

Environmental risk assessment of antibiotic residues in cattle slurry used as fertiliser

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Highlights

- Two-year study on the content of antibiotics in cattle slurry from organic farms.
- Literature search on risk assessment parameters ($PNEC_{soil}$) relevant to the soil compartment.
- Environmental risk analysis of soil following slurry use as fertiliser.

Abstract: The aim of the study was to assess the environmental risk caused by active antibiotic substances present in cattle slurry from ecological farms, used for fertilisation purposes. In order to assess the environmental risk, studies were carried out on farms located in the cleanest region of north-eastern Poland. The study focused on substances most commonly found in veterinary drugs: tetracycline, bacitracin, kanamycin, dihydrostreptomycin, benzylpenicillin, cefquinome, and cephalixin. In the analysed cattle slurry, only tetracycline, benzylpenicillin, and cephalixin were detected in the analysed slurry. Their concentrations were respectively 1.53–15.1, 0.048–0.27, and nd–1.11 ng·g⁻¹. Other tested antibiotics, such as cefquinome and dihydrostreptomycin, were detected in the samples of cattle slurry at concentrations below the LOQ. Bacitracin and kanamycin were not detected in any of the samples. The environmental risk assessment, based on the risk quotient (RQ) values calculated for tetracycline and benzylpenicillin, indicated that the potential risk to soil was well below the level of concern (LoC). The risk assessment for cephalixin could not be performed due to the lack of sufficient data.

The analysis indicates that the risk to the soil compartment from the application of cattle slurry originating from organic farms is below the level of concern. However, due to data gaps in the available literature, a comprehensive analysis could not be completed.

Keywords: antibiotics, benzylpenicillin, dairy cattle, slurry, soil, tetracycline

INTRODUCTION

Pharmaceuticals are biologically active substances that are used in human and veterinary medicine. While their use provides undeniable health benefits, it is essential to acknowledge the associated environmental risks. One of the most significant concerns is the release of pharmaceutical residues and their metabolites into the environment.

Particular attention should be paid to the use of drugs in veterinary medicine, which often exceeds the scale found in human medicine. One of the main pathways through which pharmaceuticals and their metabolites enter the environment is the agricultural application of manure. The circular economy promotes the use of natural animal fertilisers over synthetic alternatives (Muscat *et al.*, 2021). However, due to the limited absorption of pharmaceuticals animals' digestive system, it is estimated that up

to 90% of the applied dose may be excreted unchanged (Miciński *et al.*, 2015). As a result, the concentration of antibiotics in manure ranges from trace levels to 200 mg·kg⁻¹, with typical average values between 1 and 10 mg·kg⁻¹ (Kumar *et al.*, 2005; Dolliver, Gupta and Noll, 2008; Nightingale *et al.*, 2022).

Pharmaceuticals present in soil can enter surface waters through runoff and reach groundwater through leaching. The impact of veterinary pharmaceuticals is considered to be more significant on soil and groundwater than on surface waters (Halling-Sørensen *et al.*, 1998; Sacher *et al.*, 2001; Balizs and Hewitt, 2003; Sarmah, Meyer and Boxall, 2006; Nightingale *et al.*, 2022).

Antibiotic residues play a particularly important role, contributing to the increase in antibiotic resistance and the potential emergence of superbugs resistant to most known antibiotics (Chowdhury *et al.* 2021; Emes, Kagambèga and Dione, 2024; Massaccesi *et al.*, 2024; Yordanova, Platikanova and Hristova, 2024). According to various estimates, antibiotic-resistant bacteria are responsible for approximately 23,000–35,000 deaths annually in the United States alone (Okon *et al.*, 2023). Globally, AMR is linked to more than 700,000 deaths per year, making it one of the leading causes of mortality (Xu *et al.*, 2024). Another consequence of antibiotic residue presence is the disruption of soil microbiological functions, such as those related to the circulation of nutrients in nature, and consequently the impact on climate change. Soil microorganisms are essential for the decomposition of organic matter and the transformation of nitrogen, phosphorus, sulphur, and carbon. Antibiotic residues can kill sensitive microbial populations, reducing the diversity of soil microbiota. The resistant bacteria may not perform nutrient cycling functions as effectively as the displaced microbes. Bacteria involved in nitrogen fixation (e.g., *Rhizobium*, *Azotobacter*), nitrification (e.g., *Nitrosomonas*, *Nitrobacter*), and organic matter degradation (e.g., *Actinobacteria*, *Bacillus*) are often sensitive to antibiotics. This mechanism may also affect the nitrous oxide emissions due to the altered nitrogen cycling (Cycoń, Mrozik and Piotrowska-Seget, 2019; Fang *et al.*, 2023, Yang *et al.*, 2024). Residues of veterinary drugs have also been found in insects, e.g. black soldier fly larvae, including those that are a potential source of protein (Meyer *et al.*, 2021; Dongen van *et al.*, 2024).

Due to their wide application in veterinary medicine, tetracyclines, sulphonamides, and fluoroquinolones are listed among the most significant groups of antibacterial drugs. These substances largely accumulate in manure due to their poor metabolism in animals (Kemper *et al.*, 2008). These compounds have been detected in slurry and manure in concentrations as high as 46 mg·kg⁻¹ for chlorotetracycline, 29 mg·kg⁻¹ for oxytetracycline and 23 mg·kg⁻¹ for tetracycline (Martínez-Carballo *et al.*, 2007).

Repeated application of manure as fertiliser can result in localised contamination of agricultural soils, leading to the accumulation antibiotic residues over time. Studies investigating antibiotic concentration in various manure-amended soils reported maximum levels of sulphonamides 0.015 mg·kg⁻¹ (Christian *et al.*, 2003) and fluoroquinolones 0.37 mg·kg⁻¹ (Martínez-Carballo *et al.*, 2007; Spielmeier, Ahlborn and Hamscher, 2014; Spielmeier *et al.*, 2015; Widyasari-Mehta, Hartung and Kreuzig, 2016). In soil samples fertilised with manure in Turkey, tetracyclines were detected at a concentration of about 35.5 mg·kg⁻¹ and sulphonamides reached up to 0.50 mg·kg⁻¹ (Karcı and Balcıoğlu, 2009). Wolters *et al.* (2016) reported a maximum concentration of

tetracycline reaching 300 mg·kg⁻¹ dry matter in pig manure. Similarly, Zhao *et al.* (2010), analysing fertilisers derived from poultry waste, found enrofloxacin and norfloxacin at exceptionally high concentrations of 1,420 mg·kg⁻¹ and 225 mg·kg⁻¹, respectively.

The presence of veterinary antibiotics in fertilisers and soils has been described in many publications worldwide (Christian *et al.*, 2003; Boxall *et al.*, 2004; Hamscher *et al.*, 2005; Martínez-Carballo *et al.*, 2007; Karcı and Balcıoğlu, 2009; Hu, Zhou and Luo, 2010; Zhao, Dong and Wang, 2010; Spielmeier *et al.*, 2015). Among the most commonly detected antibiotics are sulphadimidine, tetracycline, and chlortetracycline (Wohde *et al.*, 2016).

In Poland, as in the entire European Union, the authorisation, use, and monitoring of veterinary medicinal products are governed by Regulation (EU) 2019/6. The legislation, which came into effect on 28 January 2022, aims to improve both public and animal health, while addressing the growing threat of antibiotic resistance. This regulation prohibits the prophylactic use of antibiotics in healthy animals, except in exceptional circumstances. It also imposes restrictions on the use of antibiotics that are critically important for human medicine and requires detailed monitoring and reporting of their sale and use.

According to the 2023 Statistical Yearbook for Poland (GUS, 2024), approximately 23,500 Mg of pharmaceuticals were sold, corresponding to approximately 0.6 kg per capita. A large part of these pharmaceuticals was used in veterinary medicine. In Poland, antibiotics may only be administered to cattle under the supervision of a licenced veterinarian, who determines the appropriate treatment based on clinical diagnosis. The use of antibiotics for growth promotion is prohibited, while their prophylactic use is restricted to situations where there is a high risk of infection and no alternatives are available. The cited literature as well as our own (unpublished) research indicate that the use of various types of organic waste as fertilisers may lead to elevated levels of active pharmaceutical substances in the environment. The research hypothesis assumed that the use of pharmaceuticals on organic farms is significantly lower compared to conventional systems; therefore slurry from organically raised cattle should be free of antibiotics and, as a result, pose no significant threat to the soil environment.

The aim of the study was to assess the environmental risk posed by the active antibiotic substances present in cattle slurry from organic production when applied as fertiliser.

MATERIALS AND METHODS

Samples of dairy cattle slurry were collected for analysis to determine the presence of selected active substances of veterinary antibiotics. These included tetracycline (CAS 60-54-8), bacitracin (CAS 1405-87-4), kanamycin (CAS 8063-07-8), dihydrostreptomycin (CAS 128-46-1), benzylpenicillin (CAS 61-33-6), cefquinome (CAS 84957-30-2), and cephalixin (CAS 15686-71-2). Slurry samples were collected over two consecutive years from five certified farms located in Siedlce County, Mazowieckie Province, Poland.

Five traditionally operated farms, each covering an area of 5–9 ha, were selected for the study. These farms were classified as small and medium-sized agricultural holdings. Detailed characteristics are presented in Table 1.

These farms are typical for an agricultural region where cereal crops, potatoes, and the raising of pigs and dairy cattle dominate.

Table 1. Basic information about the studied farms

Location	Number of dairy cows	Number of cattle
1	35	72
2	45	95
3	46	94
4	25	50
5	38	75

Source: own elaboration.

According to information from the attending veterinarian, dairy cows were treated with antibiotics primarily during the dry period, particularly for the treatment of mastitis and the prevention of new intramammary infections caused by bacteria. The antibiotics used during the dry-off period included Cobactan LC, Mastijet Forte, Ubrolexin, and Tetra-delta, whose active substances were analysed. Samples of slurry were taken according to the Polish standard PN-B-12098:1997. The samples were taken directly from channels with continuous self-drainage in a tied-stall animal husbandry system.

Primary slurry samples were taken at regular intervals of 5 m along the entire length of the channel to the outlet to the reservoir. The first and last sampling points were located 2–5 m from the channel outlet. Prior to that, the slurry was mixed within the channel. From each farm, between 7 and 10 primary samples were collected and combined into a single 5 dm³ polyethylene canister. After mixing, a 3 dm³ subsample was extracted for laboratory analysis.

The cattle were primarily fed with fodder produced on-site, originating from permanent grassland (meadows and pastures). During spring and summer seasons, the cattle grazed on pastures and were supplemented with concentrated feed. In the late autumn season, their diet consisted mainly with silage from the same farm, along with concentrated feed. The determination of antibiotic residues in the slurry samples was conducted using the analytical method described by Czarny *et al.* (2024).

The analysis of antibiotic active substances was performed using the LC-MS method with a Shimadzu LC-MS 8050 chromatograph, with Stationary Column/Phase: Kinetex® 2.6 µm C18 (100×4.6 mm) and mobile phase: methanol (MeOH)/0.1% HCOOH; 0.01 min – 10% MeOH; 3.00 min – 95% MeOH; 6.00 min – 95% MeOH; 7.00 min – 10% MeOH; 10.00 min – 10% MeOH. The flow rate was 0.5 cm³·min⁻¹ and the column temperature was 30°C.

Table 3. Calibration curves

Compound	MRM scan mode	Retention time (min)	Range of calibration curve (ng·g ⁻¹)	Calibration curve equation	R ²
Tetracycline	445.2–154.1	3.494	0.010–10.0	$y = 1474169x + 14592$	0.9976
Bacitracin	711.95–356.25	3.932	10.8–195.0	$y = 133205x - 89729$	0.9966
Kanamycin	485.35–324.3	1.428	11.9–91.0	$y = 5723x + 1499$	0.9985
Dihydrostreptomycin	584.35–263.20	1.474	0.60–9.56	$y = 3348826x - 78256$	0.9983
Benzylpenicillin	335.20–176.1	3.785	0.019–19.8	$y = 1617833x - 4,813$	0.9994
Cefquinome	529.3–134.15	3.119	0.024–24.0	$y = 899860x + 515$	0.9986
Cephalexin	348.0–158.05	3.423	0.40–25.1	$y = 2756055x - 19994$	0.9997

Explanations: MRM = multiple reaction monitoring, R² = determination coefficient.

Source: own elaboration.

Hydrophilic-lipophilic balanced (HLB) columns were conditioned with 6 cm³ of acetonitrile and water, while MCX columns were conditioned with 3 ml of methanol and water. A sample of slurry (40–45 cm³) was placed in a Falcon tube and centrifuged for 30 min at 12,000 rpm. The solution was decanted and extracted into the solid phase using Oasis HLB and Oasis MCX columns at the same time.

Analytes from HLB columns were extracted using a 6 cm³ mixture of acetonitrile with 5% formic acid, while 3 cm³ of a mixture containing 50% methanol and 25% ammonia was used for MCX. All extracts were enriched in a nitrogen stream at 40°C. The enriched extracts were reconstituted to a final volume of 1 cm³ with 0.1% formic acid, centrifuged and chromatographically analysed using LC/MS.

Acetonitrile with 5% formic acid was added to the precipitate left after decanting, and then shaken intensively for 5 min. Later, the mixture was centrifuged at 6,000 rpm for 10 min. The resulting solution was decanted, and the sludge was re-extracted twice. The decanted solutions precipitate were pooled and enriched in a nitrogen stream. The residue was dissolved in 1 cm³ of 0.1% formic acid. The filtered extract was chromatographically analysed using LC/MS. The total concentration of each pharmaceutical in the sample was calculated as the sum of the analyte concentrations in the liquid and solid fractions.

Validation and calibration parameters are presented in Tables 2–3.

Table 2. Validation parameters

Compound	Content		Recovery (%)	SD
	LOD (ng·g ⁻¹)	LOQ (ng·g ⁻¹)		
Tetracycline	0.002	0.008	68	1.7
Bacitracin	2.4	8.1	85	4.1
Kanamycin	2.7	8.8	86	3.4
Dihydrostreptomycin	0.15	0.49	75	4.2
Benzylpenicillin	0.005	0.016	78	2.4
Cefquinom	0.006	0.019	84	4.2
Cephalexin	0.098	0.32	64	2.1

Explanations: LOD = limit of detection, LOQ = limit of quantification, SD = standard deviation.

Source: own elaboration.

RESULTS AND DISCUSSION

The concentrations of the analysed antibiotics detected in cattle slurry samples are presented in Table 4.

Quantitative analysis of active antibiotic substances in slurry samples collected from five farms showed that tetracycline was the predominant compound, detected in all samples. Its concentrations ranged from 1.53 to 15.1 ng·g⁻¹ of slurry.

Tetracyclines are among the most commonly used classes of antibiotics approved for veterinary use. In Poland, they account for approximately 40% of total veterinary medicinal products sales and are also one of the most frequently applied antibiotic groups in medicated feed. Their primary indications in livestock include bacterial diseases of the gastrointestinal tract, respiratory system, and urogenital system, especially mastitis, as well as bacterial skin infections and systemic diseases. Additionally, Patyra *et al.* (2019) investigated the presence of tetracyclines in animal feed. Their study, conducted in Poland in 2015–2017, detected the presence of tetracycline antibiotics in 31 out of the 286 feed samples tested.

It should be assumed that the presence of tetracycline in the analysed cattle slurry results primarily from the veterinary treatment of animals, rather than from contaminated feed. On the selected farms, cattle were mainly fed with fodder produced on-site, sourced from permanent grasslands. Moreover, the surveyed farms are certified as organic and are located in one of the least industrially impacted regions of Poland.

The obtained results are consistent with global data on the use of veterinary antibiotics. Tetracyclines remain the most commonly used antimicrobials, with a reported global usage of 33,305 Mg in 2020 and predicted increase by 9% until 2030 (Mulchandani *et al.*, 2023).

The concentration of tetracycline in dairy slurry varies substantially across countries, as reported in the literature. In

Japan, the investigated range was 0–1.2 ng·g⁻¹, in the United States, it ranged from 0 to 1,200 ng·g⁻¹, and in China, concentrations ranged from 0.45 to 26,900 ng·g⁻¹ (Oliver *et al.*, 2020; Gaballah *et al.*, 2021). The results obtained in this study fall within the range reported in previous studies, placing Poland in an intermediate position globally.

After tetracycline, cephalixin was the second most frequently detected antibiotic in the cattle slurry, with concentrations up to 1.11 ng·g⁻¹. In veterinary medicine, cephalixin is used in cattle to treat metritis, intercutaneous inflammation, wound infections and abscesses, and septic mastitis.

Cephalixin, which is one of the most commonly prescribed antibiotics in veterinary medicine, has not been investigated in dairy cattle slurry to date. However, it has been detected in significant concentrations in the aquatic environment, which justifies its inclusion in this study (Lu *et al.*, 2014). The existing literature on the subject is extremely limited, despite its frequent use in the treat of cattle.

Of the seven antibiotics tested, benzylpenicillin was detected in all slurry samples at levels of 0.048–0.27 ng·g⁻¹. This antibiotic is commonly used in cattle to treat respiratory tract infections, soft tissue infections, abscesses, joint infections, endometritis, and mastitis.

Benzylpenicillin, investigated by Huygens *et al.* (2022), has not been detected in any of the analysed slurry samples.

Other antibiotics, such as cefquinome and dihydroxystreptomycin, were detected in the tested samples of cattle slurry at concentrations below the LOQ. Bacitracin and kanamycin were not detected in any of the samples.

Bacitracin content was analysed in slurry samples collected from animals treated with this antibiotic. The detected concentration was 0.78 mg·kg⁻¹ (Joy *et al.*, 2013).

The review of the literature shows that research on antibiotic residues in slurries used as natural fertilisers is

Table 4. Content of analysed antibiotics in cattle slurry

Location	Year	Content (ng·g ⁻¹)													
		Tetracycline		Bacitracin		Kanamycin		Dihydro-streptomycin		Benzyl-penicillin		Cefquinome		Cephalixin	
		value	SD	value	SD	value	SD	value	SD	value	SD	value	SD	value	SD
1	1	15.1	0.7	ND		ND		ND		0.059	0.002	<LOQ		1.03	0.01
	2	2.5	0.2	ND		ND		ND		0.214	0.003	<LOQ		0.99	0.01
2	1	2.8	0.3	ND		ND		<LOQ		0.12	0.02	<LOQ		<LOQ	
	2	2.8	0.2	ND		ND		ND		0.27	0.02	<LOQ		1.1	0.1
3	1	10.7	1.1	ND		ND		<LOQ		0.12	0.01	<LOQ		1.11	0.3
	2	2.9	0.2	ND		ND		ND		0.08	0.06	ND		ND	
4	1	5.3	0.3	ND		ND		ND		0.048	0.02	<LOQ		ND	
	2	1.6	0.1	ND		ND		<LOQ		0.079	0.06	ND		0.73	0.03
5	1	2.0	0.3	ND		ND		ND		0.053	0.07	<LOQ		ND	
	2	1.53	0.05	ND		ND		ND		0.07	0.03	ND		ND	

Explanations: SD = standard deviation, ND = not detected, LOQ = limit of quantification.

Source: own study.

currently very limited. The present study contributes valuable data to fill this knowledge gap.

An environmental risk assessment was carried out for antibiotic active substances detected in cattle slurry at concentrations above the LOQ. These substances included tetracycline, cephalixin and benzylpenicillin.

The calculations were based on the maximum legally recommended slurry application rate for nitrogen, which should not exceed 170 kg N. Therefore, an annual application of two doses of 25 m³·ha⁻¹ was assumed.

For the purposes of the analysis, a worst-case scenario approach was adopted due to the lack of reliable degradation half-life (DT₅₀) in the soil. Therefore, it was assumed that DT₅₀ values could be sufficiently high to allow accumulation of antibiotics in the soil. Consequently, a single application dose of 50 m³·ha⁻¹·year⁻¹ of slurry was used in the calculations. The following assumptions were applied: soil depth of 0.2 m, soil bulk density of 1.5 g·cm⁻³, and a slurry density of 1 Mg·m⁻³. One square meter of soil to a depth of 0.2 m weighs approximately 300 kg, which multiplied by 10.0 thous. m² equals 3,000,000 kg (or 3,000 Mg) per hectare.

To calculate the risk quotient (RQ), the predicted no-effect concentration (PNEC_{soil}) for tetracycline was assumed to be 57 µg·kg⁻¹, as reported in the literature (Lihong *et al.*, 2021). Based on this value and the highest detected tetracycline concentration of 15.1 µg·kg⁻¹ in slurry, the predicted environmental concentration (PEC_{soil}) for tetracycline was calculated as 0.025 µg·kg⁻¹. The RQ was defined as:

$$RQ = \frac{PEC_{soil}}{PNEC_{soil}} \quad (1)$$

The calculated RQ for tetracycline is 0.00044, which is well below the level of concern (LoC).

Analogously to tetracycline, the predicted no-effect concentration (PNEC_{soil}) for benzylpenicillin was also adopted from the literature, with a value of 20 mg·kg⁻¹ (Ciucă *et al.*, 2023).

For PEC_{soil} 0.00045 µg·kg⁻¹, calculated for the highest determined amount 0.27 µg·kg⁻¹, the resulting RQ is close to 0 and considered negligible. Results are presented in Table 5.

Table 5. Risk assessment for soil compartment

Substance	<i>PNEC</i> _{soil}	<i>PEC</i> _{soil}	<i>RQ</i>
	µg·kg ⁻¹		
Tetracycline	57	0.025	0.00044
Benzylpenicillin	20,000	0.00045	≈0

Explanations: PNEC_{soil} = predicted no-effect concentration, PEC_{soil} = predicted environmental concentration, RQ = risk quotient.

Source: own study.

This indicates that the application of the assumed dose of cattle slurry, i.e. 50 m³·ha⁻¹·year⁻¹, does not pose an environmental risk.

No environmental risk assessment was carried out for cephalixin due to lack of available literature data and the absence of a derived own PNEC_{soil} value.

CONCLUSIONS

The presence of antibiotics in cattle slurry has attracted significant interest among researchers and has been the focus of numerous studies. Literature analysis has shown that the use of cattle slurry as fertiliser can introduce antibiotics into the soil, potentially posing an environmental risk. However, there is a notable lack of studies focusing on organic farming. Therefore, the aim of this study was to investigate whether dairy cattle slurry from organic farms, where necessary veterinary antibiotics may still be used for therapeutic purposes, can be considered environmentally safe fertiliser. The results showed that residues of certain antibiotics used during the drying-off period were found and identified in cattle slurry. The analysis indicates that probably of environmental risk to the soil compartment from the application of cattle slurry from organic farms is likely to be below the risk level. However, due to gaps in the available literature, a comprehensive risk assessment could not be fully conducted.

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CONFLICTS OF INTEREST

All authors declare that they have no conflict of interests.

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