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Research article

Changes in layers of laboratory vermicomposting using spent mushroom substrate of *Agaricus subrufescens* P

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ABSTRACT

World mushroom production in 2018 was approximately 8.99 million tonnes. The most commonly cultivated species in the Czech Republic are *Agaricus* spp., which are sold fresh or canned. In 2017, 2018 mushroom production in the Czech Republic was approximately 540 tonnes. Vermicomposting is an easy and less ecologically harmful way to process the spent mushroom substrate. Earthworms, which are referred to as the engine of the process of vermicomposting, and microorganisms, help convert organic waste into fertilizer. This study is concerned with laboratory vermicomposting in a system of continuous feeding of earthworms *Eisenia andrei*. It compares the differences between variants with and without earthworms. The dry matter percentage was approximately 20% or more in both variants. The variant with earthworms showed a significant decrease in electrical conductivity. The C/N ratio was very low in both variants. The highest total P was recorded in the variant with earthworms, but the highest values of K and Mg were found in the control. Both variants recorded higher content of bacteria than fungi. All values of microorganism contents were higher in the vermicomposter without earthworms, but the bacterial/fungal ratio was higher in the variant with earthworms. The highest content in both variants shows the bacteria especially G-bacteria, on the other side, the lowest content shows the actinobacteria. The highest activity of β-D-glucosidase and acid phosphatase was measured in the vermicomposter with earthworms, but the activity of other enzymes was higher in the control. In both vermicomposters laccase activity was below the detection limit. The method of classical vermicomposting can be used for processing the spent mushroom substrate. However, in terms of higher content of total and available nutrients, there seems to be a better method of processing the substrate without earthworms.

1. Introduction

Agaricus subrufescens (Peck) grows wild in Brazil and this mushroom has had various names, such as *A. blazei* (Heinemann) and *A. brasiliensis* (Wasser) (Kerrigan, 2005). *A. subrufescens* is cultivated on the same substrate as *A. bisporus* (Antonín et al., 2013). To ensure high yield a casing layer must be added to the surface of the substrate; this casing layer must be porous for gas exchange, have good water holding capacity and not be toxic (Zied et al., 2012).

World production of mushrooms in 2018 was approximately 8.99 million tonnes, approximately 384 thousand tonnes in eastern Europe. The most commonly cultivated species in the Czech Republic are *Agaricus* spp., which are sold fresh or canned. In 2017, 2018 mushroom production in the Czech Republic was approximately 540 tonnes and approximately 680 tonnes of canned mushrooms were exported from the Czech Republic (FAO, 2020). The production of 1 kg of mushrooms generates approximately 5 kg of spent mushroom substrate (SMS) (Tu and Huang, 2005). In 2018 approximately 44.95 million tonnes of SMS was generated around the world, of which approximately 2.7 thousand tonnes came from the Czech Republic. This substrate must be processed and vermicomposting is an easy and ecologically less damaging method of achieving this.

Vermicomposting is defined as a mesophilic aerobic fermentation method of organic materials which uses certain types of earthworm to produce the (Hanč and Plíva, 2013); much of the primary organic matter is mineralized to carbon dioxide (CO_2), ammonia (NH_3) and water

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(H_2O) during the vermicomposting process. The remaining part is converted to stable organic matter similar to humus (Romero et al., 2007). Earthworms, which are referred to as the engine of the whole process, and microorganisms, which interact with earthworms, help convert organic waste into fertilizer (Champar-Ngam et al., 2010). Vermicomposting, like composting, takes place in different phases. In the case of vermicomposting, the active phase is where organic material is processed and converted, changing the physical properties and microbial composition (Lores et al., 2006). The maturation phase involves microorganisms only because the earthworms move into a new, fresher layer of bio waste (Aria et al., 2007). The length of both phases depends on the composition of the bio waste and the type of earthworm (Domínguez et al., 2010).

Vermicomposting can be performed either on a large or small scale. On a small scale it is possible to vermicompost waste directly in the home using vermicomposters, which contain individual trays that can be stacked. In home vermicomposting, it is necessary to maintain a constant temperature of approximately 20 °C and to irrigate the vermicomposted waste continuously. It is possible to filter out the vermicompost flow through the perforated bottom and collect it in the lower part of the vermicomposter. Vermicompost extracts can also be used as a fertilizer (Plíva et al., 2016).

Earthworms have a positive effect on plant growth: First, they assist in the decomposition processes and thereby accelerate mineralization and, secondly, they improve the overall physical, chemical and biological properties of the soil. They have a positive effect on the maintenance of crumbly aggregates, which improves the air and water regime of the soil, and this can reduce water consumption by up to 40% (Sinha et al., 2010). Earthworms affect the amount of nutrients in the soil, especially N, P, K and Ca, which are easily utilized by plants. A large proportion of these nutrients are contained in slime and earthworm excrements (Arthur et al., 2012). The activity of earthworms also affects the occurrence of soil microorganisms, which give vermicomposts a finer structure (Garg et al., 2006).

The nutrient content in vermicomposts is often much higher than in traditional composts (Dickerson, 2001) and it contains humus, growth hormones and still-active enzymes (Sinha et al., 2010). Vermicompost is mainly used as a fertilizer, for grass and other garden or house plants, or it can be used as a mulch (Dickerson, 2001), but it can also be used in combination with phytoremediation to remove polyaromatic hydrocarbons from contaminated soil (Wang et al., 2012). Vermicompost can also be used to prevent the occurrence of plant pathogens and plant parasites. According to Edwards and Arancon (2004), it reduces damage from aphids or caterpillars. The total nitrogen content in vermicomposts is statistically significantly higher than that in green fertilizers or manure, according to the findings of Bhadauria and Ramakrishnan (1996). Fertilization with vermicompost positively affects microbial activity in the soil and increases the formation of plant root systems (Wang et al., 2012). It also saves growers money by reducing the need for industrial fertilizers and pesticides (Sinha et al., 2010).

Although there are some studies concerning the vermicomposting of SMS (Song et al., 2014; Tajbakhsh et al., 2008a, Tajbakhsh et al., 2008b, etc.), many of them do not define the composition of the substrates or the species of mushrooms concerned. Most of them consider vermicomposting of combination of SMS and other bio waste in a single feeding system.

The aim (and novelty) of this study is in its determination of a large number of agrochemical and biological parameters in layers of different ages. Based on vermicomposting in a system of continuous feeding of earthworms *Eisenia andrei*, it compares the differences between the variants (variant with earthworms and control variant without earthworms) and their layers of different ages. The type of SMS used, and its preparation and cultivation are specified and the enzymatic activity of ten enzymes are determined, rather than just the basic agrochemical parameters, and the content of microorganisms are measured using phospholipid fatty acids (PLFA).

2. Material and methods

2.1. Experimental design

SMS was obtained from the mushroom growing room of the Department of Horticulture at the Czech University of Life Sciences (CULS) in Prague. Fermented wheat straw was used as cultivation substrate. A casing layer of sapropel peat was added on top when the substrate was fully colonized (after three weeks). *A. subrufescens* was cultivated at 24 °C and after the first flush of fruiting bodies the substrate was vermicomposted.

The vermicomposting experiment was set up in the laboratory of the Department of Agroenvironmental Chemistry and Plant Nutrition (CULS Prague) in May 2017 at Červený Újezd, in the Czech Republic. The vermicomposting process took place in vertical continuous feeding vermicomposters (Worm Factory). The experiment had two variants: with earthworms (*E. andrei*, density 50 pcs/kg) and without earthworms (the control).

The bedding layer, with 10 L of substrate and earthworms, was put into position first, then a layer of 10 L of SMS was added. Every 6 weeks a new layer of spent substrate was added. After the end of the experiment (in December 2017) three samples were collected from each layer except the bedding layer; both vermicomposters contained four layers. Earthworms were separated from each sample.

2.2. Agrochemical and biochemical analysis

The pH/H_2O and electric conductivity (EC) were measured using a WTW pH 340i and a WTW Cond 730 (1:5 w/v), according to BSI EN 15933. Percentage dry matter was determined after drying the samples to constant weight in a dryer at 35 °C. A CHNS Vario MACRO cube (Elementar Analysensysteme GmbH, Germany) was used to determine the C/N ratio, according to Hanč et al. (2017). The total macronutrient content (P, K and Mg) were determined using the wet method of decomposition in a closed system with microwave heating in an Ethos 1 system (MLS GmbH, Germany). The N–NH_4^+, N–NO_3^- and dissolved organic carbon (DOC) content and the available macronutrient (P, K and Mg) content were measured using CAT solution (0.01 mol l^{-1} $CaCl_2$ and 0.002 mol l^{-1} diethylene triamine pentaacetic acid (DTPA)) at the rate of 1:10 (w/v), in accordance with BSI EN 13651. Total and available element contents were determined using Inductively Coupled Plasma Optical Emission Spectrometry with an axial plasma configuration (ICP-OES, VARIAN VistaPro, Varian, Australia). The N–NH_4^+, N–NO_3^- and DOC contents were analysed colourimetrically using a Skalar San Plus System®.

The earthworms were separated from the vermicompost and counted manually, they were also washed and weighed in order to calculate biomass. Groups of microorganisms were detected using PLFA analysis, according to Hanč et al. (2017). The enzymatic activity of hydrolytic (β–D–glucosidase, acid phosphatase, arylsulphatase, lipase, chitinase, cellobiohydrolase, alanine aminopeptidase, leucine aminopeptidase) and ligninolytic enzymes (Mn-peroxidase and laccase) were measured in 96-well microplates: 0.2 g of lyophilized vermicompost was extracted using 20 mL of acetate buffer (pH 5.0, c = 50 mmol/L) in an Erlenmayer flask. The mixture was homogenized using an Ultra-Turrax® instrument (IKA Labortechnik, Germany) for 30 s at 8000 rev/min. The substrates used for the hydrolytic enzymes are shown in Table 1. Ligninolytic enzymes (Mn-peroxidase and laccase) were measured according to Košnář et al. (2019). Individual enzyme activities were measured in four replications using the Tecan Infinite® M200 (Tecan, Austria), according to Baldrian (2009) and Štursová and Baldrian (2011).

2.3. Statistical analysis

All the results are the means of three replicates. Analysis of variance was performed using the non-parametric Kruskal-Wallis test with

Table 1
Substrates used for the analysis of enzymatic activity.

Enzyme	Substrate	Concentration (mmol.L^{-1})
β – D – glucosidase	4-methylumbellyferyl-β-D-glucopyranoside (MUFG)	2.75
acid phosphatase	4-methylumbellyferyl-phosphate (MUFP)	2.75
arylsulphatase	4-methylumbellyferyl sulphate potassium salt (MUFS)	2.50
lipase	4-methylumbellyferyl-caprylate (MUFY)	2.50
chitinase	4-methylumbellyferyl-N-acetylglucosaminide (MUFN)	1.00
cellobiohydrolase	4-methylumbellyferyl-N-cellobiopyranoside (MUFC)	2.50
alanine aminopeptidase	L-alanine-7-amido-4-methylcoumarin (AMCA)	2.50
leucine aminopeptidase	L-leucine-7-amido-4-methylcoumarin (AMCL)	2.50

STATISTICA 12 software (StatSoft, Tulsa, USA).

3. Results and discussion

SMS dry matter was 25.5 ± 2.1%, pH was 8.0 ± 0.2, EC 4523.3 ± 559 μS/cm, C/N was 11.1 ± 0.3, the NH_4^+-N/NO_3^--N ratio was 6.9 ± 3.9 and DOC was 37,261 ± 4360 mg/kg. González-Marcos et al. (2015) composted SMS after cultivation of *A. bisporus*, which was cultivated on compost consisting of straw, poultry manure, gypsum and urea in plastic bags in a growing room. They found dry matter to be 47.4% and their pH value was 7.2. The EC was found to be very high in their experiment (6200 μS/cm). They measured a C/N ratio of 15.1, which some scientific literature points to as inadequate (Edwards et al., 2011), but it was a little higher than our value. Tajbakhsh et al. (2008a) vermicomposted a mixture of nonspecific spent mushroom compost (SMC) and cow dung. Their pH value of SMC before the experiment was lower than ours (6.83), and the C/N ratio was almost the same (11.39).

In both vermicomposters dry matter percentage decreased with the age of the layers, which was caused by the infiltration of water into the lower layers. pH values in the vermicomposter with earthworms increased with the age of the layers and ranged from 7.3 (Layer III) to 7.7 (Layer I). In the case of vermicomposter without earthworms it ranged from 7.2 (Layer II) to 8.6 (Layer III) (Table 2). EC values were lower in the vermicomposter with earthworms; in this vermicomposter it decreased with the age of the layers, the highest value (4833.3 μS/cm) was found in Layer IV (vermicomposter with earthworms) and the lowest (3533.3 μS/cm) in Layer I. In the vermicomposter without earthworms EC values were higher than 4000 μS/cm, which means that treatment with earthworms reduces the EC. This is probably due to processing by the earthworms themselves and passing through their digestive tract. The C/N ratio decreased from Layer III to Layer I, in both variants. However, in Layer IV (the youngest layer) the value was lower than in Layer III, in both cases. The highest ratio in the vermicomposter with earthworms was 13.4 (Layer III) and in the vermicomposter without earthworms it was 13.9 (Layer III). On the other hand, the lowest value was 11.6 in the vermicomposter with earthworms (Layers II and I) and 11.3 in vermicomposter without earthworms (Layer I). Suthar (2009) says that if the value of C/N ratio is lower than 20:1, than the compost is mature and if the C/N ration is equal or lower than 15:1, it indicates that the compost is higher agronomic value. However, all layers of both variants and the substrate itself used in our research had values lower than 15:1. Even so, based on the results of other parameters, especially the fact that in the variant with earthworms, earthworms survived in large numbers, it can be said that the process went correctly. Purnawanto et al. (2020) measured an initial C/N value for vermicomposting of a mushroom substrate of 24: 1, which is almost twice as much as us. However, they vermicomposted the substrate after growing oyster mushrooms (on a substrate of unspecified composition) with the addition of nitrogen. González-Marcos et al. (2015) composted SMS after the cultivation of *A. bisporus* in combination with both SMS after the cultivation of *Pleurotus ostreatus* and winery sludge (2:1:1) for 42 days in aerated compost bins and found that dry matter content was almost three times higher than ours; they measured values of approximately 56% and in our vermicomposters it was over 20%. The pH value in their compost was 7.3 after 42 days, which was almost the same as in our experiment. The EC decreased with the time of composting from 5400 μS/cm to 2100 μS/cm. Their C/N ratio was 12.5 after the end of composting. Tajbakhsh et al. (2008a) vermicomposted a mixture of nonspecific SMC with cow dung and other materials (potatoes, fruit and vegetables, pomegranates, tomatoes) in different ratios for three months in plastic containers in a single feeding system. They also reported an increase in pH values during the vermicomposting period, for all combinations. The EC in their experiment fell approximately 27%–45%, depending on the mixture, in our experiment EC decreased 27% in the variant with the earthworms. The C/N ratio significantly decreased, approximately 25%–57%, in their experiment. In our experiment it also decreased with layer age in both variants (about 13.4% in the variant with earthworms; approximately 18.7% in the control).

The NH_4^+-N/NO_3^--N ratio in our experiment was almost constant in the variant with earthworms but the highest value was 0.34 (Layer III) and the lowest was 0.01 (Layers II and I) in the control. In both cases no statistically significant differences were found, due to the high standard deviation in Layer III of the vermicomposter without earthworms. Atiyeh et al. (2000), who vermicomposted cow manure in laboratory conditions in a single feeding system using *E. andrei*, reported that nitrogen forms (NH_4^+-N and NO_3^--N) play an important role in the use of vermicompost as fertilizer. During the vermicomposting process earthworms accelerate the mineralization of nitrogen, so nitrogen is present in vermicomposts in the form of nitrates, primarily, and this is in line with our NH_4^+-N/NO_3^--N values, because the ratio was below one in both variants. Abouelwafa et al. (2008) suggested value of <1.0 for NH_4^+-N/NO_3^--N ratio for mature compost, and Benito et al. (2003) suggested value < 1.9 for a mature compost composed of a woodchip base, which is closer to the composition of the mushroom substrate. However, our values were lower in all layers than the values determined by them, which was mainly due to the composition of the initial substrate, but also probably to the high mineralization of nitrogen. The DOC values in

Table 2
Selected parameters of layers vermicomposters with and without earthworms.

Variant	Layer	Dry matter (%)	pH	EC (μS/cm)	C/N	NH_4^+-N/NO_3^--N	DOC (mg/kg)
With earthworms	IV	25.3 ± 2.7 a	7.4 ± 0.3 a	4833.3 ± 729.5 a	12.2 ± 2.1 a	0.03 ± 0 a	8951 ± 2059 a
	III	26.3 ± 2.2 a	7.3 ± 0.2 a	3974.3 ± 38.6 a	13.4 ± 1.2 a	0.02 ± 0 a	6121 ± 740 a
	II	23.1 ± 0.8 a	7.5 ± 0.2 a	3553.3 ± 127.0 a	11.6 ± 0.2 a	0.02 ± 0 a	7040 ± 615 a
	I	22.3 ± 0.1 a	7.7 ± 0 a	3533.3 ± 61.1 b	11.6 ± 0.2 a	0.02 ± 0 a	7151 ± 242 a
Without earthworms	IV	25.8 ± 0.7 a	7.9 ± 0.5 ab	4920.0 ± 183.3 ab	10.8 ± 0.1 ab	0.03 ± 0 a	14,312 ± 1466 a
	III	22.5 ± 0.2 ab	8.6 ± 0 a	4043.3 ± 90.2 ab	13.9 ± 0.1 ab	0.34 ± 0.3 a	13,657 ± 991 b
	II	20.9 ± 0.3 ab	7.2 ± 0.1 b	4296.7 ± 61.1 a	11.4 ± 0.4 a	0.01 ± 0 a	11,341 ± 701 ab
	I	18.6 ± 0.3 b	8.4 ± 0.2 ab	4706.7 ± 314.7 b	11.3 ± 0.9 b	0.01 ± 0 a	27,428 ± 1419 ab

Values are the means ± SD (n = 4). Different letters in a column indicate significant differences between layers (Kruskal-Wallis test, P ≤ 0.05).

the vermicomposter with earthworms ranged from 7040 mg/kg (Layer II) to 8951 mg/kg (Layer IV). In the variant without earthworms the values were 1.5–3.8 times higher than in the variant with earthworms, ranging from 11,341 mg/kg (Layer II) to 27,428 mg/kg (Layer I) (Table 2). However, the highest DOC value for the variant without earthworms was measured in the layer where the C/N ratio was only 11.3. This may be due to the leaching of DOC from higher layers, as the DOC content was significantly lower in the higher (younger) layers. The lower values of DOC in the variant with earthworms were probably caused by the activity of earthworms themselves because it is organic carbon and all the organic matter is decomposed by the earthworms. Zmora-Nahum et al. (2005) determined the value 4000 mg/kg DOC as the level indicating the compost has reached maturity. However, Hue and Liu (1995) recommended a higher value: 10,000 mg/kg of DOC. Our variant with earthworms only meets the values for mature compost reported by Zmora-Nahum et al. (2005). The variant without earthworms meets the values reported by Zmora-Nahum et al. (2005) as well as the higher values required by Hue and Liu (1995).

The number and biomass of earthworms recorded in the vermicomposter were as follows: Layer I (the oldest layer) 216 pcs/kg and 16 g/kg; Layer II 150 pcs/kg and 12 g/kg; Layer III 110 pcs/kg and 9 g/kg; Layer IV (the youngest layer) 27 pcs/kg and 3 g/kg. Thus, the biomass and the number of earthworms increase with the age of the layers, probably due to the very high EC value in the younger layers of the vermicomposter since earthworms are very sensitive to salinity. In the research done by Gong et al. (2019), with vermicomposting of garden waste, spent mushroom substrate (after cultivation of *P. ostreatus*) and cattle manure in different ratios in plastic bags for 10 weeks using *E. fetida* in the system of single feeding, the biomass of earthworms reached peak in week 6–8 and after that the biomass decrease to the end of the experiment, in all variants. Which indicates the mortality of earthworms, which probably did not occur in our research, because it was a system of continuous feeding, not single feeding.

The content of total and available nutrients in SMS was as follows: Ptot 5925 ± 403 mg/kg, Pavail 676 ± 95 mg/kg (about 11% of total content), Ktot 21,083 ± 2246 mg/kg, Kavail 16,834 ± 693 mg/kg (about 80% of total content), Mgtot 5743 ± 468 mg/kg, Mgavail 2922 ± 95 mg/kg (about 51% of total content). González-Marcos et al. (2015), who composted SMS after cultivation of *A. bisporus* cultivated on compost (consisting of straw, poultry manure, gypsum and urea in plastic bags in a growing room), reported higher total contents of macronutrients: P 6900 ± 0 mg/kg, K 22,000 ± 300 mg/kg, Mg 8300 ± 100 mg/kg. Tajbakhsh et al. (2008a) vermicomposted a mixture of nonspecific SMC and cow dung and their values for P and Mg were also higher than ours (P 12,700 mg/kg); Mg 13,900 mg/kg), but the value of K was less than half of ours (8702 mg/kg). Esmaielpour et al. (2017) used unspecified SMC as a fertilizer for *Ocimum basilicum* growing and they measured also higher values of P and K content then we did (P 15, 800 mg/kg; K 15,300 mg/kg). This could be due to the different composition of the SMS (SMC) and the addition of cow dung.

The highest recorded total and available P content was measured in the Layer II in the vermicomposter with earthworms (total 9893 mg/kg; available 494 mg/kg). The highest value of total K was found in the oldest layer (25,411 mg/kg), and the highest value of total Mg was measured in Layer II (7592 mg/kg). The highest values of available K and Mg were found in the youngest layer, Layer IV (K 18,943 mg/kg; Mg 2877 mg/kg). The lowest values of all macronutrients in the vermicomposter with earthworms were measured in the younger layers (Layers IV and III), except for available Mg, which was lowest in Layer I (2326 mg/kg). There were no statistically significant differences between layers for the vermicomposter with earthworms, except for available P, where statistically-significant difference were noted between Layers III and II (Fig. 1A). For the vermicomposter without earthworms, the highest total values of P, K and Mg were measured in the oldest layer (P 9526 mg/kg; K 30,880 mg/kg; Mg 9729 mg/kg) (Fig. 1B). Also, the highest available K was measured in Layer I (27,856 mg/kg), where it was approximately 90% available. However, the highest percentage of available K was found in Layer III (92%). The highest values of available P and Mg were measured in Layer II (P 790 mg/kg; Mg 3979 mg/kg), as the highest percentage of available P and Mg (P 9.2%; Mg 49%). Higher values in this variant compared to the variant with earthworms may be due to the accumulation of nutrients in the earthworms themselves (the earthworms were separated from the samples). In this vermicomposter we found statistically-significant differences in total P and K content and available Mg content, between layers. De Bertoldi et al. (1990) presented the ideal macronutrient values for composts to be used in agricultural applications, where the P value should be more than 0.43%, K > 0.41%, Mg > 0.2%, C/N < 18. Our highest values of macronutrients were higher than the values recommended for composts for agricultural application and the C/N ratio was lower throughout the process. González-Marcos et al. (2015), who composted a combination of SMS after cultivation of *A. bisporus*, SMS after cultivation of *P. ostreatus* and winery waste (2:1:1), measured the resulting values of macronutrients: P 13,000 mg/kg, K 3000 mg/kg, Mg 10,500 mg/kg after 42 days of composting. Their final values of P and Mg were higher than ours. However, the K value in their experiment decreased with composting duration (from 25,000 mg/kg to 3000 mg/kg), so it was lower than our values of K. Tajbakhsh et al. (2008a) vermicomposted a mixture of nonspecific SMC with cow dung and some other materials (potatoes, fruit and vegetables, pomegranate, tomatoes) in different ratios for three months in plastic containers in a single feeding system. Our highest value of total P in both variants was in agreement with their final value derived from a mixture of SMC with potatoes (2:1) (9533 mg/kg). The highest value for Mg in the variant with earthworms corresponded to their value measured in a variant with SMC and pomegranate (1:1) (8000 mg/kg), in our variant without earthworms it corresponded to their variant with SMC and pomegranate (2:1) (9967 mg/kg). Our highest value of K in both variants was 2–5 times higher than their final K values. Gong et al. (2019) also vermicomposted SMS (after cultivation of *P. ostreatus*), but in the combination with garden waste 1:1 in plastic bags for 10 weeks using *E. fetida* in the system of single feeding. Their values of total P and K after 10 weeks of vermicomposting were lower than ours (P 8500 mg/kg; K 13,800 mg/kg), but it can be due to the different composition of the SMS and its combination with garden waste.

The microorganism content of the SMS was as follows: fungal PLFAs 140.8 ± 8.9 $\mu g.g^{-1}dw$, bacterial PLFAs 183.4 ± 67.5 $\mu g.g^{-1}dw$, actinobacterial PLFAs 1.5 ± 1.0 $\mu g.g^{-1}dw$, G + PLFAs 51.1 ± 13.1 $\mu g.g^{-1}dw$, G- PLFAs 113.6 ± 56.3 $\mu g.g^{-1}dw$, total microbial biomass PLFAs 421.2 ± 74.8 $\mu g.g^{-1}dw$. The bacterial/fungal ratio was approximately 1.3 due to the high abundance of fungi.

In both vermicomposters the highest ratio of bacteria/fungi was found in Layer II (with earthworms 58:1; without earthworms 25:1) and the lowest in the youngest layer (with earthworms 28:1; without earthworms 16:1). Total bacterial, fungal, and G-content and total microbial biomass PLFAs were, in both cases, highest in the youngest layers (with earthworms: bacteria 167 $\mu g.g^{-1}dw$, fungi 6 $\mu g.g^{-1}dw$, G- 81 $\mu g.g^{-1}dw$, total microbial biomass 230 $\mu g.g^{-1}dw$; without earthworms: bacteria 325 $\mu g.g^{-1}dw$, fungi 21 $\mu g.g^{-1}dw$, G- 171 $\mu g.g^{-1}dw$, total microbial biomass 457 $\mu g.g^{-1}dw$). This was expected because the substrate itself contained a high amount of microorganisms and the microorganisms, together with the earthworms, are involved in the decomposition processes of organic matter. The lowest values in the control vermicomposter were recorded in Layer II (bacterial, fungal, actinobacterial, G+ and total microbial biomass PLFAs) and in Layer I (G- PLFAs). This was due to the low percentage of organic matter to decompose, which is confirmed by the lowest measured DOC value in layer II. The lowest concentrations were actinobacteria, which ranged from 8.5 $\mu g.g^{-1}dw$ to 11.5 $\mu g.g^{-1}dw$, but the lower actinobacteria content noted may be caused by the higher pH values (Rousk et al., 2010). In the vermicomposter with earthworms the lowest values were not found in the same layers, but the highest values were all in the youngest layer, Layer IV,

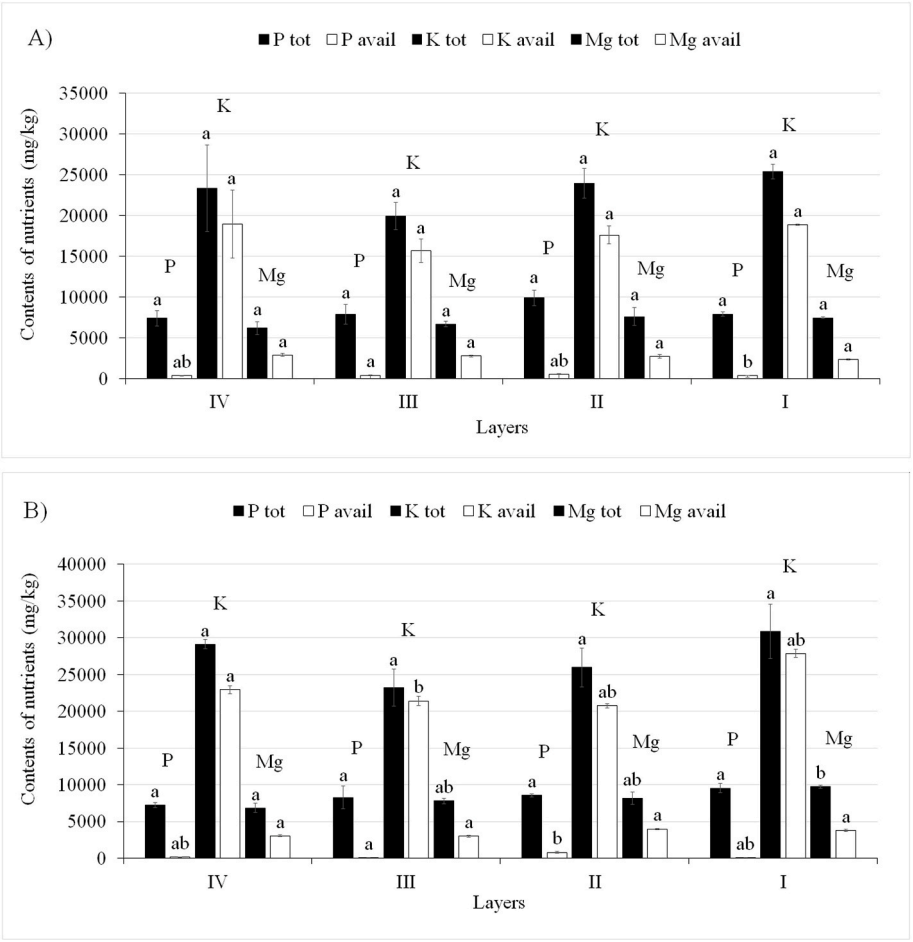

Fig. 1. Changes in total and available P, K and Mg in layers of vermicomposter with earthworms- A) and variant without earthworms- B). Values are the means ± SD (n = 4). Different lowercase letters indicate significant differences between layers (Kruskal-Wallis test, P ≤ 0.05).

except for actinobacteria, which was highest in the Layer II (10 μg.g^{-1}dw) and lowest in Layer IV (7 μg.g^{-1}dw) (Fig. 2A). All values of microorganism contents were higher in the vermicomposter without earthworms than in the vermicomposter with earthworms. However, the bacterial/fungal ratio was higher in the vermicomposter with earthworms. But this was expected, because most of the bacteria, but especially of fungi, also serve as food for earthworms. In the vermicomposter with earthworms no statistically-significant differences were found, but in the vermicomposter without earthworms there were significant differences between the layers as regards the bacteria, actinobacteria and G+ bacteria content, and also in total microbial biomass (Fig. 2B). Lower G- and G+ bacteria content, and also fungal content, can be caused by higher earthworm activity (Gómez-Brandón et al., 2012). Devi et al. (2020) also vermicomposted spent mushroom substrate, but it was a substrate after cultivation *P. ostreatus* in a combination with cow dung (3:1) processed in vermireactors for 60 days in a system of single feeding using three species of earthworms (*E. fetida, Eudrilus eugeniae, Perionyx excavatus*). They also measured very low content of actinobacteria in all variants (from about 0.5% in variant with *E. fetida* to about 6% in variant with *P. excavatus*), but the lowest content they measured in the fungi, where the values ranged from about 1.1% to 2.0%. In their control variant the percentage of fungal PLFA was lower than 0.5%, which means a very low content of fungi in the original substrate. The highest percentage of all groups of microorganisms they found in the content of G+ bacteria in the variant with *E. fetida* (about 12%), and in the content of G-in the variants with *E. Eugeniae* and *P. excavatus* (over 20%). We also vermicomposted the SMS after cultivation *P. ostreatus* (on a substrate consisted of wheat straw) in our previous research in laboratory conditions in vermicomposters for 180 days in a system of continuous feeding using *E. andrei* (Hřebečková et al., 2020). The values of the groups of microorganisms in our previous research were in most cases multiple times higher than the contents in this research with *A. subrufescens*, except the value of actinobacteria, which was lower in the experiment with *P. ostreatus* in variant without earthworms (≤7 μg.g^{-1}dw). However, these differences are due to the different composition of the culture medium and the species of mushroom grown, since *P. ostreatus* is a different type of mushroom (ligninolytic). Aria et al. (2011) vermicomposted cow manure in a metal pilot-scale vermireactor in a greenhouse in a system with continuous feeding (every three weeks). They divided the vermireactor into three parts (upper, medium, bottom) and measured bacterial, fungal and total PLFAs. In the bottom layer bacterial PLFAs were lower (about 400 μg.g^{-1}dw than in the upper layer (about 700 μg.g^{-1}dw). The fungal PLFAs were also lower in the bottom layer (about 12 μg.g^{-1}dw) than in the upper layer (about 20 μg.g^{-1}dw), as were total PLFAs. In our experiment the highest values were also recorded in the younger layers. The bacterial/fungal ratio in their experiment was approximately 30 across the profile, which is almost the same as we found in the oldest layer in the vermicomposter with earthworms. Fernández-Gómez et al. (2013) vermicomposted vegetable waste in combination with sludge from a paper mill (2:1) for 24 weeks using *Eisenia fetida*. They measured the PLFA values of microorganisms in the following order: bacteria 43 nmol PLFA. g^{-1}, G-bacteria 19 nmol PLFA. g^{-1}, G + bacteria 18 nmol PLFA. g^{-1}, fungi 8 nmol PLFA. g^{-1}, actinobacteria 4 nmol PLFA. g^{-1}. We also found the same order in most of the layers in both variants.

The enzyme activity in the SMS was measured as follows: β-D-

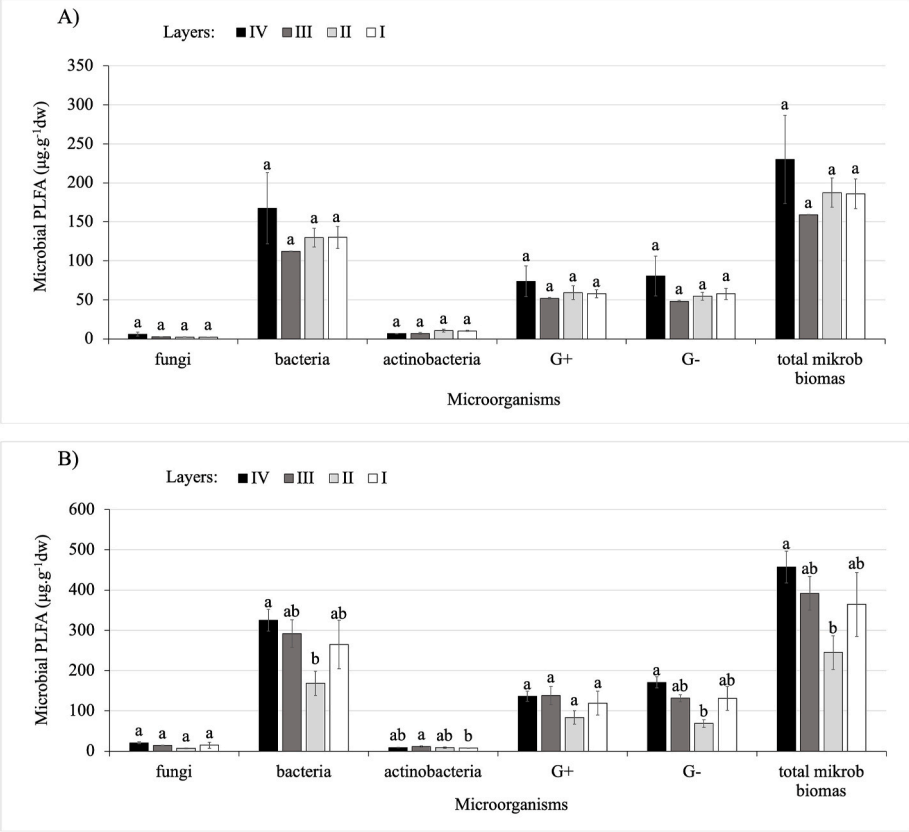

Fig. 2. Changes in microbial activity in layers of vermicomposter with earthworms- A) and variant without earthworms- B). Values are the means ± SD (n = 4). Different letters indicate significant differences between layers (Kruskal-Wallis test, P ≤ 0.05).

glucosidase 10,304 ± 3477 µmol MUFG. h^{-1}. g^{-1}, acid phosphatase 7419 ± 674 µmol MUFP. h^{-1}. g^{-1}, arylsulphatase 243 ± 70 µmol MUFS. h^{-1}. g^{-1}, lipase 7860 ± 167 µmol MUFY. h^{-1}. g^{-1}, chitinase 3534 ± 519 µmol MUFN. h^{-1}. g^{-1}, cellobiohydrolase 4659 ± 2155 µmol MUFC. h^{-1}. g^{-1}, alanine phosphatase 196 ± 83 µmol AMCA. h^{-1}. g^{-1}, leucine aminopeptidase 488 ± 184 µmol AMCL. h^{-1}. g^{-1}, and Mn-peroxidase 2.09 ± 0.09 mU.g^{-1}. Laccase was below the detection limit.

The highest β-D-glucosidase activity was in Layer II of both vermicomposters (with earthworms 2103 µmol MUFG. h^{-1}. g^{-1}, without earthworms 1586 µmol MUFG. h^{-1}. g^{-1}). The lowest value was in the youngest layer of the vermicomposter with earthworms (917 µmol MUFG. h^{-1}. g^{-1}). Activity increased with the age of the layers, from Layer IV to Layer II. In the case of the control, it was lowest in Layer III, where was also the highest value of pH, the lowest value of EC and the highest C/N ratio. However, in our previous research (Hřebečková et al., 2020) with vermicomposting SMS after cultivation *P. ostreatus*, we measured the highest value in the variant without earthworms in the youngest layer, where was the lowest value of pH, the highest value of EC and the lowest value of C/N ratio, but there were different composition of initial substrate. In this research were no statistically-significant differences between the layers, however statistically-significant differences were found between the layers of the vermicomposters, except for Layer II, where the highest activity occurred. Lazcano and Domínguez (2011) conducted an experiment assessing the effect of vermicompost on soil fertility and plant growth. They also studied the enzyme content in the soil after the application of vermicompost, conventional fertilizer and manure. The experiment showed that glucosidase content was statistically-significantly higher in the variant with vermicompost (120,000 µg p-nitrophenol. g^{-1}. h^{-1}) than in the variant with conventional fertilizer (100,000 µg p-nitrophenol. g^{-1}. h^{-1}). In our experiment the activity of β-D-glucosidase was also higher in the variant with earthworms. Unfortunately, the activity of β-D-glucosidase was multiple times higher in the unprocessed SMS. The highest level of acid phosphatase activity was in Layer II of the vermicomposter with earthworms (2819 µmol MUFP. h^{-1}. g^{-1}), as was β-D-glucosidase activity, in the vermicomposter without earthworms it was highest in the oldest layer (2238 µmol MUFP. h^{-1}. g^{-1}). The lowest values were in Layer III of both vermicomposters. In both vermicomposters no statistically-significant differences between the layers were found, but there were statistically-significant differences between the vermicomposters in the values for Layers II and III. Pramanik et al. (2007) also measured acid phosphatase activity, vermicomposting several types of biodegradable waste (cow manure, fresh grass, weeds, and municipal solid waste) for 70–85 days using *E. fetida*. They measured the highest activity after the end of experiment in the vermicompost from cow dung (about 180,000 µg p-nitrophenol. g^{-1}. h^{-1}), conversely, the lowest activity was found in the variant derived from municipal solid waste (about 120,000 µg p-nitrophenol. g^{-1}. h^{-1}). Lazcano and Domínguez (2011) performed an experiment testing the effect of vermicompost on soil fertility. They found that the variant fertilized by vermicompost had higher phosphatase content (160,000 µg p-nitrophenol. g^{-1}. h^{-1}) than the variant with conventional fertilizer (140,000 µg p-nitrophenol. g^{-1}. h^{-1}). Their value for the variant with vermicompost was lower compared to the highest value measured by Pramanik et al. (2007). In our experiment the activity of acid phosphatase was also higher in the variant with earthworms, but the highest activity was measured in the SMS, before vermicomposting. Arylsulphatase activity was highest in Layer I of the control vermicomposter without earthworms (203 µmol MUFS. h^{-1}. g^{-1}), in the vermicomposter with earthworms the highest value was found in Layer III (148 µmol MUFS. h^{-1}. g^{-1}). The lowest values were in Layer I in the vermicomposter with earthworms and in Layer II in the vermicomposter without earthworms. However, this values were higher than the values measured in our previous research with vermicomposting of three different types of biowaste (household biowaste, grape marc, sludge

from malt house) in outdoor conditions for 12 months using E. andrei (Hřebečková et al., 2019), where the values ranged from 13 μmol MUFS. g^{-1}. h^{-1} (layer I, grape marc) to 66 μmol MUFS. g^{-1}. h^{-1} (layer V, household biowaste). There were no statistically-significant differences between the layers and only one difference between the vermicomposters, in Layer I (Table 3). Mondini et al. (2004) composted cotton waste alone and in combination with garden waste and analysed arylsulphatase activity. Arylsulphatase activity was many times lower than the activity of other enzymes (β-glucosidase, acid phosphatase and alkaline phosphatase), but increased after 120 days and 160 days of composting. Arylsulphatase activity in our experiment was also multiple times lower in both variants than the activity of β-D-glucosidase or acid phosphatase. The highest activity of lipase (3091 μmol MUFY. h^{-1}. g^{-1}) and also of chitinase (351 μmol MUFN. h^{-1}. g^{-1}) was measured in the youngest layer of the vermicomposter with earthworms and in the oldest layer of the vermicomposter without earthworms (lipase 5227 μmol MUFY. h^{-1}. g^{-1}; chitinase 770 μmol MUFN. h^{-1}. g^{-1}). The highest values in our previous research with vermicomposting of SMS after cultivation of *P. ostreatus* (Hřebečková et al., 2020) were found in the younger layers (without e.- layer IV 8249 μmol MUFY. g^{-1}. h^{-1}; with e.- layer III 2881 μmol MUFY. g^{-1}. h^{-1}) and the highest chitinase activity in both vermicomposters was found in the oldest layer as it was in this research in the variant without e. (with e.- 646 μmol MUFN. g^{-1}. h^{-1}; without e.- 835 μmol MUFN. g^{-1}. h^{-1}). The values of chitinase in our previous research were higher than in this research, due to higher fungal activity. The lowest values of lipase were in Layer III of the vermicomposter with earthworms and in Layer II of the vermicomposter without earthworms. Chitinase activity was lowest in the oldest layer of the vermicomposter with earthworms and in Layer II of the vermicomposter without earthworms. A statistically-significant difference was found between chitinase activity in Layers I and II of the vermicomposter without earthworms. Statistically-significant differences were found in both lipase and chitinase activity between the vermicomposters in Layers IV, III and I. Lee et al. (2016) composted a mixture of bio waste (pig manure, poultry manure, sponged mushroom substrate, sawdust, rice residues with the addition of fertilizers containing Mo, Zn, B, Mn and Cu) for 90 days. The activity of chitinase measured in this compost decreased with composting duration, and ranged from 400 μg/g (at the end of the process) to 550 μg/g (at the beginning). In our experiment the activity of chitinase also decreased with the duration of the process in both variants, excepting Layer I in the variant without earthworms. The highest cellobiohydrolase activity was recorded in Layer II of both vermicomposters (401 μmol MUFC. h^{-1}. g^{-1}, variant with earthworms; 462 μmol MUFC. h^{-1}. g^{-1}, variant without earthworms). Conversely, the lowest value of this enzyme was, in both vermicomposters, measured in Layer III, but in the vermicomposter with earthworms this value (319 μmol MUFC. h^{-1}. g^{-1}) was almost double that of the vermicomposter without earthworms (Table 3). There were no statistically-significant differences between the layers of the vermicomposter with earthworms, but in the case of the vermicomposter without earthworms we found a statistically-significant difference between the highest and the lowest values of cellobiohydrolase. The lowest values of cellobiohydrolase activity in Layer III may be caused by coincidence with the highest values of C/N, which were found also in this layer. The highest activity of alanine and leucine aminopeptidase, and also of Mn-peroxidase, showed opposing trends between the vermicomposting variants. In the variant with earthworms the highest values of these enzymes were found in the youngest layers and the lowest alanine aminopeptidase and Mn-peroxidase values were measured in the oldest layer. Leucine aminopeptidase activity was lowest in Layer II. In the case of the variant without earthworms the highest values were measured in the oldest layers. The lowest values of alanine and leucine aminopeptidase were found in Layer II, the lowest value of Mn-peroxidase was measured in the youngest layer. This value (Mn-peroxidase) was 1.5-times lower than the lowest value measured in the variant with earthworms, but in neither vermicomposter were there any statistically-significant differences between the layers or variants. In our previous research with vermicomposting of SMS after cultivation of *P. ostreatus* (Hřebečková et al., 2020) was the Mn peroxidase activity higher in the vermicomposter without earthworms, especially in layer II, where it was 5.4 mU.g^{-1}, which is multiple times higher than in this research. However, this is due to the activity of *P. ostreatus*, a ligninolithic mushroom that directly produces Mn peroxidase. Values of leucine aminopeptidase were statistically-significantly higher in the variant without earthworms than in the variant with earthworms. Laccase activity was below the detection limit in both variants, as it was in the SMS. All values of enzymatic activity were higher in the SMS than in the vermicomposters, excepting only the highest alanine and leucine aminopeptidase values measured in the variant without earthworms.

Table 3
Enzymatic activity in variants with and without earthworms.

	Variant with earthworms	Variant without earthworms
β-D-glucosidase (μmol MUFG.h^{-1}.g^{-1})		
IV (45 days)	917.2 ± 231.9 aA	543.2 ± 96.5 aB
III (90 days)	1621.1 ± 135.6 abA	513.5 ± 194.5 aB
II (135 days)	2103.4 ± 521.7 bA	1586.1 ± 503.2 aA
I (180 days)	1463.7 ± 6.4 abA	848.9 ± 247.6 aB
Acid phosphatase (μmol MUFP.h^{-1}.g^{-1})		
IV (45 days)	2521.8 ± 626.4 aA	1995.1 ± 199.6 aA
III (90 days)	2381.6 ± 34.8 aA	1434.1 ± 43.1 aB
II (135 days)	2819.3 ± 1240.0 aA	1619.4 ± 345.6 aB
I (180 days)	2392.8 ± 444.7 aA	2237.5 ± 604.8 aA
Arylsulphatase (μmol MUFS.h^{-1}.g^{-1})		
IV (45 days)	134.9 ± 22.2 aA	118.1 ± 61.5 aA
III (90 days)	148.1 ± 16.8 aA	154.3 ± 36.7 aA
II (135 days)	147.7 ± 27.1 aA	112.5 ± 31.1 aA
I (180 days)	124.3 ± 25.0 aA	202.7 ± 18.8 aB
Lipase (μmol MUFY.h^{-1}.g^{-1})		
IV (45 days)	3091.3 ± 553.9 aA	4786.3 ± 809.7 aB
III (90 days)	2032.0 ± 379.4 aA	4249.8 ± 816.4 aB
II (135 days)	2330.7 ± 158.2 aA	3029.9 ± 639.5 aA
I (180 days)	2369.6 ± 280.8 aA	5227.2 ± 1502.9 aB
Chitinase (μmol MUFN.h^{-1}.g^{-1})		
IV (45 days)	350.5 ± 76.3 aA	495.5 ± 76.4 abB
III (90 days)	264.6 ± 35.9 aA	380.0 ± 42.1 abB
II (135 days)	244.6 ± 37.8 aA	293.2 ± 46.1 aA
I (180 days)	215.4 ± 31.2 aA	770.1 ± 186.8 bB
Cellobiohydrolase (μmol MUFC.h^{-1}.g^{-1})		
IV (45 days)	347.4 ± 99.1 aA	294.6 ± 8.4 abA
III (90 days)	319.1 ± 45.7 aA	166.3 ± 1.5 aB
II (135 days)	400.5 ± 77.9 aA	462.0 ± 157.3 bA
I (180 days)	371.4 ± 111.2 aA	270.9 ± 74.8 abA
Alanine aminopeptidase (μmol AMCA.h^{-1}.g^{-1})		
IV (45 days)	185.7 ± 91.4 aA	127.9 ± 7.1 aA
III (90 days)	103.6 ± 12.0 aA	158.3 ± 64.2 aA
II (135 days)	89.3 ± 28.4 aA	122.6 ± 75.5 aA
I (180 days)	76.8 ± 24.0 aA	281.8 ± 154.2 aB
Leucine aminopeptidase (μmol MUFG.h^{-1}.g^{-1})		
IV (45 days)	170.8 ± 92.8 aA	182.6 ± 38.2 aA
III (90 days)	81.0 ± 13.3 aA	227.9 ± 84.8 aB
II (135 days)	66.9 ± 31.9 aA	182.3 ± 36.4 aB
I (180 days)	73.8 ± 34.6 aA	512.0 ± 295.7 aB
Mn-peroxidase (mU.g^{-1})		
IV (45 days)	1.82 ± 0.18 aA	0.90 ± 0.90 aA
III (90 days)	1.44 ± 0.54 aA	1.71 ± 0.52 aA
II (135 days)	1.64 ± 0.11 aA	1.37 ± 0.96 aA
I (180 days)	1.41 ± 0.63 aA	1.94 ± 0.73 aA

Values are the means ± SD. Different lowercase letters in a column indicate significant differences between layers, capital letters indicate significant differences between variants (Kruskal-Wallis test, $P \leq 0.05$).

4. Conclusions

The percentage of dry matter was, in both vermicomposters, approximately 20% or above, which indicates the ideal humidity for the decomposition process. Electrical conductivity, indicating salinity, is one of the main problems encountered when unamended SMS is used. There was a significant decrease in electrical conductivity in the variant

with earthworms, compared to the original raw material, which means that earthworms are more suitable for processing spent mushroom substrate. The C/N ratio was very low in both cases, but the process still ran well. The highest total P was measured in the variant with earthworms, but the highest value of K and Mg was measured in the variant without earthworms, where the highest available content of these nutrients was also recorded. In both variants bacterial content was higher than fungal content. The highest content of fungi, and also of bacteria, was measured in the variant without earthworms. The highest values of enzymatic activity in the vermicomposter without earthworms were found in the oldest layers, except for β-D-glucosidase and cellobiohydrolase activity, where the highest values were found in Layer II. Enzyme activity in the vermicomposter with earthworms was the highest in the youngest layer in five cases (lipase, chitinase, alanine and leucine aminopeptidase, Mn-peroxidase). In other cases, it was in Layer II (β-D-glucosidase, acid phosphatase and cellobiohydrolase) or in Layer III (arylsulphatase). Laccase activity was below the detection limit in both vermicomposters. The highest activity levels of β-D-glucosidase and acid phosphatase were measured in the vermicomposter with earthworms, but the activity of other enzymes was higher in the vermicomposter without earthworms.

In terms of the overall and accessible content of the elements, the one that was processed without earthworms appears to be a higher quality product. However, this method does not result in such sanitation as in conventional vermicomposting using earthworms or in conventional composting, where high temperature plays a role. It would be appropriate to follow up the research by the process of composting the same implanted substrate and compare the data with this research.

The research showed that the vermicomposting method is suitable for processing the spent mushroom substrate produced by cultivation of *Agaricus subrufescens*. However, the substrate processed without earthworms showed higher values of total and available nutrients, and therefore it can be said from the point of view of plant nutrition that it is a higher quality product. However, this method does not eliminate pathogens such as conventional vermicomposting using earthworms or conventional composting with a thermophilic phase. It would be appropriate to follow up the research by composting the same spent mushroom substrate and compare the resulting data with this research.

CRediT authorship contribution statement

T. Hřebečková: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Visualization. **L. Wiesnerová:** Conceptualization, Formal analysis, Resources, Data curation, Writing - original draft. **A. Hanč:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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