



Peroxide impact on the fate of veterinary drugs in fertilizers

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Abstract

The presence of veterinary medicines in organic manure causes soil contamination which contributes to increasing resistance of indigenous microflora to drugs and results in greater susceptibility of people to allergies. The main aim of the study was to assess the efficiency of inorganic peroxide mixtures (PM) with calcium peroxide content (CaO₂) in the stabilization process of manure contaminated with antiparasitic agents: albendazole (ALB) and levamisole (LEV). As a solid, CaO₂ is relatively stable against decomposition. In contact with water, however, it hydrolyzes with release of oxygen. The hydrolyzation of CaO₂ proceeds very slowly in soil, which guarantees the constant release of hydrogen peroxide that subsequently becomes the source of free radicals (chemical oxidation) and oxygen (aerobic conditions for the microbes). It may contribute to continuous elimination of drugs from manure. The study has demonstrated that there were significant differences in ALB and LEV conversion stimulated by the PM addition. PM supplementation increased the drug availability (on average 15% and 25% increase in the initial concentration for ALB and for LEV, respectively), thereby increasing the initial rate of reaction. Elimination of ALB and LEV from the manure sorption complex is followed by Ca²⁺ saturation. The initial degradation rate was affected by PM for both drugs, but the mechanisms of decomposition have been modified only for ALB. The loss of ALB in the peroxide supplemented samples was 92%, and in the samples, without the PM, it did not exceed 61%. Loss of LEV was over 90% irrespective of PM supplementation.

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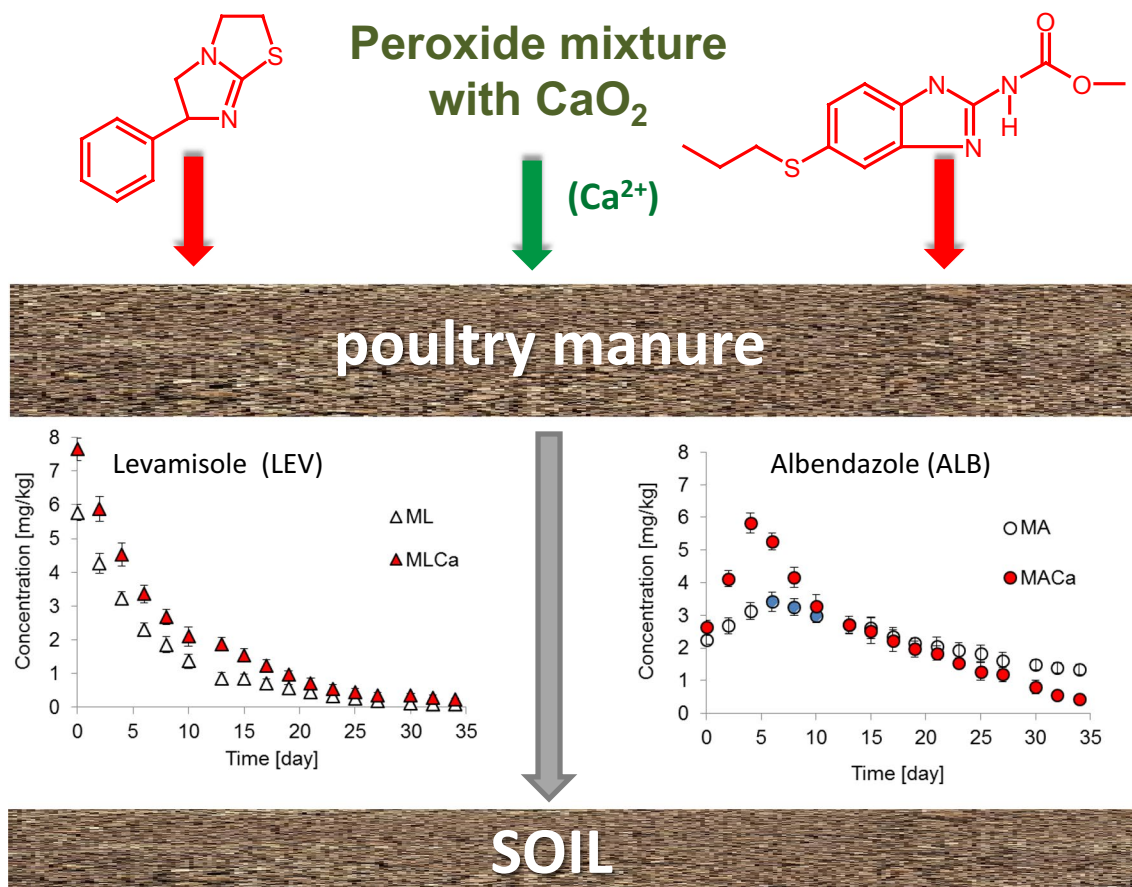
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Graphic abstract



Keywords Rate · Degradation · Albendazole · Levamisole · Calcium peroxide (CaO_2) · Poultry manure

Introduction

The use of veterinary medicines in animal husbandry increases the risk of their presence in organic fertilizers (manure and liquid manure), which results in their occurrence in soil environment (Kumar et al. 2005; Sarmah et al. 2006; Song et al. 2010). Antiviral, antifungal, and antiparasitic agents can penetrate the soil at varied rates in an unchanged or partially degraded form (Boxall et al. 2003, 2004; Capelton et al. 2006). Consequently, surface runoff from the soil and resulting water contaminated with drugs causes gradual development of drug resistance and endocrine disorders in soil micro-organisms. The build-up of drug residues in the environment makes it necessary to use drugs at higher pharmacological doses, which in effect constantly increases environmental pollution (Kools et al. 2008a; Varel et al. 2012).

The use of manure for fertilizing soils is a common agricultural technique and has been enhancing quantity

and quality of crops (Miller and Berry 2005). Polluting potential of pharmaceutical residues in soils is determined by their bioavailability, which is mostly defined by sorption, desorption, and migration with water (Hamscher et al. 2005; Popova et al. 2013). Their distribution can be influenced by a range of factors and processes, including physico-chemical properties of the environment and drugs (Jørgensen and Halling-Sørensen 2000; Chefetz et al. 2008). Drugs infiltrating into the soil with organic fertilizers influence the quality of both the soil and the crop (Davis et al. 2006; Oliver and Gregory 2015). The negative environmental impact is mainly due to organisms' ability to immobilize and accumulate medicines in their tissues (Boxall et al. 2003; Kumar et al. 2005; Marciocha et al. 2013).

Albendazole (ALB) is an antiparasitic drug with a broad activity range. It is a benzimidazole derivative and is used against roundworms and some flatworms (Dayan 2003; Prasad et al. 2010). The mechanism of action is

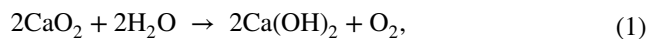
based on inhibition of specific enzymes (e.g., tubulin polymerase and fumarate reductase). ALB is eliminated from the organism mainly with urine as albendazole sulfoxide (Capece et al. 2009; Bartikova et al. 2011).

Levamisole (LEV) is used as a selective agent for the control of nematodes in the treatment of parasitic infections in humans and animals (Kamal et al. 2005). It is mainly applicable as a deworming agent designed to fight gastrointestinal and pulmonary nematodes in poultry, pigs, and ruminants (Grønvold et al. 2004). It also shows anti-cancer and immunomodulation activity (Sadeghi et al. 2007). From the body, LEV is mainly excreted in an unchanged form in the urine. Metabolites constitute about 20% of LEV (Barker 2008; Kools et al. 2008b).

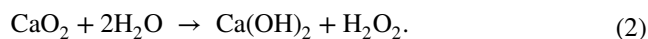
ALB and LEV were selected for investigations in this study due to their high consumption as veterinary medication, unknown metabolic paths, and migration in soil, as well as evidence in scientific literature of high concentration in the environment (Capleton et al. 2006; Spychaj-Fabisiak et al. 2007; Kools et al. 2008a). The environmental risk of these drugs has been assessed in this study, because predicted environmental concentration (PEC) for both selected drugs was in excess of 100 µg/kg. At such PEC, according to the European Medicines Agency (EMA) guidelines for ALB and LEV, the process of assessing the environmental risk should be performed (EMA 2007; Oliver and Gregory 2015). The average concentration of ALB found in soil and sediment samples was determined in the range 0.2–25 mg/kg (Thiele-Bruhn 2003). Studies showed that the environmental concentrations of pharmaceuticals are in the range of 0.034 µg/kg to 500 mg/kg (Thiele-Bruhn 2003; Babić and Mutavdžić Pavlović 2013; Wohde et al. 2016; Mutavdžić Pavlović et al. 2018). Calcium peroxide (CaO₂), a component of inorganic peroxide mixtures (PM), is a source of oxygen (aerobic condition for autochthonic microflora) and free radicals (chemical interaction) in the environment. Free radicals, especially hydroxyl radicals, have been reported to have strong chemical activity (2.8 V) which leads to a non-selective degradation of both persistent organic pollutants (POPs) and organic substances (Biń and Zieliński 2000; Liu et al. 2014; Miksch et al. 2015).

Low solubility of PM results in gradual secretion of molecular oxygen and free radicals to achieve slow yet complete dissolution (Walawska and Gluzińska 2006). Substrate generation is at its maximum in the first 7 days after introduction PM to the soil environment and then slowly declines, but usually takes about 30 days (Walawska et al. 2007; Romero et al. 2011). The disintegration rate depends on the pH and increases together with the increase of soil moisture content. Once the PM is introduced into the soil, the released oxygen improves aerobic environmental conditions and stimulates the activity of indigenous microflora, which gives rise to the rate of the biological pollutants

degradation. Due to low PM water solubility, the oxidation process occurs slowly and results in the slow and continuous formation of hydrogen peroxide (reactions 1, 2):



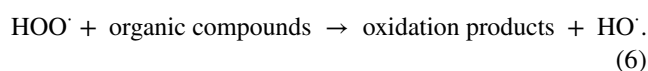
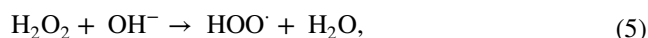
or



In turn, H₂O₂ molecules are the source of free radicals (chemical oxidation) and oxygen (due to aerobic conditions for micro-organisms and biological oxidation) (reactions 3, 4):



Hydroxyl radicals can react with organic contaminants reactions 5 and 6 (Deng and Zhao 2015; Wang et al. 2016):



During the degradation of PM and H₂O₂, reactive oxygen species (ROS) are produced, which is the source of oxygen for micro-organisms taking an active part in the biodegradation of contaminants and at the same time being the source of free radicals capable of oxidizing organic contaminants. However, it has been shown that the presence of ROS in low concentrations is essential for the proper course of many physiological processes. The apparent paradox is defining the role of ROS as a transformation regulator of the compounds. On the other hand, the toxicity of the products is mainly dependent on their concentration.

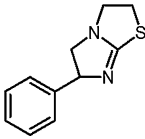
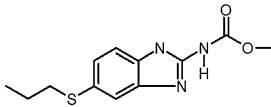
As a solid substance, the PM-containing CaO₂ is far less problematic and safer in handling than hydrogen peroxide solutions (Solvay Chemicals and Inc 2013; Malachowska-Jutysz and Neisler 2015; Wang et al. 2016). The oxidizing potential of ROS generated from CaO₂ is summarized in Table 1.

ALB and LEV as pharmaceutical products are among the “emerging compounds”. Contaminants of emerging concern (CECs) are increasingly being detected at low levels in the environment, and there is a concern that these compounds may have a negative impact on living organisms. Horvat

Table 1 The oxidizing potential of ROS (Pera-Titus et al. 2004; Deng and Zhao 2015)

Substance	Oxidizing potential (V)
OH [·]	2.80
	1.23 (pH < 7)
	0.4 (pH > 7)
O ₂	2.42
H ₂ O ₂	1.77

Table 2 Physico-chemical properties of veterinary drugs

Active substance	Levamisole hydrochloride LEV	Albendazole ALB
Systematic name (by IUPAC)	(6 <i>S</i>)-6-phenyl-2 <i>H</i> ,3 <i>H</i> ,5 <i>H</i> ,6 <i>H</i> -imidazo[2,1- <i>b</i>][1,3]thiazole ^a	Methyl <i>N</i> -(6-propylsulfanyl-1 <i>H</i> -benzimidazol-2-yl) carbamate ^c
Brand names	Levamisol, Ergamisol, Ketrax, Tetramisol, Wormicid, Decaris ^f	Valbazen, Albendazole, Zentel, Bilutac, Eskazole ^c
Molecular formula	C ₁₁ H ₁₂ N ₂ S ^a	C ₁₂ H ₁₅ N ₃ O ₂ S ^d
Structural formula		
Molar mass	204.291 g/mol ^a	265.3314 g/mol ^d
Half life	4.4–5.6 h ^a	8–12 h ^d
Melting point	264–265 °C ^a	208–210 °C ^d
Solubility in water	1.44 g/l ^a 1.116 g/l (25 °C) ^b	2.28e–02 g/l ^d 0.010 g/l (25 °C) ^b
Octanol–water partition coefficient Log <i>K</i> _{OW}	1.84 ^c 2.87 ^b	3.14 ^e 3.07 ^b
Organic carbon normalized sorption coefficient Log <i>K</i> _{OC}	1.88 ^b	2.94 ^b
Toxicity	LD ₅₀ = 40 mg/kg (pig, subcutaneously) LD ₅₀ = 180 mg/kg (rat, orally) ^a	LD ₅₀ = 2.400 mg/kg (rat, orally) ^c

^a<http://www.drugbank.ca/drugs/DB00848>^bHorvat et al. (2012)^cBoxall et al. (2006)^d<http://www.drugbank.ca/drugs/DB00518>^e<http://www.chemicalland21.com/>^f<https://pubchem.ncbi.nlm.nih.gov/compound/Levamisole#section=Synonyms>

et al. present the analysis, occurrence, and fate of anthelmintics and their transformation product including ALB and LEV in the environment. Their wide range of applications in the protection of livestock health and prevention of diseases and equally widespread practice of managing organic fertilizer justifies carrying out research on the fate of drugs in the soil environment. The key aim of this research was to determine the influence of PM on the fate of veterinary drugs from the group of antiparasitic agents (ALB and LEV). Second, the study was aimed at developing an alternative bioremediation method to increase the efficiency of manure stabilization by the addition of PM-containing CaO₂.

The degradation of pharmaceuticals have been responsible for the generation of many transformation products (TP) (Boxall et al. 2003; Sadeghi et al. 2007; Capece et al. 2009; Prasad et al. 2010; Horvat et al. 2012; Oyeduntan and Uwalaka 2015; Technical Evaluation Report 2015; Mutavdžić Pavlović et al. 2018).

The presented research justifies the desirability of combining chemical and biological processes that would lead to the disappearance of drugs in poultry manure before their deposition into the soil.

Materials and methods

The selected properties of studied drugs ALB and LEV are summarized in Table 2. Both tested drugs were purchased from Sigma-Aldrich, LEV CAS Number 16595-80-5 and ALB CAS Number 54965-21-8. The experiment used a solvent Sigma-Aldrich (methanol, dimethyl sulfoxide—DMSO, and acetonitrile) and chemical reagents purchased from POCH (K₂HPO₄, NaHCO₃, and Na₂CO₃).

In the experiments, natural chicken manure (veterinary ID 30 17 84 01) produced by PPHU CDN Poland was used. The manure properties are summarized in Table 3. The manure was activated using water that was dosed to obtain 60% of full manure water capacity, and then incubated for 7 days to keep the constant level of respiration activity. Manure activity was assessed by respiration activity SIR method determined by OxiTop[®] Control System developed by WTW. Biodegradability tests were carried out in accordance with the method DIN EN 29 408/ISO 9408/OECD 301 F.

The initial concentration of both drugs in manure was 45 mg/kg. This dose was selected to detect for a large margin above the analytical detection limit and allow for long

Table 3 Physico-chemical properties of the manure

Parameter	Value
pH	11.6
Buffering	171.6 mmol H ⁺ /kg _{d.m.}
ADW (absolutely dry weight)	62%
N _{Kjeldahl}	48.39 g/kg
Organic substance	87.2%
Organic carbon	13.2%
Hh (hydrolytic acidity)	52.9 mmol H ⁺ /kg
S (alkalis sum)	705.1 mmol H ⁺ /kg
T (sorption capacity of the fertilizer)	758.0 mmol H ⁺ /kg

reaction time to compare varied test conditions. The tested manure (10 kg dry mass, d.m.) was thoroughly mixed with ALB and LEV (45 mg of each the drug/kg_{d.m.}). ALB is insoluble in water; therefore, DMSO was used as a co-solvent for homogeneity samples with this drug. LEV is water-soluble and distilled water was used to obtain solutions at tested concentrations.

The PM dose was calculated to raise the pH of the manure by one unit assuming manure's buffering capacity of 0.172 mol H⁺/kg_{d.m.} (Table 3). The total dose of PM was 0.5 g per 1 kg of manure dry mass. This dosage of PM stimulated chemical changes by the addition of active oxygen produced from CaO₂. PM is strongly alkaline and using larger amounts might significantly change the pH and abrupt pH changes could affect the homeostasis of the environment or distress indigenous microflora by acting toxic (Turek-Szytow et al. 2015; Małachowska-Jutysz and Neisler 2015).

The research included the following test series: MA—manure with ALB, MACa—manure with ALB and PM, ML—manure with LEV, and MLCa—manure with LEV and PM.

During the experiment, the moisture level in all samples was maintained at the level of 60% fwc (full water capacity). To determine the removal rate of the drugs, the concentrations of ALB and LEV were tested on every second day for the total period of 35 days. All test series underwent three replicate tests to assure statistical significance of the results.

The influence of PM on the loss of ALB and LEV in the manure

Determination of ALB and LEV was carried out with use of the HPLC method applying the RP-18 chromatography column, length 250 mm, which was preceded by the RP-18 Hypersil Gold pre-column, length 25 mm, manufactured by Thermo Fisher. The system was provided with the UVD 340U detector by Gynkotek, the ASI-100 autosampler by Dionex, and the P 580 LPG pump by Gynkotek. The results

obtained were processed using the Chromilion software package.

To analyse tested samples for the LEV content, 1 g of the homogenized material was collected and underwent a 3-h extraction process with 10 ml of methanol (MeOH). In the ALB analyses, 1 g of the homogenized sample underwent several steps of the extraction process using firstly distilled water (200 ml for 1 h) and chloroform (20 ml for 1 h), and then a 3-h extraction process using 1-ml DMSO solution. The samples for both LEV and ALB were filtered using a glass 0.2- μ m fiber filter. Acetonitrile and phosphate buffer (15-mmol K₂HPO₄, pH 3.8) were the eluents; the flow through the column was maintained at 1 ml/min. The method recovery reached 99.6% and 89.7% for ALB and LEV, respectively. To determine the concentration of veterinary pharmaceuticals in the manure, the five-point analytic curve was plotted in the range between 0.20 and 10 mg/l. The linear affinity was 99.987 and 99.973 for LEV and ALB, respectively. It was assumed that both compounds the limit of quantification (LOQ) correspond to the first point of the calibration curve with the lowest concentration (LOQ=0.20 mg/l). Limit-of-detection (LOD) level was 0.02 mg/l.

ALB was determined using a mixture of acetonitrile and phosphate buffer at a 50:50 ratio in the mobile phase. The spectra were recorded at a wavelength of 292 nm. The analysis was conducted for 8 min, whereas the time of ALB retention was about 6.5 min.

The LEV concentration was determined using the eluent mixture at a ratio of 85:15 (buffer: acetonitrile) changeable flow gradient. The multi-gradient flow of an isocratic (variable) and ramp (constant) was used. Flow changes were as follows: (1) isocratic, i.e., decreasing from 1 to 0.4 ml/min in the time interval 4–7 min, (2) isocratic, i.e., increasing from 0.4 to 1 ml/min for the period 10–13 min, and (3) ramp, i.e., 1 ml/min at intervals of 1–4 min and 13–19.5 min and a constant 0.4 ml/min in the period 7–10 min. The analytical detection wavelength was 217 nm, and the total analysis duration was 19.5 min. The retention time for LEV was estimated at 13.8 min.

The kinetic model was used to analyse drug loss rates:

$$C(t) = C_0(e^{-k_1 t}),$$

where $C(t)$ is the concentration in the function of time, C_0 is the initial concentration, and k_1 is the kinetic constant.

The value of the constant k was also calculated by the following:

$$k = 1/t \ln [C_0/(C_0 - C)] = 2303/t * \log[C_0/C_0 - C].$$

In this way, it was possible to check whether the given reaction is the reaction of the first order.

The effect of PM on the availability of drugs was investigated. The relationship between the highest identified

concentration of drugs and Co was determined. These assessments were based on determining the correlation coefficient “*R*” Pearson’s between initial concentration rates of reaction for the ALB and LEV with and without PM addition. The value of “*a*” coefficient and statistical significance analyses were calculated using the REGLIN function in Microsoft Excel Software package.

The value of “*a*” coefficient and statistical significance analyses were calculated using the REGLIN function in Microsoft Excel Software package. Duncan’s test (program XLSTAT) was used for the statistical analysis of all results. Both Duncan’s test and REGLIN function methods were performed with 5% error margin.

The influence of PM on the physico-chemical properties of manure contaminated with veterinary drugs ALB and LEV

Physico-chemical parameters were measured at two intervals, namely at the start day (i.e., day 0) and the experiment end (day 35). Based on previous studies (Walawska and Gluzińska 2006; Małachowska-Jutcz and Neisler 2015), 35 days of test duration was chosen as adequate to assure stabilization of chemical reactions related to sorption and desorption, and biochemical activity associated with peroxide decomposition. Each sample was homogenized prior to the analysis by grinding in a Testchem’s mill for 3 min.

The following parameters were measured and analysed at selected time intervals:

- organic substance and absolute dry mass—using the gravimetric method,
- pH—using potentiometric method,
- Kjeldahl *N*—using Kjeltac 8200 system,
- organic carbon—using Turin’s method,
- nitrite and nitrate nitrogen concentration—were determined in soil solution (PN-ISO 14255:2001) using ion chromatography (IC) by the Dionex system ICS-900, with conductivity detector DS5, applying Rfic TM IonPac® AG22, length 2 × 50 mm Guard column. The results were analysed using the Chromilion software package. The eluent was used to the analysis, and it contained 4.5 mM Na₂CO₃ and 1.4 mM NaHCO₃; applied flow was 0.25 ml/min. The retention time for nitrite ions was 6.7 min, while that for nitrate ions was equal to 9 min.

Results and discussion

The application of PM as a chemical oxidant in manure can contribute to changes in physico-chemical properties of both the selected substances (ALB, LEV) and the manure itself.

The gradual progression of the reaction makes the PM an ideal stimulant of aerobic processes in manure. The manure stabilization lasts usually up to 35 days and occurs in the period between its removal from the breeding room to the release onto the soil. Therefore, the tests conducted as part of this research lasted for 35 days and corresponded to the most intense PM decomposition process. Apart from influence of PM on the dissolution velocity of drugs, the effectiveness and their removal are linked with soil solution–manure split factor and solubility constants for all reaction intermediates.

The initial concentration of LEV and ALB was twice as high of magnitude above the determined average concentration in the environment. It was the simulation of events when the drugs are applied to all animals on the farm and due to the precision of the analytical methods. At the same time, the results of the study at such a high initial concentration of pharmaceuticals (45 mg/kg manure) can be used for intervention cases, such as those when the fertilizer originate from excrements of treated animals should be stabilized. The analysis showed the desirability of the use of PM in such cases. We found that the positive effect obtained for ALB, which is slightly soluble in water and has a high coefficient of *K_{ow}* (Table 2). The use of PM significantly increases the desorption of this compound and its removal. In contrast, LEV is well soluble in water, which has a low *K_{ow}* (Table 2), and the use of PM, in this case, increases desorption.

Probably, the kinetics described in high concentrations may occur in the environment due to the consumption of ALB and LEV. Benzimidazoles (among them ALB) are introduced into the environment when they are excreted by animals treated with this compound. It is expected that 100% of the prescribed dose is excreted within 7 days. For cattle farms of ten animals per acre, it is conventionally recommended to treat the animals three times a year using approximately 3.5 g of benzimidazoles per animal per treatment. The amount of benzimidazole excreted onto 1 acre is about 110 g/year. Because the benzimidazoles bind to humic material, they are not expected to run off into aquatic environments (Technical Evaluation Report 2015).

The influence of PM (with CaO₂) on the physico-chemical properties of manure contaminated with veterinary drugs

The test result analysis presented in Fig. 1 has demonstrated that for samples at day 35, with and without PM addition, the determined physical–chemical parameters of manure are not significantly different (taking variation coefficients into account).

The basic parameter of soil fertility is the pH; it determines the organic and mineral transformations, conversion of humic substances, and availability of nutrients and

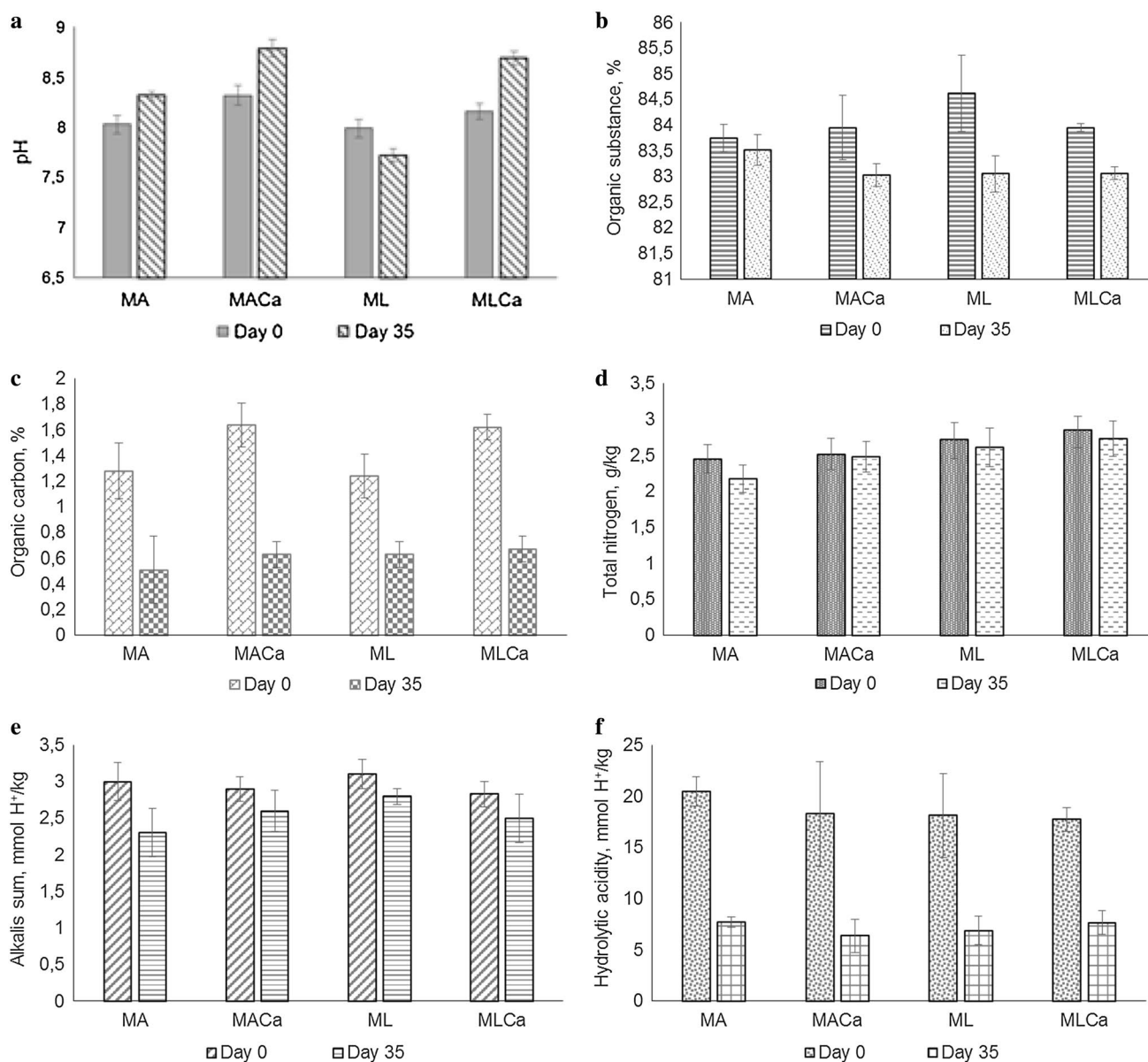


Fig. 1 Change of chosen physico-chemical parameters of manure contaminated with LEV and ALB over time under influence of PM; **a** pH, **b** organic substances, **c** organic carbon (%), **d** total nitrogen (g/kg), **e** alkalinity (mmol H⁺/kg), and **f** hydrolytic acidity (mmol H⁺/kg)

toxic components. Based on stoichiometric calculations, a calcium peroxide dose of 0.5-g PM per 1 kg of manure dry mass was determined; this dose was responsible for the increase of pH by one pH unit. It was determined that such dosage of the PM should not significantly influence important biological processes in the manure. Hence, it would not be toxic to the micro-organisms that live in the manure, but it would accelerate the aerobic processes of biological transformations and degradation of the studied drugs (LEV and ALB) due to better oxygenation of the environment. At day 35, pH values were lower than calculated based on theoretical assumptions. In the ALB

samples, an increase of pH was about 0.3 units, and in the case of LEV samples, it was 0.17 units. Most probably, the buffering capacity of the manure caused such a result.

The obtained results indicate a beneficial effect of the PM on the loss of the studied veterinary drugs (ALB and LEV) during manure stabilization, without a negative influence on the physico-chemical parameters characterising organic matter transformation (Fig. 1).

Neutralization of distinctive, unpleasant odor released during stabilization of organic fertilizers can be an additional benefit of the PM addition process (Walawska et al. 2007).

The influence of PM (with CaO₂) on the LEV and ALB detection

During the fertilizer stabilization process, the concentrations of drugs were measured. The initial concentration (day 0, time 0) was measured to be much lower than the introduced dose of 45 mg/kg_{d.m.}. Drug concentration did not exceed 7.66-mg LEV/kg_{d.m.} and 2.65-mg ALB/kg_{d.m.} in the PM samples. Samples without PM contained 5.76-mg LEV/kg_{d.m.} and 2.25-mg ALB/kg_{d.m.} (Fig. 2). At the start of the experiment, higher drug concentration was determined in the series consisting of PM with ALB and LEV than in the series without the PM addition. This could have been related to the separation of the compounds from the manure sorption complex due to saturation with calcium (Ca²⁺) ions, which resulted in the compounds being more available and detected in higher concentrations. The PM addition resulted in an increase of the solution ionic strength. Consequently, it affected the liquid-manure partition coefficient and increased the solubility of drugs. The percentage loss of drugs was calculated as a difference in the concentration at day 0 and day 35, and accounted for sorption and PM activity. The net rate of drug loss was 95%, 94%, 87%, and 83% for samples MA, MACa, ML, and MLCa, respectively. Due to high removal rates

achieved, it has been assumed that a dominant part of the drugs loss is associated with sorption. However, the sorption process promotes accumulation (Kümmerer 2008), which in effect only delays the adverse impact of drugs as biologically active substances still persist in the environment (Halling-Sørensen et al. 1998).

The sorption mechanisms and kinetic properties of ALB were investigated through sorption equilibrium and sorption rate experiments in the range of concentrations between 1- and 20-mg/kg soil/sediments (Mutavdžić Pavlovic et al. 2018). This study demonstrates that the ALB sorption affinity is strongly governed by physico-chemical characteristics of the sediment matrix (soil, sediment) as well as physico-chemical characteristic of ALB. The benzimidazoles are generally insoluble in water, sticks to humic material in terrestrial and aquatic environments, and are readily photodegradable (Horvat et al. 2012).

Levamisole is highly soluble in water and can runoff into the aquatic environment. It may decompose non-enzymatically. Depending on the temperature and pH, its decomposition results in information of one of three degradation products (Technical Evaluation Report 2015). One of the products shows the immunomodulatory activity.

The presence of pharmaceutical compounds beyond the sorption complex, e.g., in the soil water, increases the likelihood of their loss as a result of biological and chemical reactions. Based on the obtained results, differences in physico-chemical properties of both drugs were observed. The ALB reached a maximum concentration in the studied solution much later than the LEV. The highest concentration of LEV in manure was observed at the start of the experiment in MLCa samples (with PM) and ML samples (without PM); whereas, in ALB tests, the concentration was the highest on the 4th day in MACa samples (5.83-mg ALB/kg_{d.m.}) and on the 6th day in the MA series (3.42-mg ALB/kg_{d.m.}). The PM addition increased the ALB release to the environment. In the series with added PM, the ALB concentration increased twofold between day 0 and day 4, and then, it was gradually decreased (Fig. 2). The manure sorption complex saturation with calcium ions was significantly slower for ALB than for LEV, which suggests lower ALB mobility in relation to the LEV. Until day 4, the increase of ALB concentration was attributed to two consecutive processes: desorption and degradation. From day 6 onwards, a decrease in ALB concentration was observed, which implies that the degradation process occurred at a faster rate than the desorption process.

Desorption is of great importance for removal processes. The presence of substances in the solution increases their availability and thus increases the possibility of degradation, both chemical and biological.

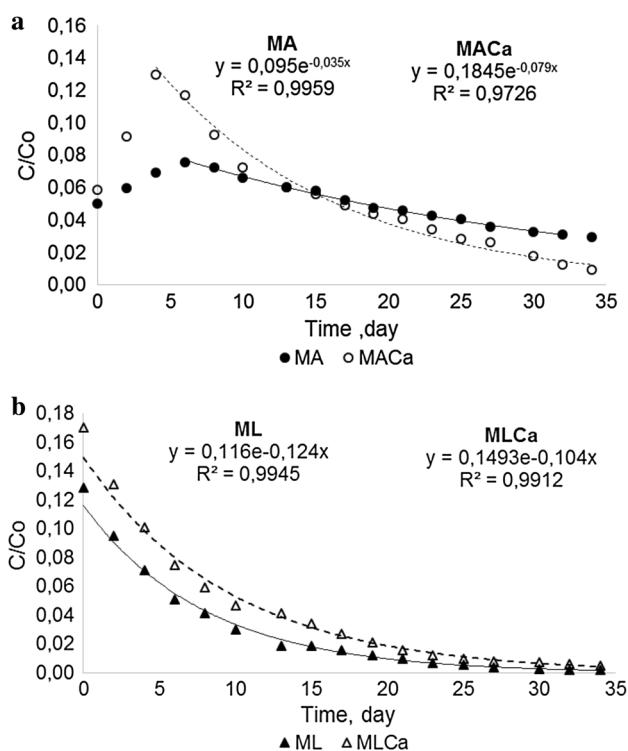


Fig. 2 The disappearance of pharmaceuticals (C/C_0) over time and fitting the kinetic equation to the disappearance of pharmaceuticals for **a** ALB (MA series without PM, MACa series with PM) and **b** LEV (ML series without PM, MLCa series with PM)

Loss of drugs during manure stabilization

During the experiment, a reduction in the ALB content over 35 days was found in all series (Fig. 2). The initial ALB concentration was 2.25 mg/kg_{d.m.} of manure for the MA series without the PM and 2.65 mg/kg_{d.m.} of manure for the MACa series with the PM. The ALB loss in the series with peroxide addition (MACa) was 84% (0.43-mg/kg_{d.m.} on day 35). In the MA series, the ALB removal did not exceed 41% (1.36-mg/kg_{d.m.} on day 35), which was three times less compared to the MACa series.

The effect of PM on drug loss was calculated taking into account the highest determined concentrations of drugs (time 0 for ML and MLCa, day 4 for MACa, and day 6 for MA). Estimated 92% loss of ALB in PM series in comparison to 61% loss in non PM series is an evidence of a measurable effect of PM.

In the LEV case, the beneficial effect of PM used was less prominent. This study has shown a 1.4% difference in LEV removal (losses 98.4% for series ML and 97.0% for series MLCa). It could be assumed that in the case of this drug, the use of PM cannot be justified. The LEV loss exceeding 97% was obtained in both ML and MLCa series. However, given that the drug dose was the same (45 mg/kg_{d.m.}) in samples with and without the PM, the observed amount of LEV at time 0 was significantly below the dose (7.66 mg/kg_{d.m.} in samples with PM and 5.76 mg/kg_{d.m.} in samples without PM). Availability of LEV was 25% higher when the PM was added. These results indicate a supportive role of the PM (with CaO₂) in the capacity to remove LEV from manure.

Time series of drug concentrations over time enabled to establish tangents to the initial losses of drugs from the equation $y = ax + b$ (Fig. 3). Based on the determined slope (inclination angle $a = -\text{tg}\alpha$) of the tangents, initial reaction rates were compared. Based on the calculated rates, the influence of PM on the loss of LEV and ALB was determined.

For both ALB and LEV, the PM (with CaO₂) addition resulted in changes of the initial degradation rate. Negative α tangents were 0.0985 for ALB and 0.137 for LEV, whereas for the samples without PM, these values were lower for ALB ($\text{tg}\alpha = 0.034$) and higher for LEV ($\text{tg}\alpha = 0.152$). The above values confirm the augmenting influence of the PM on the loss of ALB and LEV in manure.

The obtained results suggest a stronger PM influence on the degradation of a hydrophobic substance (ALB), as the rate of drug loss (value of $\text{tg}\alpha$) was near twofold higher in comparison with the sample without the PM addition. In the case of a substance with mainly hydrophilic properties (LEV), the change of $\text{tg}\alpha$ value was not statistically significant (coefficient of variation was 7% between ML and MLCa).

The k_F constant as k_{LEV} for LEV and k_{ALB} for ALB was calculated according to Eq. (2). The resulting

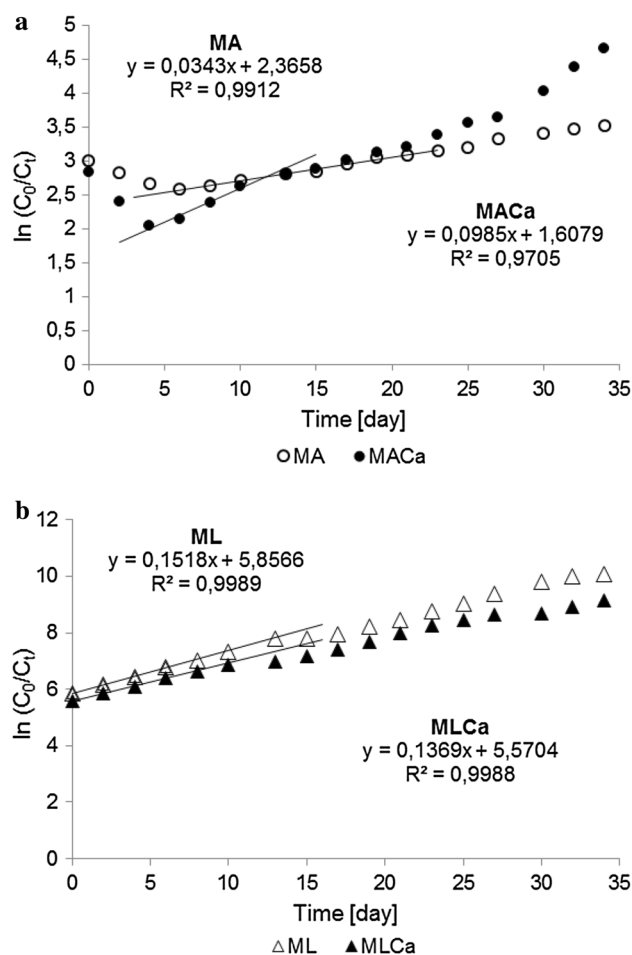


Fig. 3 Determination of the initial reaction rate for **a** ALB (MA series without PM and MACa series with PM) and **b** LEV (ML series without PM and MLCa series with PM)

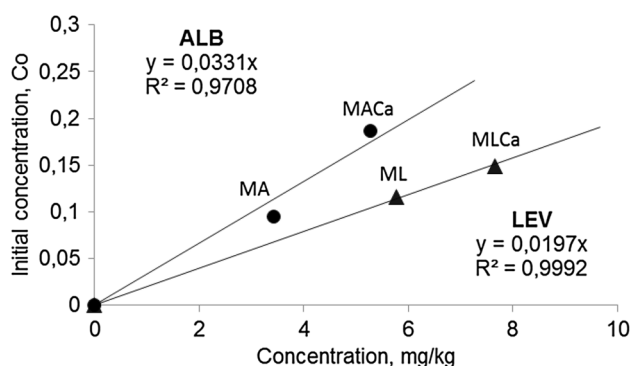
values of k_{ALB} were variable and did not show any trend with its concentration. In contrast, k_{LEV} in the range of 4–21 days was 143.5 day⁻¹ and 86.7 day⁻¹ for ML for MLCa, respectively.

To demonstrate the impact of PM on ALB and LEV conversions in poultry manure, the influence of determined initial concentration (C_0) as a change in “ a ” coefficient values in the formula $y = ax + b$ was assessed (at confidence level 95% and critical value $t\alpha = 3.1824$) (Table 4). Statistically significant differences in conversions of ALB and LEV influenced by the addition and absence of inorganic PM were observed.

Further investigations focused on assessing the impact of PM on the reaction rates. Linear correlation of initial conversion rates separately for the samples with and without PM was also tested by determining Pearson’s “ R ” coefficient. It was assumed that in the absence of drugs, the reaction rate would be 0, and hence, the intersection was at point 0 (Fig. 4).

Table 4 Value of “a” coefficient

Samples	MA	MACa	ML	MLCa
Value of “a” coefficient	3.430E-02	9.851E-02	15.18E-02	13.69E-02
SD	0.228E-02	0.121E-02	0.349E-02	0.336E-02
Confidence limit				
Δ	0.363E-02	1.930E-02	0.556E-02	0.537E-02
Min	3.067E-02	7.920E-02	14.62E-02	13.151E-02
Max	3.793E-02	11.782E-02	15.733E-02	14.221E-02

**Fig. 4** Correlation of initial concentration in reaction C_0 for drugs (MA and ML) and drugs with PM addition (MACa and MLCa)

The effect of PM on drug conversion factors was verified by drawing a linear correlation between the highest identified concentration in the MA and MACa and ML and MLCa samples and the designated C_0 (Fig. 4).

The effect of PM on drug conversion factors was verified by drawing a linear correlation between the highest identified concentration in the MA and MACa and ML and MLCa samples and the designated C_0 .

The PM influence on drug conversion rates was verified by drawing linear correlation between initial concentration in samples MA and MACa and ML and MLCa. Pearson’s “R” coefficient was 0.9708 for ALB and 0.997 for LEV. The linear relationship of initial rates was obtained for LEV, which suggests no impact of PM on the reaction mechanism. The addition of PM increased the availability of the drug causing an increase in initial concentration and thus an increase in the initial rate of the reaction. In the ALB case, no linear relationship was found. For that reason, it can be confirmed that ALB decomposition has occurred in PM’s presence. It can be explained by biological and chemical transformations occurred during ALB decomposition. In the case of LEV, however, the environment was oxygenated, and the activity of micro-organisms stimulated, intensifying biological transformations.

The Duncan analysis of the differences between the categories with a confidence limit of 95% demonstrated significant statistical differences with the PM supplementation for ALB from day 0 to day 10 and from day 30 to day 35. The influence of PM on the loss of LEV occurred from day 1 to day 21 and then ceased; no statistically significant differences were observed between samples of ML and MLCa.

In complex test media, such as poultry manure, there are several simultaneous, multidirectional, and alternating processes of decay that consist of sorption, desorption, degradation, complexation, complexes disintegration, etc. It is difficult to specify direct and unambiguous causes of changes in concentration of a single pharmaceutical in such conditions (Gomez-Hermosillo et al. 2006). In the natural environment, pharmaceuticals can be degraded thanks to abiotic processes such as photodegradation, phototransformation, photolysis, hydrolysis, or thermolysis. These processes may occur separately or simultaneously depending on the conditions (Werner et al. 2006). However, they are of little importance in matrices as soil and fertilizer.

Conclusions

The study has demonstrated that physico-chemical properties of the tested drugs influenced the degree of drug elimination in the studied environment. Lower LEV sorption in manure and hence a higher availability of the pharmaceutical resulted in higher elimination than in the ALB case.

The introduction of PM (with CaO_2) proved to be more beneficial in the case of ALB elimination, as compared to the LEV elimination. Not only did the PM addition increase the availability of drugs, but it also had a beneficial impact on the elimination rate.

High sorption of ALB and LEV in manure and enriched soil causes their continuous presence in the environment. The PM liming effect gives rise to greater loss of pharmaceutical substances (92% for ALB). Drugs’ availability increased by 15% for MACa samples when compared to the MA series, and by 25% for MLCa samples in relation to the ML series. It can be concluded that the PM addition reduces the risk of accumulation of drugs classified as persistent organic pollutants (POP) in manure used as an organic fertilizer for soil enrichment. Studies on the use of peroxide substances can lead to the development of alternative strategy for the stabilization of organic fertilizers to limit the risk of veterinary drug migration into the soil environment.

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