Slov Vet Res 2013; 50 (4): 173-82
UDC 636.09:631.86:637.966:579.6:615.284

**Original Scientific Article**

# DORAMECTIN DEGRADATION AND BACTERIAL COMMUNITY DURING SHEEP MANURE COMPOSTING

Martin Dobeic[1,2]*, Matjaž Ocepek[3], Nevenka Kožuh-Eržen[5], Štefan Pintarič[1,2], Vesna Cerkvenik-Flajs[4], Ivan Gobec[5]

[1]Institute for Environmental and Animal Hygiene with Ethology, Veterinary Faculty University of Ljubljana, Gerbičeva 60, Center of Excellence NAMASTE, Jamova 39, Institute for Microbiology and Parasitology, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, Reziduum, Residue Consulting, Chengdujska 22, 1000 Ljubljana, Krka d.d., Novo mesto, Šmarješka cesta 6, 8501 Novo mesto, Slovenia

*Corresponding author, E-mail: martin.dobeic@vf.uni-lj.si

**Summary:** In the present study doramectin degradation and bacterial community was investigated in sheep manure in experimental bioreactors during the most bioactive-thermophile phase of composting. Samples were artificially fortified with doramectin and composted for 21 days. On average, doramectin analysed by chromatographic determination (HPLC) was significantly reduced in all samples (K1-K3) by 31.8-41.8% during the 21 days of composting. The correlation between declination of doramectin and reduction of moisture in compost mixture samples (K1–K3) was also observed. It was assumed that the mean doramectin concentrations in samples were reduced in accordance with the decrease in water content. For this reason a parallel laboratory test was performed which resulted in lower mean values of doramectin in samples with lower contents of water. Doramectin indicated the influence on viable bacteria numerosity which was highest in the samples without doramectin compared to the samples fortified with doramectin. PCR amplification and phylogenetic analysis (T-RFLP) revealed the presence of the phyla *Fibrobacteres* and *Bacteroidetes*, while *MspI* restriction analysis indicated the potential presence of the genus *Pseudomonas* and *Rhodococcus* as potential decomposers of doramectin. This study indicated the effectiveness of biophysical factors on degradation of doramectin and its influence on the abundance of viable bacteria, but not on thediversity of the bacterial community.

**Key words:** composting; doramectin degradation; bacteria

## Introduction

In the environment, biological degradation is the key process in the assimilation and reduction of waste, or hazardous substances (1, 2). Aerobic degradation, e.g. composting, is the fastest and most complete biotechnological process of actively controlled decomposition, often used as an important hygienic measure of organic waste and contaminated soil (3).

Received: 17 December 2012
Accepted for publication: 18 June 2013

Doramectin is an exceptionally potent endectocide drug with a broad spectrum of activity against nematodes and arthropod parasites in livestock. It belongs to a group of avermectins, a subfamily of macrocyclic lactones. Up to 98% of macrocyclic lactones are excreted through the faeces of medicated animals in a non-metabolised form as parent compounds or active metabolites (4, 5). Limited information is available about their fate and persistence in the environment (6). Their degradability and dissipation in the environment depends strongly on their physicochemical characteristics and environmental conditions (7).

Composting of livestock manures may lead to a reduction in veterinary drug residue levels before land application (8, 9, 10, 11). However, little is known about the reciprocal effect of pharmaceuticals on the composting process. As far as is known by the authors, the only such data available is for some veterinary antimicrobials, demonstrating their possible role in the inhibition of composting (12).

Due to the frequency of doramectin use, its high ecotoxicity (13) and its highly probable appearance as a parent compound in compost, this study was aimed at the examination of degradation of doramectin in relation to moisture levels, the impact of doramectin on the presence of different bacterial species, their number, viability, and the extracellular enzymatic activity in the process of composting sheep manure containing different concentrations of doramectin. Hence the test samples were fortified with doramectin before the onset of composting due to its known metabolism involving large excretion of a parent compound via faeces (14). The study was designed to maximally approximate the field situation during composting. However, tests of doramectin degradation were also carried out under laboratory conditions in sterilised compost mixtures to exclude the impact of biological processes and, in additionally moistened compost mixtures, to determine the influence of the compost moisture on doramectin degradation.

## Methods

### *Doramectin inoculation in sheep manure compost mixtures in bioreactors*

The experimental composting process in bioreactors was investigated during the most bioactive-thermophilic phase (21 days), using a mixture of sheep barn deep litter and pine bark chips (<30mm) with a moisture content of 60 to 65% (w/w) and a density of 570kg/m$^3$. To prepare the test samples, the compost mixture was fortified with doramectin, as the commercial drug Dectomax® (Pfizer, France), at a target test concentration of 2.0mg/kg corresponding to a maximum concentration of 2186µg/kg, which was determined in dry sheep faeces after a single subcutaneous administration of 0.2mg/kg b.w. of doramectin/kg b.w. (5). Two other investigated concentrations of doramectin in the tested compost mixtures were one half of, and double, the target test concentration. Therefore after homogenization, the final concentrations of doramectin in the compost mixtures were approximately 1.0mg/kg (sample K1), 2.0mg/kg (sample K2) and 4.0mg/kg (sample K3). Control compost mixture samples were not supplemented with doramectin (sample K0). The mixtures were homogenized, transferred to test boxes made of stainless steel mesh of dimensions 15 x 15 x 5cm and placed in the central layers of compost material in three 1m$^3$ polypropylene bioreactors. The bioreactors were insulated with a 6cm thick layer of expanded polystyrene and aerated using forced ventilation with radial fans enabling a positive pressure of 3kPa in compost to ensure effective aeration of the compost by ensuring at least 5% of oxygen in the material. Radial fans delivered air uniformly through the base of the entire compost, under control of oxygen concentration sensors Dräger Multivarn II (Dräger, Lübeck, Germany), and air speed sensors Testo 450 (Testo, Lanzkirch, Germany) certifying that each batch received the same volume of air. Collection of samples during the three composting batches (P1, P2, P3) as three separate experimental composting repetitions under the same conditions with a duration of 21 days within the thermophilic phase is presented in Table 1.

**Table 1:** Sampling of the compost mixtures (samples K0-K3) prepared with sheep manure and processed in composting reactors in three batches (P1, P2, P3) and of sterilised compost mixtures (samples K0-K3) processed in one experimental batch (P1)

| | **K0** (0.0 mg/kg*) | **K1** (1.0 mg/kg) | **K2** (2.0 mg/kg) | **K3** (4.0 mg/kg) | **Sterilised compost** (1.4 mg/kg) |
|---|---|---|---|---|---|
| 0/7/14/21 | **n=6/6/6/6 | n=6/6/6/6 | n=6/6/6/6 | n=6/6/6/6 | n=6/6/6/6 |

*doramectin addition in mg/kg of dry compost
**n=6 (number of samples of the sheep manure in composting reactors and in sterilised compost mixture samples in three experimental batches = P1(n=2)+P2(n=2)+P3(n=2)

*Laboratory analysis of doramectin degradation in sterilised compost mixtures and in compost mixtures irrespective of additional amounts of water*

A compost mixture was sterilised by autoclaving for 40 minutes at 200kPa and 121°C. After cooling, the suspension of doramectin was mixed into the sterilised compost mixture (mean value 1.4mg/kg of dry weight). Homogenized mixtures were placed in 250ml containers and incubated at 68°C. The samples were collected within 21 days according to Table 1.

In order to determine the influence of the compost moisture on doramectin degradation, four (4) amounts of water (0.0, 0.375, 0.75 and 1.25ml) were added into four (4×2g) samples of homogenised compost mixture from experimental bioreactors which already contained the moisture of 342g/kg. Thus samples contained 0.34ml/g, 0.53ml/g, 0.71ml/g and 0.96ml/g of water. Samples were incubated at room temperature for 42 hours. The moisture level remained almost constant throughout the investigation of the sterilised compost since the air in the room, where composting was performed, had at least 80% of relative air moisture, likewise, containers were covered with semipermeable coverings during incubation.

*Doramectin analysis - extraction, clean-up and derivatization*

Homogenized, moist compost samples (2g) were extracted with 25ml of acetonitrile (Merck, Darmstadt, Germany, p.a.) by shaking for 1 min using a vibromix and for 5 min using a horizontal shaker at 400rpm (Vibromix 313 EVT, Tehtnica, Železniki, Slovenia). After centrifugation at 2100×g for 10min (centrifuge Rotixa/RP, Hettich, Germany) the supernatant was filtered through paper with pores < 2µm. 50µl of triethylamine was then added to 15ml of supernatant. The mixture was diluted with deionized water to 50ml volume and cleaned-up using a solid phase extraction (SPE), Bakerbond columns with $C_8$ sorbent (500mg, 6ml, J.T. Baker, Phillipsburg, NJ, USA), previously conditioned by 10-15ml of acetonitrile and 10ml of a mixture of acetonitrile, water and triethylamine (30:70:0.1, v/v/v). After applying the extract, the columns were washed with 15ml of a mixture of acetonitrile, water and triethylamine (50:50:0.1, v/v/v). Doramectin was eluted with 5ml of acetonitrile and concentrated at 50°C under a stream of nitrogen (evaporator Organomation: N-evap No111, Berlin, MA, USA). Dry extracts were derivatized at room temperature with 100µl N-methylimidazole solution in acetonitrile (1:1, v/v) and 150µl trifluoroacetic anhydride solution in acetonitrile (1:2, v/v). After 30s 750µl of acetonitrile was added to the formed conjugated fluorescent derivative, of which 50µl was injected into the HPLC system.

*Chromatographic determination*

The HPLC system was a Thermo Separation Product (Thermo Scientific, Waltham, MA, USA) and consisted of the Spectra Systems P2000 pump and an AS300 auto injector. The fluorescence detector was RF-535 from Shimadzu (Nakagyo-ku, Kyoto, Japan). The chromatographic separation was performed at 28°C on the Phenomenex Luna C18 (2) 150 ×4.6 mm (3µm) reversed analytical column with a 4cm pre-column filled with C18 stationary phase (5µm) (Phenomenex, Torrance, CA, USA). The mobile phase was a mixture of ultra HPLC grade acetonitrile, methanol and water (475:475:60, v/v/v), pumped at a flow rate of 1.1 ml/min. Excitation and emission wavelengths were 365 and 470 nm, respectively. The results were evaluated according to the external standard method.

The analytical method was validated. A detection limit of 1.0µg/kg of dry sample, reproducibility of the analytical method (RSD < 15%), linearity in the range between 20 and 500 µg/kg of the moist sample ($r > 0.98$) and the mean recovery of the analytical method > 80% were assured for the analysis. To approach the real values, the measured concentrations were corrected for mean recoveries of the respective series and used as final results.

*Bacterial count, PCR amplification and T-RFLP analysis for identification of microbial communitie.*

Bacterial cell counts were determined by quantitative estimations of direct counting using fluorescence microscopy. For this purpose LIVE/DEAD® BacLight™ (Invitrogen, USA) dye was used, which selectively stained live and dead bacteria.

Microbial communities were identified by

means of a polymerase chain reaction (PCR) with subsequent restriction analysis (15). Prior to PCR, microbial DNA was extracted using the PowerSoil™ DNA Isolation Kit (Mo Bio Laboratories, USA). Fluorescence-labelled eubacterial primers 27F (16) and 1392R (17, 18) were used in PCR, targeting the 16S rRNA gene. PCR amplification was performed using the GeneAmp PCR System 2700 (Applied Biosystems, USA). The obtained PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Germany) according to the manufacturer's instructions and were subjected to *HhaI*, *MspI* and *RsaI* (Promega, USA) restriction. Restriction fragments were cleaned using the QIAquick Nucleotide Removal Kit (Qiagen, Germany). The length of fluorescence-labelled terminal restriction fragments was determined using the ABI PRISM 310 genetic analyser (Applied Biosystems, USA), employing standard size marker 2500 ROX, and the GENEMAPER 2.0 analysis software programme (Applied Biosystems, USA). Results were compared with restriction patterns from the public database using the web based tool MiCA (Microbial Community Analysis, http://mica.ibest.uidaho.edu/trflp.php) (MiCA) (19).

### *Temperature, moisture, dry matter, pH value, nitrogen and carbon determination*

Temperature and air flow in the composted substrate were monitored using PT 100 and hot wire probe (Testo 450, Germany) directly in the compost and analysed using the computer software VisiDAQ™ runtime (Advantech®, Sunnyvale, USA). The moisture content and dry matter of the compost were analysed by drying (for 24 hours at 105°C) and weighing. The content of nitrogen and carbon were determined by a Vario MAX CNS analyzer (Elementar, Hanau, Germany) after incineration of the sample at 900°C. The pH values of a liquid phase of the material were measured by a pH meter (Hanna HI 221, Germany).

### *Data evaluation*

Statistical evaluation of results was carried out by ANOVA, t-test and correlation analysis using the SPSS (Statistical Package for the Social Sciences) 17.0 statistics software (Rainbow Technologies, USA). The Pearson product-moment correlation and linear regressions ABS versus time were accepted for $r$ >0.95, and values of $P$ <0.05 of the slopes were considered statistically significant.

## Results

### *Temperature profile, moisture content, carbon, nitrogen, and pH value characteristics*

Temperatures of the compost in bioreactors varied from 24 to 69.3°C. (Table 2). The mean moisture content of the compost was 612g/kg before composting (day 0) and decreased to 461g/kg at the end of composting (day 21) (Table 2).

The ratio of carbon (C) : nitrogen (N) in the compost mixtures decreased significantly ($P$<0.05) from the start to the end of composting: day 0 = 27:1, day 21 = 21:1. The pH value in the compost mixtures within 21 days of composting ranged from 7.4 to 6.4.

Temperature / moisture levels in the compost samples with additional water were constant for 21 days (21 ± 0.5°C/ 0,34, 0,53, 0,71 and 0,96 ± 0,05 ml/g). The C : N ratio and the pH value were the same as in the compost in bioreactors.

Temperature in the sterilised compost samples was constant for 21 days (68 ± 0.2°C), The mean moisture level in the sterilised compost was 670.9g/kg before composting (day 0) and decreased to 653.0g/kg by the end of composting (day 21). The C : N ratio in the sterilised compost mixtures was the same as in the compost in bioreactors. The pH in the sterilised compost mixtures during 21 days of composting ranged from 6.8 to 7.7.

### *Degradation of doramectin in compost mixtures*

The results of tests showed that the mean concentration of doramectin in all samples (K1-K3) declined significantly ($P$<0.05) by 31.8-41.8% during 21 days of composting. The mean concentrations of doramectin in samples with initial concentration K1 and K2 declined significantly ($P$<0.001) with the time of composting, thus from day 0 to day 21 the mean concentration of doramectin declined by 37.0-37.8%. The most pronounced decrease in doramectin ($P$<0.001) in the samples K1 and K2 was observed between day 0 and day 14 of composting (41.9-42.6%).

**Table 2:** Temperature profile and moisture content of the compost mixtures (K0-K3) in three experimental batches (P1-P3).

| | | **Temperature** (°C) | | | | **Moisture content** (g/kg) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Batch | n | Lowest | Highest | Average | Standard deviation | day 0 | 7 | 14 | 21 |
| **P1** | 21 | 24.0 | 69.3 | 55.0 | 12.9 | 634.9 | 511.6 | 545.6 | 486.8 |
| **P2** | 21 | 25.7 | 68.3 | 56.3 | 11.5 | 632.8 | 466.7 | 459.1 | 555.4 |
| **P3** | 21 | 29.2 | 65.1 | 48.9 | 12.8 | 572.0 | 517.6 | 517.3 | 342.1 |

**Table 3:** Doramectin concentrations in the compost and sterilised compost mixture samples (n) with initial concentrations K1-K3, according to the sampling time (day 0 – day 21)

| **Doramectin concentration** day | n | Lowest | Highest | Average | Standard deviation | Average doramectin reduction from day 0 (%) |
|---|---|---|---|---|---|---|
| | | (µg/kg dry matter) | | | | |
| **K1** | | | | | | |
| 0 | 6 | 962 | 1232 | 1108.3 | 100.5 | / |
| 7 | 6 | 777 | 1202 | 1022.3 | 161.1 | 7.7 |
| 14 | 6 | 606 | 714 | 643.2 | 41.2 | 41.9 |
| 21 | 6 | 595 | 845 | 698.7 | 90.4 | 30.8 |
| **K2** | | | | | | |
| 0 | 6 | 1709 | 2134 | 1878 | 152.3 | / |
| 7 | 6 | 1147 | 1969 | 1470 | 272.6 | 21.8 |
| 14 | 6 | 936 | 1352 | 1078 | 171.6 | 42.6 |
| 21 | 6 | 1014 | 1330 | 1167 | 127.1 | 37.8 |
| **K3** | | | | | | |
| 0 | 6 | 3896 | 6056 | 4625 | 998 | / |
| 7 | 6 | 2772 | 3146 | 2980 | 154 | 35.5 |
| 14 | 6 | 2245 | 3444 | 2976 | 486 | 35.6 |
| 21 | 6 | 1112 | 3584 | 2688 | 1102 | 41.8 |
| **Sterilised compost mixture** | | | | | | |
| 0 | 6 | 906 | 1986 | 1428 | / | / |
| 7 | 6 | 748 | 1932 | 1316 | / | 7.8 |
| 14 | 6 | 660 | 1667 | 1312 | / | 8.2 |
| 21 | 6 | 697 | 1863 | 1398 | / | 2.1 |

The highest decrease of doramectin (41.8%) was observed in the compost samples with initial concentration K3 but this reduction was insignificant. Nevertheless, doramectin decrease in samples K3 was significant ($P$<0.05) (35.6%) from day 0 to day 14 of composting (Table 3).

### *Degradation of doramectin in sterilised compost mixtures*

The degradation of doramectin in sterilised compost mixtures was 2.1% and within the accuracy of the respective analytical method.

### *Degradation of doramectin in relation to compost moisture*

Declination of doramectin and reduction of moisture in compost mixture samples (K1 – K3) during the composting (0 – 21 day) were in significant correlation ($P$<0.001; $r$=0.512), thus higher concentrations of doramectin were found in compost with higher concentrations of water. The same was asceratained in the laboratory test since mean values of doramectin were significantly ($P$<0.05) lower in samples with the addition of water.

### *Number of viable bacteria in compost mixture samples*

Irrespective of doramectin concentration (K1-K3) in all groups of samples, the mean count of viable bacteria was lowest prior to composting (day 0) and highest on day 7 of composting. The difference was significant ($P$<0.05). The number of viable bacteria gradually decreased in the period between days 7 and 21. The mean total count (n = 7.65 x $10^8$) of viable bacteria in the samples without doramectin (K0) was higher in comparison to the samples with doramectin (K1-K3) and steadily grew from the day 0 to day 21 (Fig. 1).

### *Phylogenetic analysis of bacterial community in compost mixture samples*

Most taxonomic groups of bacteria in the samples (K0-K3) belonged to the phyla *Fibrobacteres.* Other bacteria belonged mostly to the rumen bacteria from the phylum *Bacteriodete*s and its genera *Porphyromonas, Tannerella, Prevotella, Cytophaga, Bacteroides, Alistipes, Microscilla* and *Rikenella* and to the unidentified bacteria as presented in Figure. 2.

Regarding the T-RFLP analysis among all thedoramectin supplements (K0-K3), 2.7 to 11.6 times higher number of molecular fragments were found using the *MspI* enzyme compared to the number of fragments acquired by the enzyme *HhaI*, and 53.6 to 128.8 times higher than that acquired by the enzyme *RsaI*. Identification of fragments using the enzyme *MspI* indicates the presence of numerous potential bacterial taxa belonging to the phylum *Proteobacteria,* genus *Pseudomonas*, phylum *Actinobacteria,* genus *Rhodococcus* and to the phylum *Firmicuites,* genus *Streptococcus, Enterococcus* and *Lactobacillus.* The potential presence of bacteria belonging to the phylum *Spirochaees* was also determined.

## Discussion

The results of the experiment indicate a gradual reduction of doramectin in thermophilic phase of composting and suggest an impact on the number of live bacteria, but not on their taxonomic diversity. Namely the average concentration of doramectin in all the samples (K1-K3) significantly ($P$ < 0.05) decreased during 21 days of composting (mainly between days 7 and 14) meanwhile doramectin reduction did not differ significantly among samples K1–K3. Doramectin degradation was obviously associated with the biological processes of composting, since the test results of doramectin degradation in sterilised compost mixtures demonstrated that in the absence of viable bacteria the degradation of doramectin was

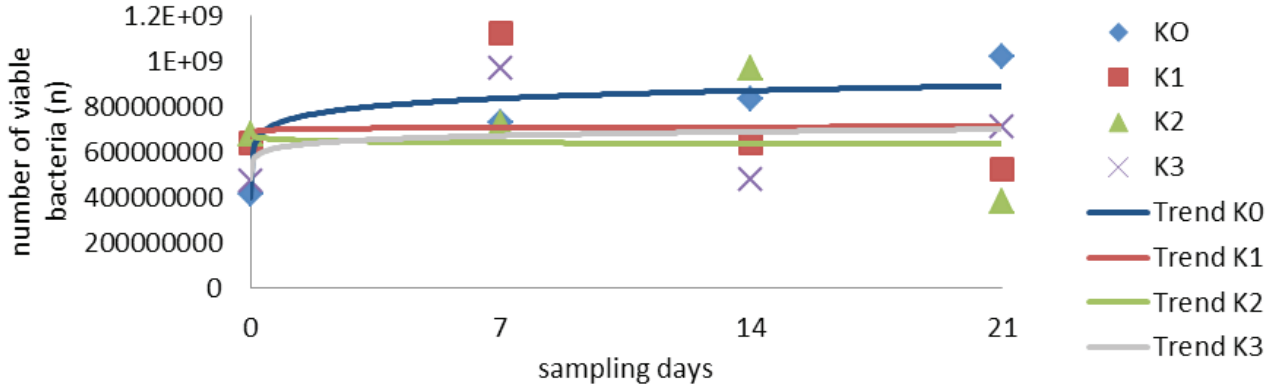


**Figure 1:** Count of viable bacteria (n) in the compost mixture samples depending on doramectin concentration (K0-K3)

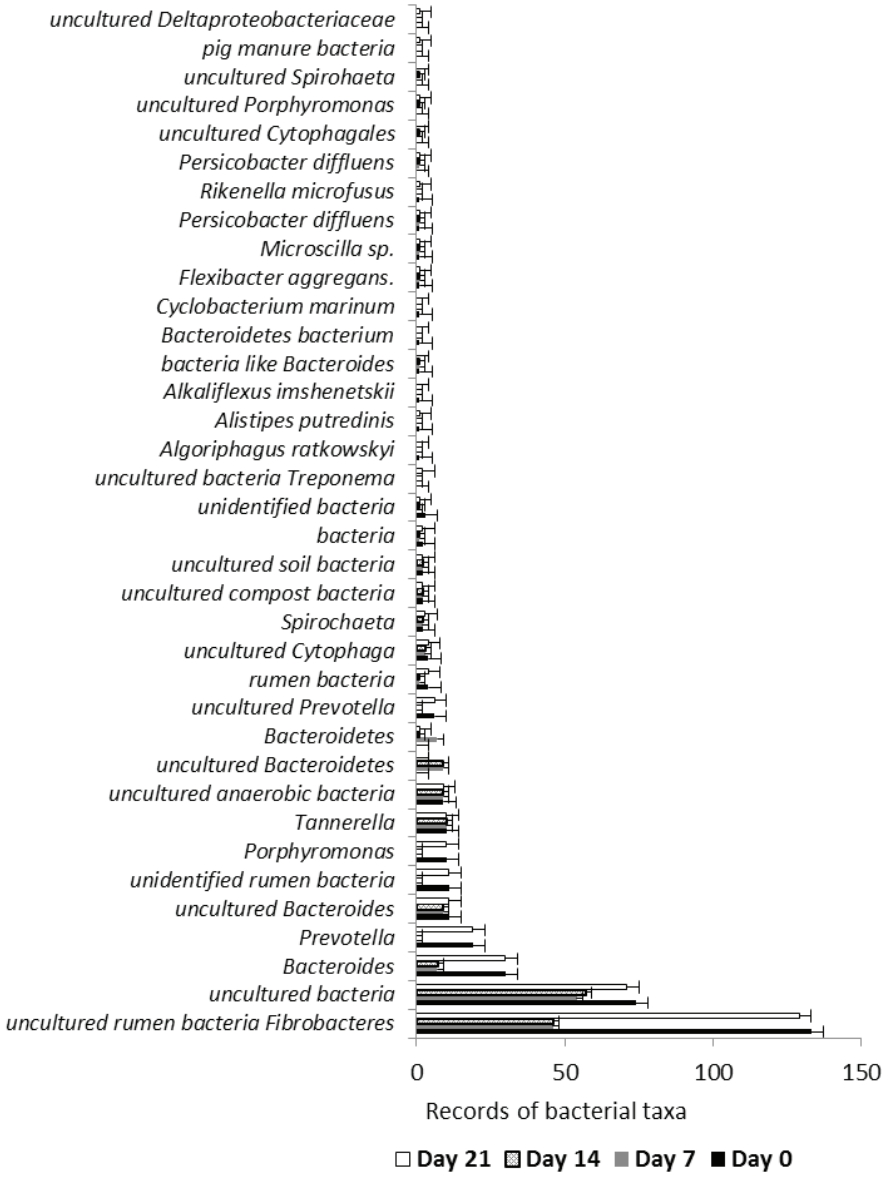


**Figure 2:** Records of bacterial taxa (taxonomic units) in the compost mixture samples according to the time of composting

very low. The highest counts of viable bacteria in bioreactors were observed in the samples without doramectin (K0) compared to the samples with doramectin (K1-K3). Namely the viable bacterial population did not alter significantly in the samples with doramectin addition (K1-K3) while the number of viable bacteria in the samples without addition of doramectin (K0) slightly increased with the time of the composting process (day 0 to the 21st day).

Beside biological degradation of doramectin it can be hypothetically concluded that declination of doramectin was related to the moisture content of the compost. It is assumed that the reason for this relationship is based on the physicochemical properties of the compost, even though doramectin has low water solubility and low vapour pressure (20). Therefore it was assumed that the mean doramectin concentrations were reduced in accordance with decreasing water content in the compost. This hypothesis was confirmed by significant ($P$<0.05) correlation between doramectin content and moisture content in the samples K3 ($r$=0.969) K1 and K2, although in insignificant positive correlation ($r$>0.80). This was also proved in the laboratory

test in which the mean values of doramectin correlated significantly ($P$<0.05) with the amount of added water, since doramectin concentrations in the samples with no addition of water were lower by 63-118% than those in the samples to which some water was added. On the other hand, concentrations of doramectin in sterilised compost mixtures remained almost the same where the mean moisture level remained almost constant throughout the investigation. These results are comparable to the previous studies (21, 7) which demonstrated a parallel increase in the concentrations of avermectins and the moisture content in sheep faeces. However, those samples were not treated as in the composting process.

Results of this study showed that among all the taxonomic groups of bacteria determined in test samples of compost mixtures (K0-K3), most of them belonged to the phyla *Fibrobacteres, Bacteroidetes* and to the unidentified bacteria. Very similar results were reported by Green et al. in the research on cattle manure composting, which found that as many as 19 (out of 31) groups belonged to the phylum *Bacteroidetes*. However, regarding the potential degradation of the environmental pollutants, bacteria from genera *Pseudomonas* and *Rhodococcus* were described as extremely important decomposers (3). The experiment showed that the highest number of molecular fragments were found using the enzyme *MspI* indicating the potential presence of bacterial taxa belonging to the phylum *Actinobacteria,* genus *Rhodococcus*. This is an important indicator to the assumption of biological degradation of doramectin in the experiment. For this reason, reliable future research is essential to confirm the presence of bacteria from the genus *Rhodococcus* in sheep manure composts.

To conclude in this study the degradation of doramectin in relation to biophysical composting factors was demonstrated, and reciprocally the impact of doramectin on the count of viable bacteria was proved. In addition, the correlation between doramectin degradation and compost moisture was proved. Due to the complexity of the composting process, a multi-variable analysis should be performed in the future to elucidate the mutual impacts of avermectin drugs and composting processes. Moreover, the development of specific micro-organisms for avermectin decomposition in the composting process should be examined in the future.

## Acknowledgements

We would like to thank the Ministry of Higher Education, Science and Technology, and the Ministry of Agriculture, Forestry and Food of Slovenia, for funding this research. The main source of data for this paper was the doctoral thesis of Ivan Gobec, who was awarded a Doctoral Fellowship from the Slovenian Research Agency (ARRS no. 33104-831611). We would also like to thank Mr. Andrej Škibin, DVM from the Centre for Sustainable Recultivation Vremščica of the Veterinary Faculty, for helping, permitting and facilitating this study.

## References

1. Haruta S, Nakayama T, Nakamura K, et al. Microbial diversity in biodegradation and reutilization processes of garbage. J Biosci Bioeng 2005; 99: 1–11.

2. Martinez J, Dabert P, Barrington S, Burton C. Livestock waste treatment systems for environmental quality, food safety, and sustainability. Bioresour Technol 2009; 100: 5527–36.

3. Fritsche W, Hofrichter M. Aerobic degradation of recalcitrant organic compounds by microorganisms. In: Jordening HJ, Winter J, eds. Environmental biotechnology: concepts and applications. Weinheim: Wiley-VCH, 2005: 203–27.

4. McKellar QA, Benchaoui HA. Avermectins and milbemycins. J Vet Pharmacol Ther 1996; 19: 331–51.

5. Kolar L, Cerkvenik Flajs V, Kužner J, et al. Time profile of abamectin and doramectin excretion and degradation in sheep faeces. Environ Pollut 2006; 14: 197–202.

6. Montforts MHMM, Kalf DF, Vlaardingen PLA, Linders JBHJ. The exposure assessment for veterinary medicinal products. Sci Total Environ 1999; 225: 119–33.

7. Virant Celestina T, Kolar L, Gobec I, et al. Factors influencing dissipation of avermectins in sheep faeces. Ecotoxicol Environ Saf 2010; 73:18–23.

8. Dolliver H, Gupta S, Noll S. Antibiotic degradation during manure composting. J Environ Qual 2008; 37:1245–53.

9. Kim KR, Owens G, Kwon SI, So KH, Lee DB, Ok YS. Occurence and environmental fate of veterinary antibiotics in the terrestrial environment.

Water Air Soil Pollut 2011; 214: 163–74.

10. Kožuh Eržen N, Kolar L, Gobec I, Pogačnik M. Degradation of avermectins in contaminated sheep faeces under various conditions. J Vet Pharmacol Ther 2006; 29: 327–8.

11. Ramaswamy J, Prasher SO, Patel RM, Hussain SA, Barrington SF. The effect of composting on the degradation of a veterinary pharmaceutical. Bioresour Technol 2010; 101: 2294–9.

12. Cessna AJ, Larney FJ, Kuchta SL, et al. Veterinary antimicrobials in feedlot manure: dissipation during composting and effects on composting processes. J Environ Qual 2011; 40: 188–98.

13. Boxall A, Fogg LA, Kay P, Paul A, Pemberton EJ, Croxford A. Prioritisation of veterinary medicines in the UK environment. Toxicol Lett 2003; 142: 207–18.

14. Shoop WL, Mrozik H, Fisher MH. Structure and activity of avermectins and milbemycins in animal health. Vet Parasitol 1995; 59: 139–56.

15. Ogram A, Feng X. Methods of soil microbial community analysis. In: Knudsen GR, Thomashow L, eds. Manual of environmental microbiology. Washington: American Society for Microbiology, 1997: 422–30.

16. Stahl DA, Flesher B, Mansfield HR, Montgomery L. Use of phylogenetically based hybridization probes for studies of ruminal microbial ecology. Appl Environ Microbiol 1988; 54: 1079–84.

17. Kutzner HJ. Microbiology of composting. In: Klein J, Winter J, eds. Biotechnology. 2nd ed. New York: Wiley-VCH, 2000: 35–100.

18. Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M, eds Nucleic acid techniques in bacterial systematic. New York: John Wiley & Sons, 1991: 115–75.

19. MiCA. Microbial Community Analysis III. University of Idaho, 2007. (last update 8. April 2011) http://mica.ibest.uidaho.edu/trflp.php

20. Bloom RA, Matheson JC. Environmental assessment of avermectins by the U.S. Food and Drug Administration. Vet Parasitol 1993; 48: 281–94.

21. Kolar L, Kožuh Eržen N. Degradation of abamectin and doramectin in sheep faeces under different experimental conditions. Int J Environ Pollut 2007; 31: 22–33.

# DEGRADACIJA DORAMEKTINA IN BAKTERIJSKE ZDRUŽBE MED KOMPOSTIRANJEM OVČJEGA GNOJA

M. Dobeic, M. Ocepek, N. Kožuh-Eržen, Š. Pintarič, V. Cerkvenik-Flajs, I. Gobec

**Povzetek:** Raziskana je bila razgradnja doramektina in bakterijskih združb v ovčjem gnoju med najbolj bioaktivno-termofilno fazo kompostiranja v poskusnih bioreaktorjih. V vzorce komposta, ki smo ga kompostirali 21 dni,so bile dodane različne koncentracije doramektina. V povprečju so se koncentracije doramektina v vseh vzorcih (K1-K3), analiziranega po postopkih kromatografske analize (HPLC), s časom kompostiranja (21 dni) znižale za 31,8 - 41,8 %. Ugotovljena je bila značilna korelacija ($P$<0,001; r=0,512) med koncentracijo doramektina in znižanjem vlage v vzorcih (K1-K3). Domnevamo, da se povprečne koncentracije doramektina v vzorcih znižujejo skladno z zniževanjem vsebnosti vode. Iz tega razloga je bil vzporedno izveden laboratorijski preskus, v katerem so bile ugotovljene značilno ($P$<0,05) nižje povprečne vrednosti za doramektin v vzorcih z nižjo vsebnostjo vode. Doramektin je vplival na številčnost bakterij, ki je bila najvišja v vzorcih brez doramektina v primerjavi z vzorci, obogatenimi z doramektinom. PCR in filogenetska analiza (T-RFLP) sta pokazali prisotnost bakterij iz rodov *Fibrobacteres* in *Bacteroidetes*, medtem ko je analiza z restrikcijskim encimom *MspI* pokazala morebitno prisotnost bakterij iz rodu *Rhodococcus*, ki so odgovorne za potencialno razgradnjo doramektina. Študija je pokazala učinkovanje biofizikalnih dejavnikov na degradacijo doramektina in njegov vpliv na številčnost živih bakterij, ne pa tudi na raznolikost bakterijske združbe.

**Ključne besede:** kompostiranje; degradacija doramektina; bakterije