Elimination of microbial pollution of domestic animal excrements using vermicomposting

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Abstract. Based on the theoretical knowledge of vermicomposting, a project was realized for the construction of a three-chamber domestic wooden vermicomposter, in which aerobic degradation of three types of animal excrements (cow, pig, dog) using the earthworm Eisenia andrei was carried out. Before laying the individual excrements to the compost batch, the appropriate input samples were taken for the microbiological examination of the biopathogens. After six months, final samples of the final substrate were taken to determine whether proper compost sanitization took place during the vermicomposting process; according to valid legislation, the bacteria Escherichia coli, Enterococcus sp. and Salmonella sp. were identified as indicator micro-organisms. After the evaluation of the performed laboratory analyses, it was proved that the use of earthworm bioactivity resulted in elimination or at least significant reduction of the concentrations of these bacterial strains in the final vermicompost samples.

1. Introduction

From the point of view of the microbiological evaluation of the compost, Enterobacteriaceae, Pseudomonadaceae and Bacillaceae are generally considered the most important groups of bacteria, however, a variety of other bacterial types and strains are always present in all input biowaste. Although they are mostly mesophilic bacteria, which are supposed to perish when they the temperature of 60°C is reached in the compost fill, there is always a risk of their survival in some layers of the maturing substrate or their secondary input, for example by transfer on bodies or digestive tracts of insects (Insecta), etc. [1]. It is also clear that the spores of bacteria are able to survive even higher temperatures [2, 3]. Therefore, both legal and technical standards require the implementation of a quality assessment of composts due to the presence and concentration of so-called indicator microorganisms, i.e. thermotolerant coliform bacteria Salmonella sp., Enterococci and Escherichia coli [4].

Therefore, it is necessary to ensure the proper sanitization of ripening substrates as well as composting output products, in particular by strictly adhering to all the established working and technological procedures while meeting all legislative requirements [5, 6]. However, it is always desirable to research alternative methods of optimizing the quality of composts. One of the most interesting options seems to be vermicomposting, which is essentially biodegradation and stabilization of input biologically degradable waste by the bioactivity of the Oligochaeta, which represent a significant part of the soil macrophage communities. Of many species of Lumbricidae, it is generally advisable to use the earthworm Eisenia andrei (red Californian hybrid), in some cases also Eisenia fetida, or other species for vermicomposting [7]. All species of earthworms thrive best in the substrate with a moisture content of about 75 % and at a temperature in the range of 18 - 25 °C, preferably in neutral pH environment and with a relatively high content of oxygen in the composting pile, at minimum 15 %.
The process of vermicomposting itself can be divided into two phases; in the first phase, the input biowaste in the digestive tract of the earthworms is processed, including the fundamental modification of the condition and composition of the present microflora. In the following phase, most earthworms move to the newly deposited layers of fresh biodegradable materials, while in the layers they have left, microbial degradation of the maturing substrate and its stabilization occurs [8].

Since the excrements of domestic and farm animals are also usually deposited in the pile of vermicomposts, either separately or as part of manure with litter, while the variety of surviving microorganisms is immense, it is necessary to closely monitor the presence of bacterial species in the final products, which are demonstrably or potentially hazardous to human, animal and plant health. In the course of vermicomposting, the temperature inside the pile is maintained in the mesophilic range; in fact, this prevents the necessary sanitisation of the compost, which requires a substantially higher temperature. However, in the professional literature, there may be quite convincing claims that earthworms can eliminate the presence of many biopathogenic microorganisms during the digestion process, or at least significantly contribute to the inhibition of their colony growth [9, 10, 11]. Therefore, the experiment, whose characteristics and results are presented in the following text, focused on the research of a real reduction in the number of so-called indicator biopathogens in vermicomposting solid animal excrements originating from cattle farming (*Bos primigenius f. taurus*), pig farming (*Sus scrofa f. domestica*), and domestic dog keeping (*Canis lupus f. familiaris*).

2. Materials and methods

Due to the predetermined conditions of strictly separated vermicomposting of three animal excrement types and according to the available suitable materials, a vermicomposter made of squared timbers and laths was designed for the experiment; it was designed as a set of three separate boxes measuring 0.9*0.9*0.5 meters whose bottom had a trough-shaped gradient, and in the middle part it was provided with a perforation to remove excess moisture from the heap. The drained water (so-called “earthworm tea”) was collected in the collecting containers under the vermicomposter and was again used for the necessary the substrate moisturizing. The top covers of the boxes were made up of three openable lids.

The heap itself was laid in mid-July 2017, with 35 litres of compost that had already been decomposed (plant waste, grass, flowers, leaves) mixed with 1 litre of wood shavings to improve the ratio of C:N.

Stocking the pile with earthworms (*Eisenia andrei*) subsequently took place in two stages. At the beginning of June 2017, 10 litres of adult earthworms together with cocoons were added to each individual box with compost. After seven days, animal excrements started to be added to the pile (5 litres of cow manure were placed in the first box every week, 5 litres of pig manure were placed in the second box and 3 litres of dog excrements were added to the third box, previously wetted with water and mixed with 0.5 litres of corn meal and 3 litres of decayed grass to promote the fermentation process). Subsequently, after the next six weeks, the original earthworms were supplemented by other approximately 8,350 individuals per box, each box being prepared to decompose one type of excrement – cow, pig, and dog. The first type of vermicompost excrements was cow manure consisting of excrements and remains of hay and straw from bedding that came from the cattle farming (fifteen cows) in a small agricultural farm in northern Moravia. At the time of sampling, the animals were grazed on the meadow and provide with a supplemental feed of oat and wheat grout. Samples of pig manure were collected in the same farm from four pigs, which were fed mainly by boiled potatoes, grout and surpluses from the production of dairy products (milk, maas). The last sampled animal excrements were solid excrements of dogs who were at that time kept in the household of the project’s co-researcher and who were predominantly fed with the Royal Canin Maxi adult feed.

First, 5*25 ml of cow manure, 5*25 ml of pig manure and 5*25 ml of dog excrements were sent to the laboratory for bacteriological examination. According to a valid laboratory methodology the samples were inoculated into a basic set of solid agar plates allowing culturing intestinal bacterial pathogens (Columbia blood agar with 5 % of ram’s defibrinated blood, MacConkey agar, Hektoen agar, Slanetz-Bartley agar, etc.) and into the culture fluid medium (selenite). After culturing, readings and
specifications of the growing bacterial colonies were carried out again in accordance with the relevant methodologies used in the laboratory concerned.
Throughout the feeding of earthworms with excrements, the vermicomposter was placed in a garden open space. At the beginning of October 2017, due to temperature drops, it was insulated with polystyrene boards placed around the perimeter of the basic wooden structure to maintain the recommended constant internal temperature. In the middle of November 2017, when the outside temperature sporadically dropped to the freezing point in the morning, the undegraded top layers of the heap of the individual boxes together with the earthworms were wintered in the garden greenhouse, where the experiment continued until the beginning of February 2018. Then the experiment was finished, and the final samples for the microbiological examination of the intestinal pathogens found in the final vermicompost product were taken. Output samples were taken from each box, two on the surface layer of the compost mass and two on the inner bed of the heap, while preserving the conditions for the correct sampling of the composts. The samples were then passed to an accredited laboratory, which was followed by bacteriological laboratory testing, like in the case of the input samples according to the given methodology.

3. Results and discussion
From the obtained results of the examination of the input samples of the animal excrements and the output samples of the final vermicomposts it was evident that the indicator microorganisms decreased by at least one order in *Escherichia coli* and *Enterococcus* sp. in all kinds of substrates. Equally, the intestinal bacteria *Campylobacter* sp. was completely eliminated in the final samples of the compost, which was probably due to its microaerophilic nature. *Salmonella* sp., a third indicator microorganism monitored to determine the sanitization of the composting process, was not found in any of the input or output samples.
Bacterial strains that were found only in the output samples of the final product can be considered as microbial contamination originating from additional biowaste initially deposited in the compost heap, or as secondary substrate contamination caused by insects during the vermicomposting process. A summary listing of specified microbial taxa is given in Table 1.

| Taxon                      | Pig samples | Cow samples | Dog samples |
|----------------------------|-------------|-------------|-------------|
|                            | input       | output      | input       | output      | input       | output      |
| *Escherichia coli*         | yes         | yes         | yes         | yes         | yes         | yes         |
| *Proteus* sp.              | yes         | yes         | yes         | yes         | yes         | yes         |
| *Bacillus* sp.             | yes         | yes         | yes         | yes         | yes         | yes         |
| *Enterococcus* sp.         | yes         | -           | yes         | yes         | yes         | yes         |
| *Campylobacter* sp.        | yes         | -           | -           | -           | -           | -           |
| *Morganella morganii* sp.  | yes         | -           | -           | -           | -           | -           |
| *Sphingomonas paucimobilis*| yes         | -           | -           | -           | -           | -           |
| *Oligella ureolytica*      | -           | -           | yes         | -           | -           | -           |
| *Rhizobium radiobacter*    | -           | -           | yes         | -           | -           | -           |
| *Comamonas testosteroni*   | -           | -           | yes         | -           | -           | -           |
| *Acinetobacter lwaffii*    | -           | -           | yes         | -           | -           | -           |
| *Pantoea* sp.              | -           | -           | -           | -           | yes         | -           |
| *Acinetobacter ursingii*   | -           | -           | -           | -           | yes         | -           |
| *Candida albicans*         | -           | -           | -           | -           | yes         | yes         |
Citrobacter freundii - yes - yes - yes
Enterobacter cloacae - yes - yes - yes
Pseudomonas putida - yes - yes - yes
Citrobacter brakii - - - yes - -
Lelliottia amnigena - - - yes - -
Aeromonas hydrophilia - - - - - yes

Bacterial strains of *Escherichia coli*, *Proteus* sp. and *Bacillus* sp. were present in the input and output samples of all types of excrements and vermicomposts. In the case of the bacterial strain *Enterococcus* sp., the presence was eliminated in the final substrate from cow and pig manure, while in the case of dog vermicompost, this bacterial strain was also present in the output samples, however, in a lower quantity.

In a particular case, the quantitative determination of colonies in primo-cultures of the individual samples was based on the individual cultivation soils where observation with the naked eye could be done. Already the initial comparison revealed a significant decrease in the quantity of colonies on cultivation soils. Conversions of concentrations for the three indicator micro-organisms are shown in Tables 2 and 3.

### Table 2. Concentrations of indicator microorganisms before vermicomposting.

| Sample          | *Escherichia coli* [CFU·g⁻¹] | *Enterococcus* sp. [CFU·g⁻¹] |
|-----------------|-------------------------------|-------------------------------|
| Cow manure      | 10⁶                           | 10⁴                           |
| Pig manure      | 10⁵                           | 10⁶                           |
| Dog excrements  | 10³                           | 10⁴                           |

*Legend to Table 2: CFU - colony-forming unit*

### Table 3. Concentrations of indicator microorganisms after vermicomposting.

| Sample          | *Escherichia coli* [CFU·g⁻¹] | *Enterococcus* sp. [CFU·g⁻¹] |
|-----------------|-------------------------------|-------------------------------|
| Vermicompost (cow) | 10³                          | 0                            |
| Vermicompost (pig) | 10²                          | 0                            |
| Vermicompost (dog) | 10³                          | 10²                          |

*Legend to Table 3: CFU - colony-forming unit*

In this context, there is an interesting comparison with the experiment carried out in 2016 at the Yazd Shahid Sadooghi University of Medical Sciences in Iran, where the possibility of reducing the number of faecal coliform bacteria by vermicomposting was verified. The input material used was sludge from a sewage treatment plant and biodegradable waste from households (especially fruit and vegetable residues), with the use of manure worms added to the heap (*Eisenia fetida*). The concentration of faecal coliform bacteria was determined at the beginning of the vermicomposting process and then at the end. After the first four weeks, the microbial biopathogens actually decreased significantly. At the end of the next four weeks, the number of bacterial colonies was evaluated from the samples of the final substrate. Although the concentrations of pathogenic bacteria were significantly reduced, the required limits were not reached [12]. It may be considered whether the period of vermicomposting was long enough for the proper course of microbial sanitization.

The results of the experiment also agree with the conclusions of the Edwards and Arancon (2010), which described the reduction of biopathogens in vermicomposting manure by up to 98% [9]. Rodríguez-Canché and Vigueros also found that vermicomposting of faecal material from septic tanks resulted in a significant reduction in microbial pathogen concentrations [13]. Also Karimi et al. (2017) observed vermicomposting of cow manure as a massive decrease in the number of faecal bacteria to
values permitted by Iranian fertilizer legislation [14]. Contreras-Ramos et al (2005) observed a similar fact in vermicomposting cow excrements [15].

4. Conclusion
The final vermicomposting products are generally considered to be high quality and stable natural fertilizers that not only increase crop yields but also improve arable land properties. For this reason, the research of the methods of vermicomposting is highly desirable; its importance increases with the promotion of the trend of material use of biodegradable waste. During the experiment with several animal excrements, a significant reduction in the number of pathogenic microorganisms (especially the bacteria \textit{Escherichia coli} and \textit{Enterococcus} sp.).

It can be concluded that domestic vermicomposting for degradation of animal excrements in terms of the environmental risk of contamination of the final product with microbiological pathogens proved to be effective as there was a real reduction in primarily found microorganisms by at least one order. However, it must be borne in mind that the results of the quantity of bacterial concentrations were detected and classified only approximately and in a rather small set. However, it was essential to find that colonies of indicator micro-organisms increased in none of the samples. For this reason, the solved experiment of the application of animal excrements as a vermicomposting additive can be evaluated as suitable for domestic use. However, prior to the industrial use of solid excrements of domestic animals in larger vermicomposters, further research is suitable and needed to evaluate the sanitization of biowaste contaminated with bacteria.

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