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CONCENTRATION AND PERSISTANCE OF ANTIBIOTICS IN PIG MANURE AND AMENDED SOIL

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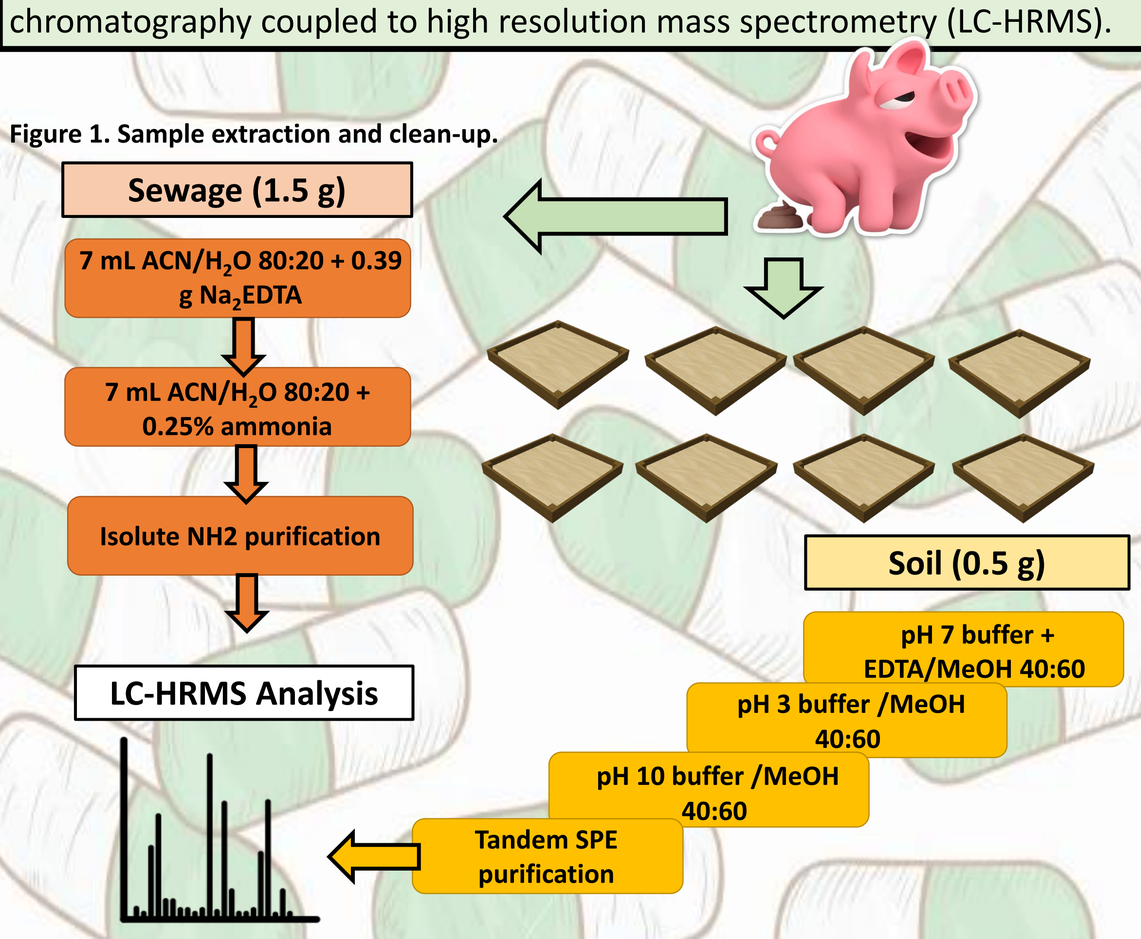
Introduction

Antimicrobial drugs are broadly used to treat diseases in livestock breedings [1]. These substances are mainly excreted via animal urine and feaces, which are often used in agriculture as soil fertilisers. Due to the growing concerns in the antibiotic resistance (AR) topic, it is important to evaluate the impact and persistence of antibiotics in the environment. The aim of this project was to quantify a wide number of drugs in pig sewage monitoring their degradation in manured soil during a nine-month period. For this purpose a multiresidue method for 58 veterinary antibiotics, belonging to ten families, was developed and validated applying liquid-chromatography coupled to high resolution mass spectrometry (LC-HRMS).

Figure 1. Sample extraction and clean-up.



Experimental

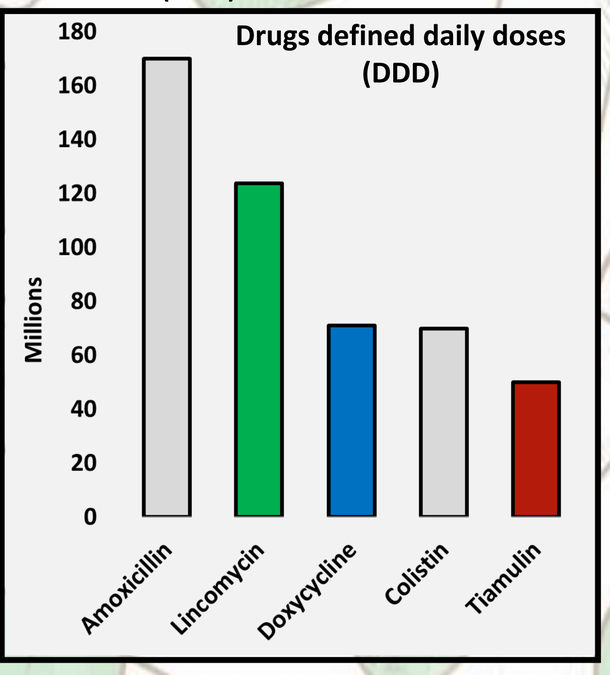
Fifty liters of pig sewage were recovered from a storage pond of a swine local farm and used to manure eight boxes containing superficial soil. At the same time four boxes containing the same (unamended) soil were prepared as control. Soil samples were collected in different days during a nine-month period (September 2019-May 2020). Sewage and soil samples were extracted and purified as summarised in Figure 1. After evaporation, the dry residues were reconstituted and injected in a Thermo Ultimate 3000 Ultra High Performance Liquid Chromatography system coupled to Q-Orbitrap analyser (Thermo Scientific, San Jose, CA, USA). Instrumental analysis was carried out in Full MS-ddMS^2 mode [2]. The collected sewage and soil samples were also tested for the presence and characterization of microbial population.

Table 1. Antibiotic concentrations in pig sewage.

Class	Analyte	Concentration (µg/kg)[b]
Sulfonamides	Sulfanilamide	29
	Sulfadimetoxhine	5
	Sulfamethazine	4
Tetracyclines	**Doxycycline**	3605
	Oxytetracycline[a]	46
	Chlortetracycline[a]	20
	Tetracycline[a]	3
Quinolones	Enrofloxacin	13
	Flumequine	9
	Ciprofloxacin	3
Lincosamides	**Lincomycin**	1196
Pleuromutilines	**Tiamulin**	369

a: Sum of epimers; b: Mean of two measurements

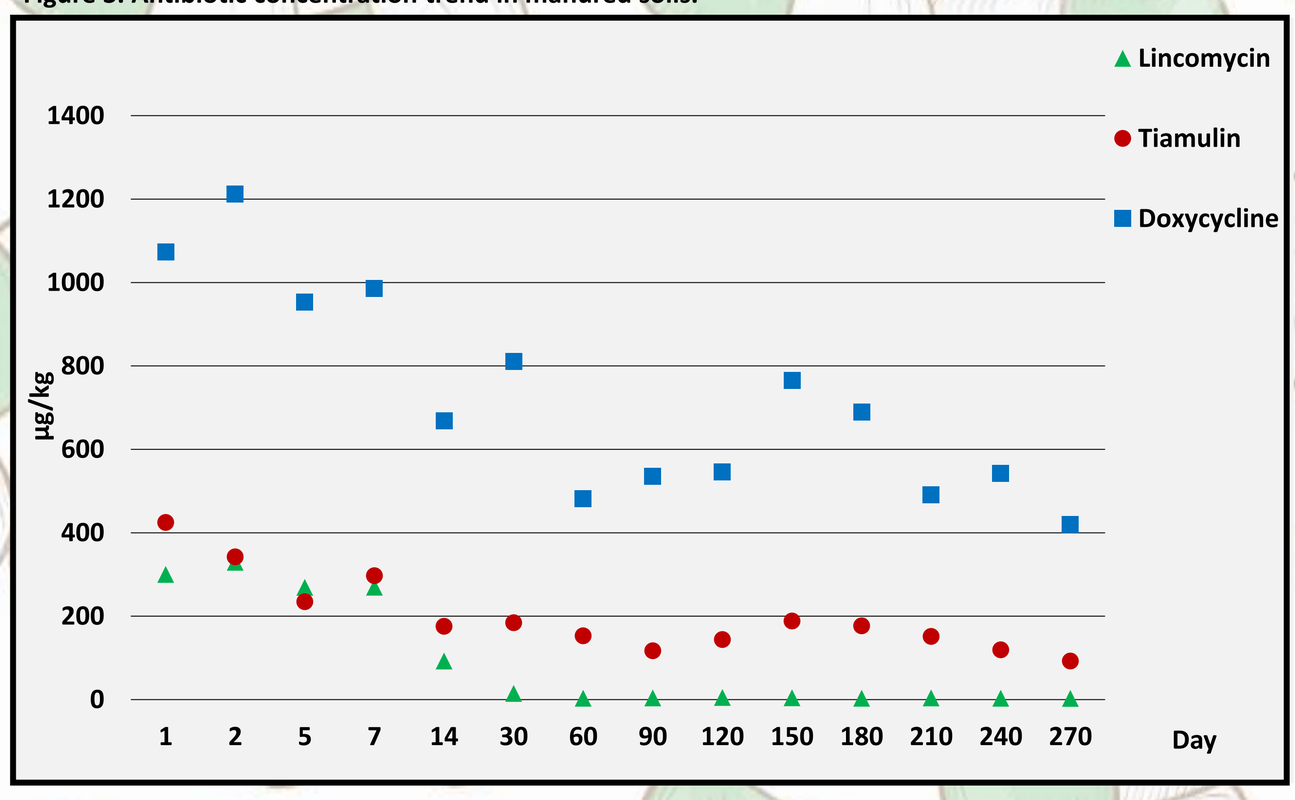
Figure 2. The most used antibiotics in Umbrian swine farms (2016).



Results and Discussion

It is worth to report the high number of veterinary antibiotics detected in pig sewage collected from a local farm (Table 1). Doxycycline, lincomycin and tiamulin were the most abundant detected drugs (from 369 to 3605 µg/kg) followed by oxytetracycline, chlortetracycline and quinolones (Table 1). Despite the large usage of sulfonamides in pig farming, sulfanilamide and sulfadimethoxine were found at concentration lower than 50 µg/kg. This observation agreed with the data of Berendsen et al. [3] who observed fast dissipation of this drug family in manure of different livestock animals (pigs, calves and broilers). Accordingly, these researchers demonstrated that tetracyclines, lincomycin and tiamulin dissipated more slowly. Among the three most abundant compounds, lincomycin decreased rapidly in soil, whereas doxycycline and tiamulin persisted with about 20 - 40% of the native compound measured after the nine-month period (Figure 3). Although amoxicillin is widely used in Umbrian swine farms (Figure 2), it was never detected because the well-known unstability of β-lactams in environmental matrices. On the other hand, colistin was not included among the searched analytes. No antibiotic residues were found in boxes with unamended soil.

Figure 3. Antibiotic concentration trend in manured soils.



Conclusions

The high levels and persistence of tetracyclines in amended soils is well documented due to their chelating properties [4]. Less data are available for tiamulin and lincomycin. Interestingly, although sulfadimethoxine and sulfadiazine have been administered in the swine farm from which manure was collected, no treatments with sulfanilamide have been recorded by the farmer. Therefore, sulfanilamide presence is not easily explainable. Since a plethora of different antimicrobials is used in pig farming, the application of multiclass methods can be useful to explore degradation/transformation pathways of the various substances supporting possible correlation among residues and AR phenomena.

Acknowledgements

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