Original Article

WM&R

Waste Management & Research
2023, Vol. 41(2) 328–336

DOI: 10.1177/0734242X221123143
journals.sagepub.com/home/wmr
SAGE

Problems associated with vermicomposting of dog excrement in practice using *Eisenia andrei*

Tereza Hřebečková[1], Natálie Králíková[2], Aleš Hanč[1] and Lucie Wiesnerová[3]

Abstract

One 25-kg dog produces about 500 g of excrement per day. Excrement is a potentially hazardous material, as it may contain pathogenic microorganisms. Our samples were tested for the presence of thermotolerant coliform bacteria, *Enterococcus* spp., *Escherichia coli* and *Salmonella* spp., which are indicators of faecal contamination, as well as for the presence of helminths and their eggs. During the experiment, it was observed whether these microorganisms could be eliminated by vermicomposting. There were two variants of vermicomposting piles: one test pile (with continuous feeding) and one control pile (with a single feeding). The vermicomposting process was run in outdoor conditions in park for 51 weeks using *Eisenia andrei* earthworms. The vermicomposting of dog excrement with waste from park maintenance (1:2) can produce a good quality fertiliser. During the process of vermicomposting, there was a gradual decrease in the content of pathogenic bacteria. At the end of the vermicomposting process, there were no eggs or adult helminths. The vermicompost was very rich in microorganisms and enzymatic activity. The pH value was slightly alkaline, and the C:N ratio corresponded to value of mature vermicompost.

Keywords

Vermicomposting, dog excrement, PLFA analyses, pathogenic organisms, endoparasites, enzymatic activity

Received 3rd January 2022, accepted 21st May 2022 by Editor in Chief Arne Ragossnig.

Introduction

The issue of handling dog excrement is a very extensive topic. Excrement is a potentially hazardous material, as it may contain pathogenic microorganisms. Many pets are bred in the Czech Republic. For example, in Prague (Czech Republic), there were more than 83 thousand dogs registered in 2018 (Portal of the capital city of Prague, 2018). A medium-sized dog (approx. 25 kg) eats about 600–800 g of food per day, depending on its type, and one 25-kg dog produces about 500 g of excrement per day (Felsmann et al., 2017). Both smaller and larger breeds of dogs are registered in Prague. The average production is therefore approximately 300 g of excrement per dog per day, which means a total production of over 24,900 kg of excrement per day.

Many potentially dangerous microorganisms can be found in excrement. Bacteria are an integral part of the digestive processes. Pathogenic bacteria in excrement mainly include species of the family Enterobacteriaceae. These bacteria can ferment glucose and other sugars. They do not form spores; they are facultatively anaerobic bacteria. These bacteria can be divided into three groups: exclusively pathogenic, occasionally pathogenic and nonpathogenic bacteria. *Escherichia coli* and *Salmonella* spp. belong to exclusively pathogenic bacteria, which mainly inhabit the environment of the digestive tract (Quinn et al., 2004).

Other dangerous organisms in excrement include parasitic nematodes. Adult nematodes are parasites most often in the digestive tracts of vertebrates. The endoparasites mainly found in excrement are *Toxocara canis, Trichuris vulpis, Ancylostomatidae, Taenia* spp., *Toxascaris leonina, Capillaria* spp., *Dioyidium caninum* and *Coccidia* (Papajová et al., 2014). Many cases of toxocarosis in humans are caused by ingesting infected eggs from the soil or infected larvae from uncooked meat (Strube et al., 2013). Another group of endoparasites found in excrement are *Trichuris* spp. *Trichuris trichura* commonly parasitises humans; however, humans can also become infected with the parasite of dogs,

[1]Department of Agroenvironmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, The Czech University of Life Sciences Prague, Prague, Czech Republic
[2]Department Seeds and Planting Materials, Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic
[3]Institute of Medical Chemistry and Biochemistry, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic

Corresponding author:
Tereza Hřebečková, Department of Agroenvironmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, The Czech University of Life Sciences Prague, Kamycka 129, Prague 16521, Czech Republic.
Email: hrebeckova@af.czu.cz

T. vulpis. Human infection occurs exclusively orally (Dunn et al., 2002). In addition, helminths are a very common cause of parasitic diseases in humans (Pipiková et al., 2017). Infection with helminths from the ground or soil is what most often occurs. It is estimated that more than a quarter of the world's population is infected with some type of helminthiasis (Awasthi et al., 2003). The infection of the dog population with these endoparasitic worms is also relatively high (Dubná et al., 2007). Given the amounts of helminth eggs that may be present in dog excrement and the ability of eggs to survive in soil, it must be emphasised that they pose a significant problem, even when the source of contamination is individual animals (Felsmann et al., 2017).

Our samples were tested for the presence of thermotolerant coliform bacteria, which are an indicator of faecal contamination from the digestive tracts of warm-blooded animals as well as for the presence of helminths and their eggs. Furthermore, the presence and amounts of *Enterococcus* spp., *E. coli* and *Salmonella* spp. were verified. During the experiment, it was determined whether these microorganisms could actually be eliminated by vermicomposting, because according to Mudrunka et al. (2020) earthworms can eliminate the presence of pathogenic microorganisms or contribute to limit the growth of their colonies.

Vermicomposting is a process of decomposition of organic waste to the formation of a stable organic fertiliser, vermicompost. The basic principle of vermicomposting is the interaction between earthworms, which fragment and moisten the substrate, and microorganisms. Vermicomposting is the transformation of organic materials by a stabilization and bio-oxidation process, and it is a maximally environmentally friendly method (Lim et al., 2016). It consists of an active phase, during which earthworms and microbes gently process the substrate, and a maturation phase, which involves the activity of microorganisms. The maturation of the vermicompost occurs when the earthworms move to a layer with a fresher material or when the material is removed from the vermicompost container. The length of the active phase depends on the amount and species of earthworms (Pathma and Sakthivel 2012). In our conditions, the most widely used species of earthworm for vermicomposting is *E. andrei*. Compared to another species (e.g. *Eisenia fetida*), which is also used for vermicomposting, *E. andrei* is a generally more accepted species, because its reproductive cycle is faster (Domínguez et al., 2005).

In the scientific literature states, that earthworms can eliminate the presence of many pathogenic microorganisms or at least significantly contribute to the inhibition of the growth of their colonies. The novelty of the study is in the vermicomposting in the system of single and continuous feeding of dog excrement, which is a problem in public spaces, especially in parks, due to the content of pathogenic microorganisms. Due to this potentially hazardous material, it is necessary to carefully monitor the presence of bacterial species during vermicomposting especially in the final vermicompost. The study determines a number of biological parameters, such as the presence of pathogenic microorganisms and parasitic organisms as well as the content of groups of microorganisms by phospholipid fatty acids (PLFA) analyses, enzymatic activity and many agrochemical parameters. This study shows how to manage potentially hazardous biowaste using a gentle method, vermicomposting, and analyses the whole process of vermicomposting, from the input raw material to the final product, vermicompost, in practice in outdoor conditions (in the park) in two variants: one with a single feeding and one with continuous feeding.

Material and methods

Experiment design

The experiment with the vermicomposting of dog excrement in combination with other biodegradable material was set up in the Ecocentrum Vyšehrad (Prague, Czech Republic). The outdoor temperature was not measured during vermicomposting, as the experiment took place directly in practice in outdoor conditions, so the temperature changed based on the season. The dog excrement came from dog excrement baskets in the Vyšehrad park area. Dog excrement was combined with biodegradable material from the maintenance of the park (grasses, leaves and similar plant-based materials) in a ratio of 1:2. The species of earthworm *E. andrei* (density 50 pcs L^{-1}–about 28,000 pcs for each pile) was used for vermicomposting. The earthworms were from the earthworm farm of Mr. Ing. Petr Filip from Lužice, Czech Republic. Two experimental piles were set up: one control pile and one test pile. The size of each of the pile was 2.5×1.5 m. At the beginning, a layer of biowaste mixture about 30 cm thick was placed on each pile. No material was subsequently added to the control pile after its establishment, but the test pile was supplemented with a mixture of excrement and biological waste from the park (about 20 cm thick layer) 1–2 times a month (depending on the season). The park staff took care of the correct course of vermicomposting. The microbiological analyses were performed at the National Institute of Public Health (Prague, Czech Republic). The analyses of agrochemical parameters and enzymatic activity were performed in the laboratories of the Czech University of Life Sciences in Prague (Czech Republic). The analyses of PLFA were performed at the Institute of Microbiology of the Czech Academy of Sciences (Prague, Czech Republic). At the end of the experiment (after 51 weeks), another six samples were taken, three from each pile, which were delivered to the Health Institute at the Department of Parasitology, Mycology and Mycobacteriology in Prague (Czech Republic), where a test for helminths and their eggs was performed. Samples were always taken from five parts of the vermicomposting pile (Figure 1). All five samples were always analysed separately for microbiological analyses. For the analyses of agrochemical parameters and analyses of enzymatic activity, a mixed sample was created and then was divided into three samples for statistical analyses.

Agrochemical analyses

The dry matter was determined gravimetrically as the difference between before and after drying in a dryer at 35 °C according to

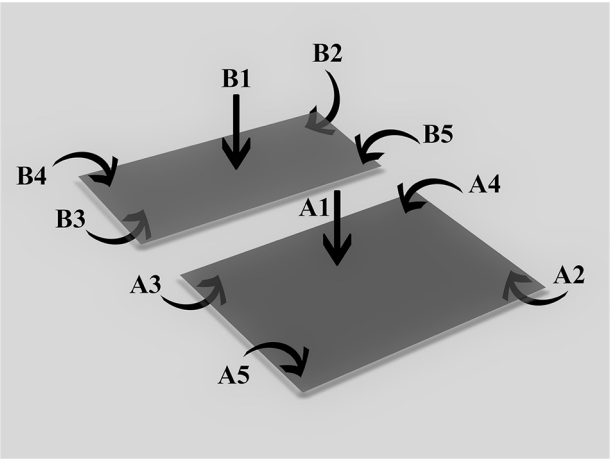

Figure 1. Vermicomposting piles sampling. A: test pile with continuous feeding (A1–A5: taken samples) and B: control pile with single feeding (B1–B5: taken samples).

Hřebečková et al. (2019b). The value of active pH and electrical conductivity (EC) was determined for fresh samples with demineralized water 1:5 according to the standard BSI EN 15933 (2012). The pH meter WTW 340i was used for measurement. The specific conductivity was measured with a WTW Cond 730 inoLab® conductivity meter. Dried samples were used to determine both the C:N ratio and the total content of macroelements. The C:N ratio was analysed on a CHNS Vario MACRO cube (Elementar Analysensysteme GmbH, Germany), which burned approximately 25 mg of the dried ground sample in a catalytic furnace, evaluated the C and N content, from which it then automatically evaluated their mutual ratio. The total content of macroelements (P, K, Ca, Mg) was determined by wet method microwave decomposition in a closed system Ethos 1 (MLS GmbH, Germany), where 0.5 g of dried sample was weighed, and added 8 ml of HNO_3 (65%) and 2 ml H_2O_2 (30%). Subsequent measurements were performed using inductively coupled plasma optical emission spectrometry (ICP – OES Varian Vista Pro) according to Hanč et al. (2017).

Biological analyses

To determine the number of earthworms, one summary sample weighing 1 kg was taken from each pile. Earthworms were manually selected and counted according to Hanč et al. (2017). For the analyses of *E. coli*, 10 g of the sample were weighed from a freshly collected sample into a polythene bag. The sample was poured into 90 ml of phosphate buffer, and the bag was placed on a shaker for 2 minutes. The solution was transferred to a glass vessel; the remaining material was squeezed thoroughly. The sample was further diluted 1–10 times according to its purity. For the determination, 1 ml of the diluted sample was taken, which was mixed with 99 ml of demineralized water. Colilert-18 reagent (IDEXX) was added to the solution, and after its complete dissolution, the solution was poured into a special Quanti-Tray/2000 plate. The plate was sealed (Quanti-Tray Sealer) and incubated for 18 hours at 44 ± 1 °C according to Matějů (2009). Samples for enterococcal analyses were prepared in Petri dishes, where 0.2 ml of a sample were plated on an agar plate with a Slanetz-Bartley medium. This amount was spread on a plate until the agar absorbed it. After 24 hours, a count can be made, and the enterococci appear as brown colonies on the surface of the agar (Matějů, 2009). For the determination of *Salmonella* spp., 50 g of a sample were weighed into peptone buffered water. After 16–20 hours at 36 ± 2 °C, revaccination to selenite was performed. Next, 0.1 ml of the culture thus obtained was transferred to a test tube containing 10 ml of RV soil (soil with $MgCl_2$ and malachite green). The sample thus prepared was exposed to a temperature of 41.5 °C for 24 hours to suppress other bacteria. Subsequently, the culture was plated with a bacteriological loop on XLT (xylose-lysine deoxycholate) and BGA (phenol red and brilliant green agar) agar plates. The dishes were turned upside down and placed in a incubator. After 24 hours it was possible to make a count; on XLT agar they appeared as black colonies, and on BGA agar they appeared by changing the colour of the media from pink to red (Matějů, 2009). The analyses of the presence of helminths and their eggs were performed in the laboratory of the Health Institute at the Department of Parasitology, Mycology and Mycobacteria in Prague. The laboratory performed the determination according to its standard operating procedure, which is proprietary. The determination was performed on samples at a compost age of 51 weeks. Groups of microorganisms were detected using analyses of free methyl esters of PLFA, according to Hanč et al. (2017). The enzymatic activity of hydrolytic enzymes (β-D-glucosidase, acid phosphatase, arylsulphatase, lipase, chitinase, cellobiohydrolase, alanine aminopeptidase, leucine aminopeptidase) were measured as the change in absorbance in 96-well microplates using Tecan Infinite® M200 (Tecan, Grödig, Austria), according to Hřebečková et al. (2019b).

Statistical analyses

Results for statistical analyses are the means of three replicates. Based on the results of the analysis of normality and homogeneity, it was chosen nonparametric Kruskal-Wallis test for an analyses of variance. Pearson's correlation coefficient and the analyses of variance were calculated using STATISTICA 12 software (StatSoft, Tulsa, USA).

Results and discussion

The dry matter content of the dog excrement was 36.23%. In the test pile, during vermicomposting, the percentage of dry matter increased up to about 50%, while the value in the control pile was almost unchanged (about 33%) (Figure 2). The pH increased with the addition of biowaste from the park because dog excrement showed a more acidic pH (6.66). In both piles, the pH was about 8.5 at the age of 29 weeks (Table 1). In both piles, the pH value decreased with the age of vermicomposting (to 7.91 in test pile and 7.59 in control pile in week 51). The EC of the raw

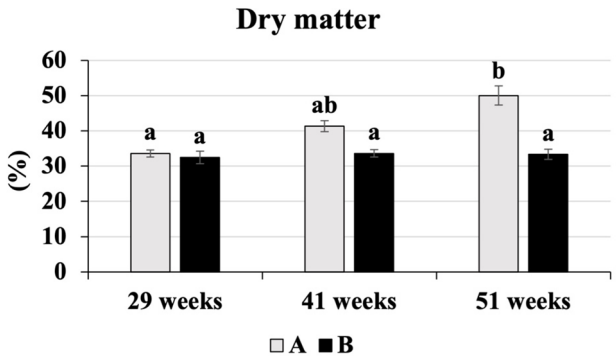

Figure 2. Dry matter content (%) in test (A) and control pile (B). Values are the means ± SD (n = 3). Different letters in indicate significant differences between weeks (Kruskal-Wallis test, $p \leq 0.05$).

Table 1. Agrochemical parameters in test (A) and control pile (B).

	Test pile A	Control pile B
pH		
29 weeks	8.45 ± 0.23 a	8.62 ± 0.30 a
41 weeks	8.00 ± 0.13 a	7.95 ± 0.12 ab
51 weeks	7.91 ± 0.14 a	7.59 ± 0.17 b
EC (μS cm^{-1})		
29 weeks	1259.7 ± 23.7 ab	1148.7 ± 51.4 a
41 weeks	1128.0 ± 45.9 a	1457.7 ± 67.9 ab
51 weeks	2102.7 ± 88.9 b	1818.0 ± 113.1 b
C/N		
29 weeks	12.26 ± 0.48 a	13.47 ± 0.7 a
41 weeks	11.64 ± 0.38 a	12.82 ± 1.09 a
51 weeks	11.85 ± 0.36 a	11.70 ± 0.82 a
C (%)		
29 weeks	29.12 ± 0.86 a	25.37 ± 1.08a
41 weeks	27.22 ± 2.34 a	24.53 ± 1.41 a
51 weeks	28.57 ± 2.68 a	25.33 ± 2.02 a
N (%)		
29 weeks	2.38 ± 0.14 a	1.89 ± 0.14 a
41 weeks	2.34 ± 0.27 a	1.92 ± 0.09 a
51 weeks	2.42 ± 0.30 a	2.17 ± 0.11 a
P (mg kg^{-1})		
29 weeks	5541 ± 872 a	8627 ± 720 a
41 weeks	6275 ± 975 a	5703 ± 993 a
51 weeks	7442 ± 990 a	5608 ± 216 a
K (mg kg^{-1})		
29 weeks	15,257 ± 342 a	14,248 ± 983 a
41 weeks	13,641 ± 606 a	13,335 ± 880 a
51 weeks	13,018 ± 978 a	11,988 ± 389 a
Ca (mg kg^{-1})		
29 weeks	40,536 ± 622 a	42,971 ± 955 a
41 weeks	41,323 ± 952 a	34,909 ± 407 a
51 weeks	47,378 ± 398 a	37,952 ± 221 a
Mg (mg kg^{-1})		
29 weeks	5589 ± 354 a	6122 ± 78 a
41 weeks	5312 ± 362 a	5121 ± 373 a
51 weeks	6239 ± 820 a	5134 ± 362 a

Values are the means ± SD (n = 3). Different letters in a column indicate significant differences between weeks (Kruskal-Wallis test, $p \leq 0.05$).

material was 2414.33 μS cm^{-1}. In both piles, in the vermicompost age of 29 weeks, this value decreased by about half, which was probably caused by the addition of biowaste from the park because this value statistically significantly increased with the age of vermicomposting. Although there was a significant increase in EC in both piles, the C and N values remained almost constant over the observed vermicomposting period. However, the increase in EC may be explained by statistically significant increased arylsulfatase activity, the value of the correlation coefficient of EC and arylsulfatase in the test pile was 0.82, in the control pile 0.75. The C:N ratio of dog excrement was 8.59:1. This value decreased in both piles; in the test pile, it decreased from 12.26:1 to 11.85:1, and in the control pile, it decreased from 13.47:1 to 11.70:1, but the differences were not statistically significant. Higher percentages of N and of C were found in the test pile, this may be due to the continuous addition of the mixture of biowaste. The C:N ratio can be affected by lipase, chitinase, alanine and leucine aminopeptidase activity according to the correlation coefficient.

Vermicomposts in the Czech Republic should meet the parameters specified in the ČSN 46 5736 (2018) standard; for example, they should have a pH in the range of 6.0–9.0, humidity 50–70%, C:N ratio less than 30:1 and percentage of nitrogen in dry matter higher than 1%. The samples taken by us complied with the range of values determined for vermicomposts. Gómez-Brandón et al. (2008) composted cattle manure for 270 days; their EC also increased with the age of composting (from 1300 to 3000 μS cm^{-1}), and the percentage of N was almost comparable to our values of N in the test pile (about 2.4%). On the other hand, their percentage of C was higher than ours was, except for the value measured in 270-day-old compost (25%). Jamaludin et al. (2012) vermicomposted goat manure with spent mushroom substrate (1:1) for 20 weeks, and their percentage of nitrogen that was comparable to our values was in the variant with 80% of goat manure (1.63–2.23% N); in the variant with 50% goat manure, their values were lower than ours. Riwandi et al. (2022) vermicomposted cow dung, buffalo dung, chicken manure and goat dung in a tray for 60 days using *Perionyx excavates*. Their C:N ratio of vermicompost from cow dung was almost same as ours (11.9:1). However, their percentage of C was lower (19.7%) and N was lower (1.65%).

The dog excrement contained 30,967 mg kg^{-1} of phosphorus. In the test pile, the value gradually increased from 5541 mg kg^{-1} (week 29) to 7442 mg kg^{-1} (week 51) (Table 1). On the other hand, in the control pile, the phosphorus content decreased from 8627 mg kg^{-1} (week 29) to 5608 mg kg^{-1} (week 51). While in the test pile the phosphorus content was affected by the reduction of the water content according to the correlation coefficient, on the other hand in the control pile it was affected by a statistically significant decrease in pH. The potassium content was significantly higher in both piles, compared to the input raw material: 4343 mg kg^{-1} (dog excrement), 15,257 mg kg^{-1} (test pile, week 29) and 14,248 mg kg^{-1} (control pile, week 29), it can be caused by the addition the waste from park maintenance, which can be rich in the content of K. During the vermicomposting, there was

a gradual reduction in potassium content, but it was not statistically significant. The potassium content of the test pile was affected by both the decreasing content of microorganisms and the decreasing activity of most of the monitored enzymes. The correlation coefficient was higher than 0.71 in all cases. In the control pile, the potassium content was most affected by the decreasing pH (correlation coefficient 0.87), lipase and aminopeptidase activity. The content of calcium in the dog excrement was 63,596 mg kg^{-1}. This value was lower in both vermicomposting piles and was about 40,000 mg kg^{-1} (week 29). This value increased in the test pile to the value 47,378 mg kg^{-1} (week 51). In the control pile, the value decreased to 37,952 mg kg^{-1}. While in the control pile a strong negative correlation was found between Ca values and cellobiohydrolase activity (−0.94), in the test pile the Ca content was affected by arylsulfatase activity (correlation coefficient 0.89) and an increase in EC (correlation coefficient 0.91), according to the correlation coefficient. The magnesium contained in dog excrement was 4376 mg kg^{-1}. The test pile contained 5589 mg kg^{-1} in week 29, which subsequently decreased to 5312 mg kg^{-1} (week 41), and at the vermicompost age of 51 weeks, the value increased to 6239 mg kg^{-1}. In the control pile, the magnesium content decreased from 6122 mg kg^{-1} (week 29) to 5134 mg kg^{-1} (week 51) (Table 1). A strong negative linear correlation was found between the Mg content and β-D-glucosidase activity in the control pile (−0.91). In the total content of nutrients, no statistically significant differences were found between the weeks of each vermicomposting pile.

The number of earthworms in the test pile was 88 pcs kg^{-1} in the week 29, 50 pcs kg^{-1} in the week 41 and 17 pcs kg^{-1} in the week 51. In the control pile, the number of earthworms in each of the samples was higher, but it also rapidly decreased (week 29: 112 pcs kg^{-1}; week 41: 77 pcs kg^{-1}; week 51: 9 pcs kg^{-1}). This could be due to the fact that the test pile was sampled as a mixture from both the bottom and the top of the pile, which was higher due to the continuous feeding, while the control pile did not refeed and was lower, so there could be more earthworms in the sample. In both piles, the number of earthworms decreased with the age of vermicomposting.

The microbial PLFAs in dog excrement were as follows: fungi 57.8 μg g^{-1} dw, bacteria 112.4 μg g^{-1} dw, actinobacteria 0.2 μg g^{-1} dw, Gram-positive bacteria 38.9 μg g^{-1} dw and Gram-negative bacteria 53.7 μg g^{-1} dw, and the total microbial biomass was 450.3 μg g^{-1} dw. The fungal PLFAs of both piles were many times lower than the content in the excrement (test pile: 2.0–5.4 μg g^{-1} dw; control pile 3.2–6.4 μg g^{-1} dw). In addition, the contents of bacteria were lower, except the actinobacteria, which were many times higher in vermicomposting piles than in the excrement (test pile: 3.9–4.8 μg g^{-1} dw; control pile 4.3–6.3 μg g^{-1} dw). Bacterial PLFAs were higher in the control variant, where they ranged from 50.8 μg g^{-1} dw (week 51) to 75.0 μg g^{-1} dw (week 41). In the test pile, PLFAs ranged from 40.2 μg g^{-1} dw (week 51) to 57.3 μg g^{-1} dw (week 29) (Table 2). Gram-positive and Gram-negative bacteria were also higher in the control variant as was the total microbial biomass. All microbial PLFAs were higher in raw excrement than in the vermicomposting piles. The only statistically significant difference was found in total microbial biomass at weeks 29 and 51. The individual groups of microorganisms interact, which is confirmed by the strong correlation coefficients found between them (e.g. correlation coefficient between fungi and bacteria: control pile 0.84, test pile 0.92).

Table 2. Microbial PLFAs in test (A) and control pile (B).

	Test pile A	Control pile B
Fungi (μg g^{-1} dw)		
29 weeks	5.38 ± 1.46 a	3.59 ± 0.12 a
41 weeks	2.57 ± 0.61 a	6.37 ± 2.11 a
51 weeks	2.03 ± 0.32 a	3.17 ± 1.31 a
Bacteria (μg g^{-1} dw)		
29 weeks	57.31 ± 5.86 a	58.90 ± 10.27 a
41 weeks	42.46 ± 3.21 a	75.00 ± 4.33 a
51 weeks	40.21 ± 9.40 a	50.76 ± 6.65 a
Actinobacteria (μg g^{-1} dw)		
29 weeks	4.78 ± 1.22 a	6.27 ± 1.76 a
41 weeks	3.85 ± 0.57 a	5.74 ± 2.42 a
51 weeks	4.04 ± 0.99 a	4.32 ± 0.78 a
G+ bacteria (μg g^{-1} dw)		
29 weeks	14.40 ± 2.85 a	12.48 ± 4.31a
41 weeks	9.88 ± 3.15 a	18.09 ± 3.54 a
51 weeks	6.86 ± 1.55 a	9.51 ± 2.15 a
G– bacteria (μg g^{-1} dw)		
29 weeks	34.94 ± 0.86 a	38.12 ± 4.73 a
41 weeks	26.93 ± 3.77 a	48.16 ± 5.55 a
51 weeks	27.81 ± 1.71 a	34.93 ± 6.22 a
Total microbial biomass (μg g^{-1} dw)		
29 weeks	96.10 ± 7.95 a	96.23 ± 8.23 a
41 weeks	70.10 ± 5.40 ab	126.76 ± 23.10 a
51 weeks	63.98 ± 3.48 b	81.24 ± 12.35 a

Values are the means ± SD ($n = 3$). Different letters in a column indicate significant differences between weeks (Kruskal-Wallis test, $p \leq 0.05$).

Dog excrement is very rich in microorganisms. The addition of biowaste from the park maintenance significantly reduced the contents of microorganisms in vermicomposting piles. No additional biowaste was added to the control pile, so there could have been a better multiplication of bacteria and fungi. Gómez-Brandón et al. (2013) vermicomposted rabbit manure in polyethene reactors for 250 days using *E. fetida*. Their content of total microbial biomass decreased with the age of vermicomposting and ranged from 350 to 800 μg g^{-1} dw PLFA. Their fungal PLFAs ranged from 1 to 3.5 μg g^{-1} dw PLFA, which was little bit lower than in our experiment. Their bacterial PLFAs were many times higher than ours were and ranged from 250 to 650 μg g^{-1} dw PLFA.

The dog excrement itself exhibited lower values of pathogens than many of the samples at the first part of vermicomposting process. The content of total coliform bacteria in dog excrement was 7.8×10^5 CFU g^{-1}, the *E. coli* content was 2.14×10^5 CFU g^{-1}, the *Enterococcus* spp. content was 3.9×10^5 CFU g^{-1}, and the presence of *Salmonella* spp. was proven, which is higher, than

Table 3. Content of thermotolerant bacteria in test (A) and control pile (B).

Test pile A:	A1	A2	A3	A4	A5
Total coliform bacteria (CFU g^{-1})					
29 weeks	>24,196	11,199	4352	>24,196	9804
41 weeks	10,462	>24,196	>24,196	4611	>24,196
51 weeks	<50	1386	<50	<50	<50
E. coli (CFU g^{-1})					
29 weeks	5298	3877	1036	2402	4569
41 weeks	20	<1	<1	<1	52
51 weeks	<50	1386	<50	<50	<50
Enterococcus spp. (CFU g^{-1})					
29 weeks	6925	975	<750	975	<750
41 weeks	11,199	5493	11,199	7701	1775
51 weeks	<750	<750	7545	4636	<750
Salmonella spp.					
29 weeks	negative	negative	negative	negative	negative
41 weeks	negative	negative	negative	negative	negative
51 weeks	negative	negative	negative	negative	negative
Control pile B:	B1	B2	B3	B4	B5
Total coliform bacteria (CFU g^{-1})					
29 weeks	>24,196	15,531	>24,196	12,997	1872
41 weeks	>24,196	>24,196	>24,196	24,196	>24,196
51 weeks	<50	<50	<50	<750	<50
E. coli (CFU g^{-1})					
29 weeks	>24,196	2577	3151	2924	2769
41 weeks	816	24,196	1081	<1	2613
51 weeks	<50	<50	<50	<750	<50
Enterococcus spp. (CFU g^{-1})					
29 weeks	<50	<750	4225	2625	<750
41 weeks	4909	19,863	2475	235,000	8341
51 weeks	<750	<750	1532	2682	<750
Salmonella spp.					
29 weeks	negative	negative	negative	negative	negative
41 weeks	negative	negative	negative	negative	negative
51 weeks	negative	negative	negative	negative	negative

A1/B1 – A5/B5: samples taken according to Figure 1.

the values measured by Mudrunka et al. (2020), who vermicomposted dog excrement in combination with corn meal and decayed grass. They measured content of *E. coli* 1×10^3 CFU g^{-1} and 1×10^4 CFU g^{-1} of *Enterococcus* spp. in raw dog excrement. However, their dog excrement samples came from the dogs of one of the authors. Our samples were more diverse and therefore the values could be higher.

In both piles, the content of thermotolerant coliform bacteria had a very similar trend; there was a gradual decrease. At the vermicompost age of 51 weeks, both piles contained almost identical amounts of thermotolerant coliform bacteria (Table 3), and the highest amount of total coliform bacteria was in sample A2. However, compared to the value at the 29th week of vermicomposting, this value was lower (in the test pile, sample A2). In other samples, the occurrences of pathogens at <50 were measured at the last sampling. Samples taken from the top of the pile did not regularly show higher values than samples taken from the bottom of the pile. All samples of the control pile showed low values in week 51; in sample B4, the value was <750, and in the other samples, the values were <50. The amount of *E. coli* decreased faster in the test pile, but at the vermicompost age of 51 weeks, the quantities of this pathogen in both piles were almost the same. The test pile contained, at the beginning of the vermicomposting process in some samples, a many times higher content of *Enterococcus* spp. than the control pile, but in both piles, the contents decreased with the age of vermicomposting. The value of *Enterococcus* spp. in the test pile increased between week 29 and 41 and then decreased between week 41 and 51, which is consistent with the development of Gram-negative bacteria values during vermicomposting, which also includes *Enterococcus* spp. The presence of *Salmonella* spp. was detected in the dog excrement, but not in both piles. Although *E. coli* decreased in our experiment, in the case of Mudrunka et al. (2020) *E. coli* content did not change (1×10^3 CFU g^{-1}). They vermicomposted dog excrement with corn meal and decayed grass in outdoor boxes for 5 months. In the contents of *Enterococcus* spp. they recorded a decrease from 1×10^4 CFU g^{-1} to 1×10^2 CFU g^{-1}. However, their slight or no decrease could

be due to the fact, that the experiment took place outdoors during the winter months (October to February). Hřebečková et al. (2019a) vermicomposted kitchen biowaste for 18 months in outdoor conditions using *E. andrei*, and all pathogens decreased with the age of vermicomposting. Total coliform bacteria decreased from 8.7×10^3 to 3.3×10^3 CFU g^{-1}, and the analyses for *Salmonella* spp. were negative. Procházková et al. (2018) and Roubalová et al. (2019) vermicomposted apple pomace and grape marc with the addition of pathogenic bacteria (coliform bacteria, *Salmonella* spp., *E. coli* and *Enterococcus* spp.). The values of all pathogen bacteria decreased with the age of the vermicompost. In the case of *Salmonella* spp. and *Enterococcus* spp., the samples were negative at the end of vermicomposting. *E. coli* were <1 CFU g^{-1} in both experiments at the end of vermicomposting. The total coliform bacteria decreased in the experiment with apple pomace from 2.5×10^5 to 2.1×10^4 CFU g^{-1}, and in the experiment with grape marc, it decreased form 3.1×10^5 to 1.3×10^3 CFU g^{-1}. Flowerdew (2011) says that dog excrement is not suitable for composting due to its health risks. The results showed that during vermicomposting the content of pathogenic microorganisms decreases, and dog excrement should therefore no longer pose any health risks to the environment or humans at the end of the process.

At the vermicompost age of 51 weeks, six samples were taken (three from the test pile, three from the control pile), in which the presence of helminths or their eggs was determined. None of the samples taken showed the presence of helminths or their eggs. Many authors have examined the presence of helminths in dog excrement samples taken from various places in the city and the countryside. Dubná et al. (2007) analysed dog excrement in Prague and the countryside of the Central Bohemian Region. The total incidence of parasites in samples of excrement from Prague was 17.6%. The most common parasite was *T. canis*. Dogs in the countryside were infected far more often than those in the city were; the total incidence of parasites in rural samples was 41.7%. The survey showed that most dogs were infected in the autumn. Papajová et al. (2014) analysed 587 samples of dog excrement from eight cities and three villages in Slovakia. Overall, 29.9% of the samples were positive, which is a higher number than in the centre of Prague, but lower than the values from dog excrement obtained in Central Bohemia. The most common parasite was also *T. canis*. Pipková et al. (2017) conducted a study in two adjoining villages in the northeast of Slovakia ('City A' and 'City B'). City A was generally characterized by a lower level of hygiene; the total incidence of parasites in 127 samples of dog excrement was 71.65%. For city B, where the standard of hygiene was much higher, the incidence of parasites in 72 samples of dog excrement was 19.44%. This study points to a connection between the occurrence of parasites in dog excrement and the level of hygiene in a city. Felsmann et al. (2017) analysed dog excrement located in seven public places in the city of Chlumno, Poland. The average contamination with helminth eggs was 36.1%, which is higher than in Prague and Slovakia, but lower than in the Czech countryside and Slovak villages with low levels of hygiene. Cvetkova et al. (2018) conducted a study at forty public and private sites in Varna, Bulgaria. They collected samples from soil and sand. Of the of soil samples, 55% were negative, and helminth eggs were found in 45% of habitats. In this study, after 51 weeks of the vermicomposting process, all samples taken were negative for the presence of helminths and their eggs. Thus, vermicomposting appears to be an effective method of removing helminths.

The enzymatic activities measured in dog excrement were as follows: β-D-glucosidase 9549.3 μmol MUFG h^{-1} g^{-1}, acid phosphatase 10,919.7 μmol MUFP h^{-1} g^{-1}, arylsulphatase 431.5 μmol MUFS h^{-1} g^{-1}, lipase 11,268.9 μmol MUFY h^{-1} g^{-1}, chitinase 5209.1 μmol MUFN h^{-1} g^{-1}, cellobiohydrolase 5530.8 μmol MUFC h^{-1} g^{-1}, alanine aminopeptidase 524.8 μmol AMCA h^{-1} g^{-1} and leucine aminopeptidase 470.8 μmol AMCL h^{-1} g^{-1}.

The activity of β-D-glucosidase in the test pile decreased with the age of the vermicompost (from 1225.2 μmol MUFG h^{-1} g^{-1} to 611.4 μmol MUFG h^{-1} g^{-1}), but the trend was the opposite for the control pile (increased from 1253.0 μmol MUFG h^{-1} g^{-1} to 2498.4 μmol MUFG h^{-1} g^{-1}). The activity of β-D-glucosidase measured by Hřebečková et al. (2019b) in the vermicomposting heaps with household biowaste, malting sludge and grape marc was lower than the activity measured in the control pile. The highest value of β-D-glucosidase in their experiment was 1642 μmol MUFG h^{-1} g^{-1} in the vermicomposting heap with grape marc. The activity of acid phosphatase, chitinase and cellobiohydrolase in our experiment decreased gradually with the age of the vermicompost in the test pile. However, only the decrease of chitinase was found to be statistically significantly different between week 29 and week 51. The activities of chitinase and cellobiohydrolase were lower in the test variant, but only in weeks 41 and 51 (Table 4). This variant also contained lower amounts of fungi, which are the main producers of these two enzymes. Pramanik et al. (2007) analysed the acid phosphatase activity of several types of biodegradable waste (cow dung, fresh grass, weeds and municipal solid waste) after 70–85 days of vermicomposting using *E. fetida* earthworms. At the end of vermicomposting, the highest activity was measured in vermicompost from cow dung (approximately 180,000 μg *p*-nitrophenol g^{-1} h^{-1}), while the lowest activity was measured in the variant with municipal solid waste (approximately 120,000 μg *p*-nitrophenol g^{-1} h^{-1}). Chitinase activity measured by Lee et al. (2016) during the process of composting a mixture of biological waste (pig and poultry manure, spent mushroom substrate, sawdust, residues after processing rice, with the addition of fertilisers containing Mo, Zn, B, Mn and Cu) decreased with the age of the compost from 550 μg g^{-1} to 400 μg g^{-1} (after 90 days of composting). There was also a decrease in our test variant from 421.5 μmol MUFN h^{-1} g^{-1} to 152.0 μmol MUFN h^{-1} g^{-1}. The only statistically significant increase in our experiment was found in the activity of arylsulphatase in the test variant (from 2.5 μmol MUFS h^{-1} g^{-1} to 85.2 μmol MUFS h^{-1} g^{-1}). Tejada et al. (2010) dealt with the influence of vermicompost from cow dung, green fodder and their combinations, on soil properties.

Table 4. Enzymatic activities in test (A) and control pile (B).

	Test pile A	Control pile B
β-D-glucosidase (μmol MUFG h^{-1} g^{-1})		
29 weeks	1225.2 ± 570.2 a	1253.0 ± 424.3 a
41 weeks	830.0 ± 176.6 a	2146.2 ± 164.5 a
51 weeks	611.4 ± 230.2 a	2498.4 ± 366.5 a
Acid phosphatase (μmol MUFP h^{-1} g^{-1})		
29 weeks	2198.9 ± 551.1 a	1499.6 ± 388.0 a
41 weeks	1467.1 ± 180.6 a	2121.3 ± 207.9 a
51 weeks	1287.8 ± 150.8 a	1905.4 ± 262.3 a
Arylsulphatase (μmol MUFS h^{-1} g^{-1})		
29 weeks	2.5 ± 1.7 a	31.9 ± 14.4 a
41 weeks	40.4 ± 16.0 ab	35.2 ± 8.7 a
51 weeks	85.2 ± 10.5 b	58.1 ± 18.2 a
Lipase (μmol MUFY h^{-1} g^{-1})		
29 weeks	14,377.9 ± 2888.9 a	8205.5 ± 304.7 a
41 weeks	5861.6 ± 372.9 b	8256.9 ± 1095.4 a
51 weeks	7071.3 ± 239.9 ab	5560.3 ± 707.2 a
Chitinase (μmol MUFN h^{-1} g^{-1})		
29 weeks	421.7 ± 144.7 a	284.2 ± 65.0 a
41 weeks	170.0 ± 11.0 ab	353.3 ± 40.1 a
51 weeks	146.5 ± 21.5 b	261.8 ± 20.9 a
Cellobiohydrolase (μmol MUFC h^{-1} g^{-1})		
29 weeks	410.5 ± 85.6 a	271.7 ± 84.3 a
41 weeks	192.1 ± 11.2 a	873.7 ± 179.9 a
51 weeks	152.0 ± 49.4 a	759.0 ± 71.0 a
Alanine aminopeptidase (μmol AMCA h^{-1} g^{-1})		
29 weeks	76.2 ± 26.7 a	40.0 ± 2.9 a
41 weeks	26.4 ± 2.4 a	39.8 ± 7.7 a
51 weeks	31.9 ± 11.1 a	27.8 ± 9.9 a
Leucine aminopeptidase (μmol AMCL h^{-1} g^{-1})		
29 weeks	96.0 ± 25.6 a	76.4 ± 11.9 a
41 weeks	26.6 ± 3.1 a	80.5 ± 10.9 a
51 weeks	34.3 ± 16.3 a	37.5 ± 2.8 a

Values are the means ± SD ($n = 3$). Different letters in a column indicate significant differences between weeks (Kruskal-Wallis test, $p \leq 0.05$).

The vermicompost from cow dung had a better effect on soil properties, where arylsulfatase values were higher by 14.2% than in other variants. The activities of aminopeptidases in the test pile in our experiment were almost the same. At first, it decreased to 26 μmol h^{-1} g^{-1}, and then it increased to a value of about 30 μmol h^{-1} g^{-1}. In the case of the control pile, the highest measured activity of lipase was 8256.9 μmol MUFY h^{-1} g^{-1} in week 41, and in the test pile, it was 14,377.9. μmol MUFY h^{-1} g^{-1} in week 29. Lipase is an enzyme of the digestive tract, which indicates the high value of its activity in dog excrement. Arylsulfatase, alanine aminopeptidase and leucine aminopeptidase showed very low values of enzymatic activity in the control vermicomposting pile, which did not exceed 100 μmol h^{-1} g^{-1}. No statistically significant differences between weeks were found in the control pile.

In the test pile, a correlation was found between β-D-glucosidase activity and G+ bacteria content (0.69); phosphatase activity and fungi content (0.67) and G−bacteria (0.70); arylsulfatase activity and fungi content (−0.73) and G+ bacteria (−0.67); lipase activity and content of both fungi (0.77) and bacteria (0.72), especially G−bacteria (0.83); chitinase activity and content of both fungi (0.72) and bacteria (0.69), especially G−bacteria (0.76); cellobiohydrolase activity and content of both fungi (0.74) and bacteria (0.75), especially G+ (0.68) and G−bacteria (0.79); alanine aminopeptidase activity and content of fungi (0.67) and G−bacteria (0.72); leucine aminopeptidase activity and fungi (0.74) and G−bacteria (0.73). By the value of the correlation coefficient was also confirmed that the levels of individual enzymatic activities affect each other. In the control pile, there was a high correlation coefficient between enzymatic activity and the content of microorganisms only for the lipase activity, which should be positively influenced by the content of all groups of microorganisms; for chitinase activity, which can be affected by bacterial content (correlation coefficient 0.81), especially G+ (correlation coefficient 0.71) and G− bacteria (correlation coefficient 0.67); for leucine aminopeptidase activity and bacterial content (correlation coefficient 0.68).

Conclusion

Dog excrement poses a relatively significant risk due to the presence of pathogenic organisms. This risk can be eliminated by collecting excrement and the regular deworming of dogs. Vermicomposting dog excrement with waste from park maintenance can be an effective way to handle dog excrement and transform it into a high quality fertiliser. During the process of vermicomposting, there was a gradual decrease in the content of pathogenic bacteria. This vermicompost was also very rich in microorganisms and enzymatic activity. The pH value was slightly alkaline, and the C:N ratio corresponded to value of mature vermicompost. The test variant, with continuous feeding, showed a higher nutrient content at the end of vermicomposting, while the control variant, with a single feeding, showed higher enzymatic activity.

Acknowledgements

The authors would like to thank Proof-Reading-Service.com for professional proofreading of the English text.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by the Ministry of Agriculture of the Czech Republic under NAZV project No. QK1910095.

ORCID iDs

Tereza Hřebečková https://orcid.org/0000-0002-9586-3065

Lucie Wiesnerová https://orcid.org/0000-0002-9937-8403

References

Awasthi S, Bundy DA and Savioli L (2003) Helminthic infections. *British Medical Journal* 327: 431–433.

BSI EN 15933 (2012) *Sludge, treated biowaste and soil – Determination of pH*. The British Standards Institution.

ČSN 46 5736 (2018) *Vermikomposty. (Vermicomposts)*. Úřad pro technickou normalizaci, metrologii a státní zkušebnictví.

Cvetkova T, Stoyanova K and Paunov T (2018) Contamination with Toxocara spp. eggs of environmental samples of public places of Varna city, Bulgaria. *Journal of IMAB – Annual Proceeding Scientific Papers* 24: 2177–2180.

Domínguez J, Velando A and Ferreiro A (2005) Are *E. fetida* (Savigny, 1826) and *E. andrei* (Oligochaeta, Lumbricidae) different biological species? *Pedobiologia* 49: 81–87.

Dubná S, Langrová I, Nápravník J, et al. (2007) The prevalence of intestinal parasites in dogs from Prague, rural areas, and shelters of the Czech Republic. *Veterinary Parasitology* 145: 120–128.

Dunn JJ, Columbus ST, Aldeen WE, et al. (2002) *Trichuris vulpis* recovered from a patient with chronic diarrhea and five dogs. *Journal of Clinical Microbiology* 40: 2703–2704.

Felsmann M, Michalski M, Felsmann M, et al. (2017) Invasive forms of canine endoparasites as a potential threat to public health – A review and own studies. *Annals of Agricultural and Environmental Medicine* 24: 245–249.

Flowerdew B (2011) *Kompost. (Compost)*. Cham, Prague: Metafora.

Gómez-Brandón M, Lazcano C and Domínguez J (2008) The evaluation of stability and maturity during the composting of cattle manure. *Chemosphere* 70: 436–444.

Gómez-Brandón M, Lores M and Domínguez J (2013) Changes in chemical and microbiological properties of rabbit manure in a continuous-feeding vermicomposting system. *Bioresource Technology* 128: 310–316.

Hanč A, Částková T, Kužel S, et al. (2017) Dynamics of a vertical-flow windrow vermicomposting system. *Waste Management & Research* 11: 1121–1128.

Hřebečková T, Hanč A and Roy A (2019a) Changes of chemical and biological parameters during vermicomposting of kitchen biowaste with an emphasis on pathogens. *Waste Forum* 3: 187–196.

Hřebečková T, Wiesnerová L and Hanč A (2019b) Changes of enzymatic activity during a large-scale vermicomposting process with continuous feeding. *Journal of Cleaner Production* 239: 118127.

Jamaludin AA, Mahmood NZ and Abdullah N (2012) Waste recycling: Feasibility of saw dust based spent mushroom substrate and goat manure in vermicomposting. *Sains Malaysiana* 41: 1445–1450.

Lee YS, Choi SY, Lee JO, et al. (2016) Comparative assessment of enzyme activities and characteristics during composting of two types of composts. *Communication in Soil Science and Plant Analysis* 47: 1845–1855.

Lim SL, Lee LH and Wu TY (2016) Sustainability of using composting and vermicomposting technologies for organic solid waste biotransformation: Recent overview, greenhouse gases emissions and economic analysis. *Journal of Cleaner Production* 111: 262–278.

Matějů L (2009) *Metodický návod pro stanovení indikátorových organismů v bioodpadech, upravených bioodpadech, kalech z čistíren odpadních vod, digestátech, substrátech, kompostech, pomocných růstových prostředcích a podobných matricích. (Methodical instructions for the determination of indicator organisms in biowaste, treated biowaste, sewage sludge, digestates, substrates, composts, growth aids and similar matrices)*. Cham, Prague: Státní zdravotní ústav v Praze.

Mudrunka J, Lyckova B, Vrublova D, et al. (2020) Elimination of microbial pollution of domestic animal excrements using vermicomposting. *IOP Conference Series: Earth and Environmental Science* 444: 012040.

Papajová I, Pipiková J, Papaj J, et al. (2014) Parasitic contamination of urban and rural environments in the Slovak Republic: Dog's excrements as a source. *Helminthologia* 51: 273–280.

Pathma J and Sakthivel N (2012) Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential. *SpringerPlus* 1: 1–19.

Pipková J, Papajová I, Šoltys J, et al. (2017) Segregated settlements present an increased risk for the parasite infections spread in Northeaster Slovakia. *Helminthologia* 54: 199–210.

Portal of the capital city of Prague (2018) *V Praze je přihlášeno více než 80 tisíc psů. Nejčastějšími jmény jsou Ben, Max a Bety. (More than 80,000 dogs are registered in Prague. The most common names are Ben, Max and Bety)*. MHMP.

Pramanik P, Ghosh GK, Ghosal PK, et al. (2007) Changes in organic – C, N, P and K and enzyme activities in vermicompost of biodegradable organic wastes under liming and microbial inoculants. *Bioresource Technology* 98: 2485–2494.

Procházková P, Hanč A, Dvořák J, et al. (2018) Contribution of *Eisenia andrei* earthworms in pathogen reduction during vermicomposting. *Environmental Science and Pollution Research* 25: 26267–26278.

Quinn PJ, Markey BK, Leonard FC, et al. (2004) *Veterinary Microbiology and Microbial Disease*. Oxford: Cham: Blackwell Science.

Riwandi R, Muktamar Z, Hasanudin H, et al. (2022) The quality of vermicompost from various sources composted with earthworm *Perionyx excavates. IOP Conference Series: Earth and Environmental Science* 1005: 012006.

Roubalová R, Procházková P, Hanč A, et al. (2019) Mutual interactions of *E. andrei* earthworm and pathogens during the process of vermicomposting. *Environmental Science and Pollution Research* 27: 33429–33437.

Strube C, Heuer L and Janecek E (2013) *Toxocara* spp. infections in paratenic hosts. *Veterinary Parasitology* 193: 375–389.

Tejada M, Gómez I, Hernández T, et al. (2010) Utilization of vermicomposts in soil restoration: Effects on soil biological properties. *Soil Biology and Biochemistry* 74: 525–532.