the medication for cattle is lower than for other animals due to the strict supervision of antibiotic residues in milk. In addition, the side effects of antibiotics also appear more frequently in cattle, thus justifying lower administration (Guo et al., 2016). The same trend was also observed by Zhao et al. (2010), who attributed it to variations in doses for growth promotion and disease prevention in different animal species.

In the present study, soils showed concentrations of antibiotics always lower than in manures, as observed in other studies (Karcı and Balcıoğlu, 2009; Hu et al., 2010). The highest concentrations were 0.6 mg kg^{-1} for TC, 0.2 mg kg^{-1} for OTC, 0.2 mg kg^{-1} for SCP and 0.1 mg kg^{-1} for SMZ (Table 4). These values are in the range of those reported in the literature for tetracyclines, which go from 0 to 3 mg kg^{-1}, although concentrations over 1 mg kg^{-1} are not frequent (Hamscher et al., 2005; Karcı and Balcıoğlu, 2009; Hu et al., 2010; Li et al., 2011). In the case of sulfonamides, the concentrations in our study are slightly higher than in other works, where values are always between 0.001 and 0.10 mg kg^{-1} (Hamscher et al., 2005; Karcı and Balcıoğlu, 2009; Hu et al., 2010; Li et al., 2011).

According to our data, it seems that accumulation of tetracyclines or sulfonamides is very limited in the agricultural soils of the two geographic areas studied. Tetracyclines are often regarded as very stable and persistent in the environment (Pikkemaat et al., 2016), so our observations could be pointing at high rates of degradation of these compounds on soils (mainly in the case of OTC), considering their lower values and presence in relation to manures, which may be due to climatic conditions or high microbial activity at some moment. As shown in previous studies, different kinds of degradation processes affect these antibiotics, including high levels of photo-degradation for tetracyclines (Liu et al., 2015; Li and Hu, 2016) and sulfonamides (Bonvin et al., 2013; Lian et al., 2015), whereas other authors pointed out that these groups of antibiotics can be eliminated simultaneously via laccase-mediated oxidation combining with soil adsorption (Ding et al., 2016). Other studies found a high biodegradation of sulfonamides (Müller et al., 2013; Yang et al., 2016) and chlortetracycline (Liao et al., 2017). In this sense, Hu et al. (2010) reported much higher concentrations of tetracyclines and sulfonamides in manures and soils sampled in winter than in summer, likely due to accelerated biodegradation at higher temperatures and higher microbial activity in summer time. In a recent study on biotic and abiotic dissipation of the tetracyclines TC, CTC and OTC (Conde-Cid et al., 2018), we found low relevance for biodegradation in soils and manures from the same two geographic areas covered in the present research, with photodegradation being the main dissipation process. It was also remarkable that increased pH values resulted in higher dissipation, with progressive reduction in cationic species of all tetracyclines, and that bacterial suspensions favored dissipation, but due to adsorption on humic acids instead of to biodegradation. In another study, Karcı and Balcıoğlu (2009) found lower amounts of sulfonamides in stored manures than in fresh manures, with prolonged time of storage facilitating degradation. For example, these authors detected a concentration of 3.76 mg kg^{-1} for sulfamethoxazole in fresh poultry manure, whereas it was reduced to only 0.10 mg kg^{-1} in stored manure.In addition, low adsorption of sulfonamides to soils cause that they are very mobile in the environment, making easy their transfer from soil to water (Pikkemaat et al., 2016; Pan and Chu, 2017). In fact, Baran et al. (2011) indicate that sulfonamides are commonly detected in surface water (concentrations of up to 19.2 µg L^{-1}), and in groundwater (concentrations up to 3.46 μL^{-1}). Another possible cause of the lower presence of antibiotics in soils than in manures is plant uptake, which has been observed for a variety of crops and different antibiotics (Tasho and Cho, 2016; Pan and Chu, 2017).

3.3 Antibiotics in plants

As shown in Table 6, antibiotics were detected in 12 of the 27 samples analyzed. However, SMP was not detected in any sample (LOD 0.020 mg kg^{-1}). The lowest concentrations showed low variation, always being in the range 0.05-0.1 mg kg^{-1}, whereas the highest concentrations ranged from 0.2 mg kg^{-1} (for OTC) to 0.6 mg kg^{-1} (for SMZ). Sulfonamides appeared more frequently than tetracyclines in plant tissues, which could be related to a facilitated uptake for the former, due to their lower molecular weight (Kumar et al., 2005b). Within sulfonamides, SDZ and SCP were those detected in a greater number of samples, although SMZ was the one showing the highest concentration (0.6 mg kg^{-1}). Five out of the 7 grass samples analyzed (71%) presented some antibiotic, the same result than in corn, while the percentage dropped to 33% (2 out of 6 samples) in wheat grains, and no antibiotic was detected in potato (0 out of 6 samples). These results are in agreement with those observed by Dolliver et al. (2007), who studied the absorption of SMZ by different crops in soils amended with manure, finding a higher concentration in corn than in potatoes. Considering the kind of manure spread on the plots, 80% of the crop samples from plots treated with pig slurry contained some antibiotic, generally in higher concentrations, and with more than one antibiotic simultaneously. High concentrations of some antibiotic were also detected in crop samples from the plots amended with poultry manure in Sarria. The concentrations detected were higher than those found by Kang et al. (2013), who reported concentrations <0.01mg kg^{-1} in 11 different crops from soils amended with turkey and hog manures or inorganic fertilizer. Pan et al. (2014), studying the presence of different antibiotics, including tetracyclines and sulfonamides, in different crops irrigated with wastewater, found that maximum concentrations of different antibiotics were lower than 0.024 mg kg^{-1}. These authors suggested different factors that may influence antibiotics uptake by plants, such as degradation processes, soil adsorption and washing of antibiotics. Concentrations similar or lower than those reported by these authors are frequently found in most studies focusing on antibiotics uptake for different crops (Kumar et al., 2005b; Chitescu et al., 2013; Azanu et al., 2016; Franklin et al., 2016). However, in a field study, Grote et al. (2007) found high concentrations for SFD (up to 0.5 mg kg^{-1}) and CTC (up to 1.1 mg kg^{-1}) in wheat roots, and around 0.043 mg kg^{-1} for CTC in wheat grains, similar to those detected for SCP in the present study. In greenhouse studies, Bassil et al. (2013) detected concentrations of antibiotics in plant tissues similar to those found in the present work (between 0.1 and 0.6 mg kg^{-1}).

In summary, our results indicate that there is a low frequency of apparition of the studied antibiotics in manures and soils, being their occurrence in soils much lower than in manures. This low occurrence could be explained by a high rate of degradation of the compounds, in particular for tetracyclines, or by a high rate of leaching under the prevailing climatic conditions in the area, at least in the case of sulfonamides, that are less adsorbed by soil components. However, crop samples showed a higher number of antibiotics and in higher concentration. This discrepancy between manure, soil and plant, may be due to different factors, such as different degree of degradation of the antibiotics in each of the three matrices, and uptake by plants resulting in accumulation of the antibiotics in their tissues, removing them from the soil. In this sense, several studies have shown a toxic effect of antibiotics in different crops (such as oats, rice, and corn), due to its potential for bioaccumulation, with greater toxicity for CTC than for SMZ (Pan and Chu, 2016), and Madikizela et al. (2018) indicated that translocation of pharmaceuticals within the plant tissues towards the edible crops remains a serious concern, although they also pointed out that results corresponding to pharmaceutical uptake by plants suggest the potential of utilizing constructed wetlands and certain plants for phytoremediation. So, despite the fact that measured concentrations of antibiotics in soil were in general low, their potential toxicity should not be ignored. Further future research should be designed and carried out to complement the present one, in order to deepen understand processes and mechanisms involved, aiding to increase knowledge on the fate of antibiotics in the environment.

4 Conclusions

The occurrence of tetracyclines and sulfonamides in pig, cattle and poultry manures and in agricultural soils receiving these manures can be considered overall low in the two agricultural areas studied, situated in Galicia (NW Spain). Both groups of antibiotics were less frequent and found at lower concentrations in soils than in manures and vegetation. The highest percentage of samples containing antibiotics, as well as the highest number of antibiotics, were detected in pig slurry and in crops grown on soils amended with these slurries. The highest concentrations of antibiotics were also found in pig slurries, while crops with the highest antibiotics concentrations were those grown on soils amended with poultry manure. Overall, the available data do not allow concluding that a high risk of soil contamination due to tetracyclines and sulfonamides exists in both geographic areas studied. However antibiotics concentrations in crops were relatively high, suggesting that plants can accumulate these compounds, with the consequent risk for consumers. These results could be of relevance taking into account public health, environmental, economic and social aspects, affecting to sustainability of the productive sectors involved.

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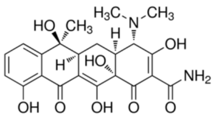
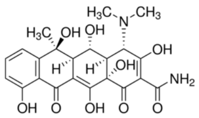
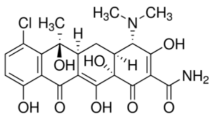
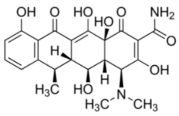
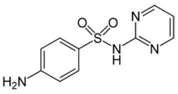
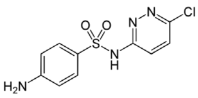
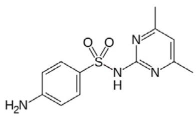
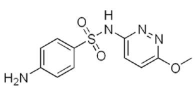
Figure captions

Fig. 1 Number of samples within different ranges of concentrations (in mg kg^{-1}) of tetracyclines and sulfonamides in slurries/manures (a), in soils (b) and in crops (c), considering the whole set of samples from Sarria and from A Limia. LOD: limit of detection (for tetracyclines, detection limits were all <25 ng g^{-1}, <50 ng g^{-1} and <40 ng g^{-1} in slurries/manures, soils and crops, respectively; for sulfonamides, detection limits were all <25 ng g^{-1}, <50 ng g^{-1} and <40 ng g^{-1} in slurries/manures, soils and crops, respectively).

Table 1. Characteristics of the antibiotics studied.

Common name	Chemical structure	Chemical formula	Molecular weight (g mol^{-1})	Log K_{OW} [1]	pK_a [2]	Water solubility (mg L^{-1}) [1]
Tetracycline		$C_{22}H_{24}N_2O_8$	444.4	-1.30	3.32 - 7.78 – 9.58	231
Oxytetracycline		$C_{22}H_{24}N_2O_9$	460.4	-0.90	3.22 - 7.46 - 8.94	313
Chlortetracycline		$C_{22}H_{23}ClN_2O_8$	478.9	-0.62	3.33 - 7.55 - 9.33	630
Doxycycline		$C_{22}H_{24}N_2O_8$	444.4	-0.02	3.02 – 7.97 - 9.15	630
Sulfadiazine		$C_{10}H_{10}N_4O_2S$	250.3	-0.09	2.10 - 6.28	77
Sulfachlorpyridazine		$C_{10}H_9ClN_4O_2S$	284.7	0.31	1.87 - 5.45	35
Sulfamethazine		$C_{12}H_{14}N_4O_2S$	278.3	0.89	2.07 - 7.49	1500
Sulfamethoxypyridazine		$C_{11}H_{12}N_4O_3S$	280.3	0.32	2.08 – 7.19	147

[1] Chen et al. (2015)

[2] Babic et al. (2007)

Table 2. General properties of the cattle (C), pig (P), and poultry/chicken (Ch) manures/slurries from Sarria (-S) and from A Limia (-L); n: number of samples; EC: electrical conductivity.

	C-S (n=5)			P-S (n=5)			Ch-S (n=5)			Ch-L (n=25)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Dry matter (%)	19	10	28	21	4	61	76	46	92	49	33	67
pH	7.9	6.6	9.1	7.1	6.7	7.9	7.6	7.5	7.7	7.5	5.8	8.3
EC (dS m^{-1})	8.78	5.33	13.96	6.35	1.37	10.59	7.85	1.58	14.52	5.32	0.59	13.46
C (g kg^{-1})	295.7	175.8	365.4	265.3	180.6	314.9	296.8	230.0	365.0	306.5	166.8	386.1
N (g kg^{-1})	21.1	16.6	28.6	26.7	17.3	34.6	24.3	1.6	3.3	28.7	13.9	36.2
C/N	14	11	17	10	8	12	13	11	15	11	9	14

EC: Electrical conductivity

Table 3. General properties of the soils amended with cattle (C), pig (P) and poultry/chicken (Ch) manures/slurries, in Sarria (-S) and in A Limia (-L). Min: minimum; Max: maximum.

	C-S (n=5)			P-S (n=5)			Ch-S (n=5)			Ch-L (n=50)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Sand (%)	40	27	49	47	31	61	30	27	39	55	39	64
Silt (%)	38	25	49	33	23	45	48	39	51	21	14	32
Clay (%)	22	17	31	20	15	23	22	21	23	24	19	31
pH_{H2O}	6.0	5.5	6.7	5.6	5.0	6.2	6.5	6.1	7.1	5.0	4.1	6.3
C (g kg^{-1})	27.7	14.1	45.9	41.5	20.2	68.8	21.1	16.3	29.3	26.5	10.7	109.2
N (g kg^{-1})	2.8	1.3	4.6	3.6	2.4	4.8	2.4	1.9	3.2	2.3	0.9	8.4
Ca^{2+} ($cmol_c$ kg^{-1})	8.81	4.21	22.11	7.60	1.84	12.79	11.52	5.66	18.71	2.93	1.02	13.29
Mg^{2+} ($cmol_c$ kg^{-1})	2.14	1.04	3.78	2.27	0.57	5.26	1.59	0.97	3.42	0.67	0.31	1.68
Na^{+} ($cmol_c$ kg^{-1})	0.29	0.23	0.41	0.29	0.19	0.41	0.29	0.20	0.38	0.28	0.12	0.57
K^{+} ($cmol_c$ kg^{-1})	1.65	0.67	4.09	1.48	0.77	3.63	0.94	0.61	1.40	1.15	0.40	2.96
Al^{3+} ($cmol_c$ kg^{-1})	0.51	0.00	2.04	0.64	0.06	2.61	0.02	0.00	0.04	1.04	0.01	2.66
eCEC ($cmol_c$ kg^{-1})	13.40	6.75	30.31	12.28	6.04	17.38	14.36	8.03	23.41	6.07	3.88	16.47
P (mg kg^{-1})	96.14	11.69	317.61	100.03	26.28	178.37	84.47	55.10	120.03	146.12	46.46	261.87
Al_0 (mg kg^{-1})	21403	10439	44759	24697	13659	50593	14901	8081	22858	2479	345	6610
Al_p (mg kg^{-1})	17059	7355	38606	26660	7098	52990	7826	6392	9125	1870	246	4590
Fe_0 (mg kg^{-1})	48046	20591	82640	51004	35782	73096	44885	33123	56424	2083	760	4910
Fe_p (mg kg^{-1})	29322	6837	57998	36728	17419	67723	34098	11196	84534	1748	676	3396

eCEC: Effective cation exchange capacity: Al_o and Fe_o: Al and Fe extractable in acid oxalic/ammonium oxalate; Al_p and Fe_p: Al and Fe extractable in pyrophosphate

Table 4. Concentrations (mg kg^{-1}) of tetracyclines (TC: tetracycline; OTC: oxytetracycline; CTC: chlortetracycline; DC: doxycycline) and sulfonamides (SDZ: sulfadiazine; SCP: sulfachlorpyridazine; SMZ: sulfamethazine; SMP: sulfamethoxypyridazine), in cattle (C), pig (P), and poultry/chicken (Ch) manures/slurries from Sarria (-S) and from A Limia (-L). Only those samples where at least one antibiotic was detected are shown (n=17).

	Source	TC	OTC	CTC	DC	SDZ	SCP	SMZ	SMP
C3-S	Cow	<LOD	2.9	<LOD	0.3	<LOD	<LOD	<LOD	<LOD
C4-S	Cow	<LOD	<LOD	<LOD	0.1	<LOD	<LOD	<LOD	<LOD
P1-S	Pig	0.2	4.6	2.7	1.5	<LOD	<LOD	<LOQ	<LOD
P2-S	Pig	<LOD	35.0	0.1	106.0	<LOD	0.05	<LOD	<LOD
P3-S	Pig	0.9	26.0	4.0	36.0	<LOD	<LOD	<LOD	<LOD
P5-S	Pig	<LOQ	28.0	<LOD	16.0	<LOD	<LOD	<LOD	<LOD
Ch1-S	Chicken	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD
Ch2-S	Chicken	<LOD	1.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ch5-S	Chicken	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD
Ch2-L	Chicken	<LOD	0.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ch6-L	Chicken	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD
Ch12-L	Chicken	<LOD	<LOD	<LOD	<LOD	<LOD	0.1	<LOD	<LOD
Ch14-L	Chicken	<LOD	<LOD	<LOD	<LOD	<LOD	0.1	<LOD	<LOD
Ch17-L	Chicken	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ
Ch19-L	Chicken	0.3	4.1	<LOD	14.0	0.6	<LOD	<LOD	<LOD
Ch21-L	Chicken	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ch25-L	Chicken	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD

Numbers after letters C, P and Ch indicate sample number; LOD: limit of detection (<0.025 mg kg^{-1} for sulfonamides and tetracyclines). LOQ: limit of quantification (<0.050 mg kg^{-1} for sulfonamides and tetracyclines)

Table 5. Concentrations (mg kg^{-1}) of tetracyclines (TC: tetracycline; OTC: oxytetracycline) and sulfonamides (SCP: sulfachlorpyridazine; SMZ: sulfamethazine) in soils amended with cattle (C), pig (P), and poultry/chicken (Ch) manures/slurries, in Sarria (-S) and in A Limia (-L). Only those samples where at least one antibiotic was detected are shown (n=17).

	TC	OTC	SCP	SMZ
C5-S	<LOD	<LOD	<LOD	0.1
P1-S	<LOD	<LOD	0.1	<LOD
Ch5-S	<LOD	<LOD	0.2	<LOD
Ch7-L	<LOD	<LOD	<LOD	0.1
Ch21-L	0.2	<LOD	<LOD	<LOD
Ch27-L	0.3	<LOD	<LOQ	<LOD
Ch30-L	<LOD	0.2	<LOD	<LOD
Ch35-L	0.6	0.1	0.2	<LOD
Ch39-L	<LOD	<LOD	0.1	<LOD
Ch44-L	<LOQ	<LOD	<LOD	<LOD
Ch46-L	0.2	<LOD	0.2	<LOD

Numbers after letters C, P and Ch indicate sample number; LOD: limit of detection (<0.050 mg kg^{-1} for sulfonamides and tetracyclines). LOQ: limit of quantification (<0.050 mg kg^{-1} for sulfonamides and tetracyclines)

Table 6. Concentrations (mg kg^{-1}) of tetracyclines (TC: tetracycline; OTC: oxytetracycline; CTC: chlortetracycline; DC: doxycycline) and sulfonamides (SDZ: sulfadiazine; SCP: sulfachlorpyridazine; SMZ: sulfamethazine; SMP: sulfamethoxypyridazine) in tissues of crops grown on soils amended with cattle (C), pig (P) and poultry/chiken (Ch) manures/slurries, from Sarria (-S) and A Limia (-L). Only those samples where at least one antibiotic was detected are shown (n=17).

	Type	TC	OTC	CTC	DC	SDZ	SCP	SMZ	SMP
P1-S	Grass	<LOD	<LOD	<LOD	<LOD	<LOD	0.2	<LOD	<LOD
P2-S	Grass	0.1	0.1	<LOD	<LOD	0.1	0.1	<LOD	<LOD
P3-S	Grass	0.1	0.1	0.1	<LOD	0.1	0.1	0.1	<LOD
P4-S	Corn	0.1	0.2	0.1	0.1	0.3	0.1	0.1	<LOD
C4-S	Grass	0.1	0.1	0.1	<LOD	<LOD	0.1	0.1	<LOD
C5-S	Grass	<LOD	<LOD	<LOD	<LOD	0.1	<LOD	<LOD	<LOD
Ch2-S	Corn	0.3	<LOD	<LOD	<LOD	0.2	0.1	<LOD	<LOD
Ch3-S	Corn	<LOD	0.1	<LOD	<LOD	0.4	0.1	0.1	<LOD
Ch4-S	Corn	0.1	0.1	<LOD	<LOD	<LOD	0.2	0.6	<LOD
Ch5-S	Corn	<LOD	<LOD	<LOD	<LOD	0.5	<LOD	<LOD	<LOD
Ch21-L	Wheat	<LOD	<LOD	<LOD	<LOD	<LOD	0.1	<LOD	<LOD
Ch30-L	Wheat	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.1	<LOD

Numbers after letters C, P and Ch indicate sample number; LOD: limit of detection (<0.020 mg kg^{-1} for sulfonamides and 0.040 mg kg^{-1} for tetracyclines). LOQ: limit of quantification (<0.040 mg kg^{-1} for sulfonamides and tetracyclines)