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# Change in agrochemical and biochemical parameters during the laboratory vermicomposting of spent mushroom substrate after cultivation of *Pleurotus ostreatus*

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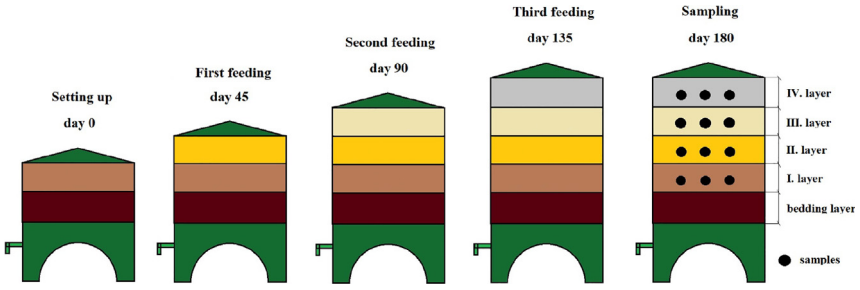
## HIGHLIGHTS

- Spent mushroom substrate after cultivation *P. ostreatus* was vermicomposted.
- Vermicomposting in the system of continuous feeding was applied.
- Ten hydrolitic and lignolitic enzymatic activities were measured.
- PLFA and other biological and agrochemical parameters were measured.

## GRAPHICAL ABSTRACT

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## ABSTRACT

*Pleurotus ostreatus* is one of the most cultivated mushrooms in the Czech Republic. The production of 1 kg of mushrooms generates about 5 kg of spent mushroom substrate. A gentle and fast method for using this substrate is vermicomposting. Vermicomposting of spent mushroom substrate using *Eisenia andrei* was conducted for seven months. For control purposes, a treatment without earthworms was also prepared. The vermicomposting process used vertical continuous feeding vermicomposters. The agrochemical and biological parameters were analysed. Values of electrical conductivity were very high in both vermicomposters (higher than 2000 μS/cm). During the vermicomposting process the C/N ratio decreased. The number and biomass of earthworms decreased with the age of the layers. The values of total P, K and Mg were higher in the vermicomposter without earthworms. There were also lower microbial phospholipid fatty acids content - than in the vermicomposter with earthworms. However, the fungal phospholipid fatty acids content were two times higher in the vermicomposter without earthworms. The highest hydrolytic enzyme activity was found in lipase, acid phosphatase and β-D-glucosidase. Most hydrolytic enzymes were more active in the vermicomposter without earthworms, with the exception of arylsulphatase. Mn-peroxidase activity was higher in the vermicomposter without earthworms and laccase activity was below the detection limit.



## 1. Introduction

Mushroom production is widespread throughout the world. Mushrooms are healthy foods, rich in vegetable proteins, chitin, vitamins and minerals and low in calories and fat (Manzi et al., 1999). The European Union produced about 1.9 million tonnes in 2016 and about

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1.3 million tonnes of mushrooms in 2017 (when the experiment ran). The most cultivated species in the Czech Republic is *Agaricus bisporus* but the second is *Pleurotus ostreatus*. In 2017, the world production of *P. ostreatus* was about 4.1 million tonnes (FAO, 2020). *P. ostreatus* is cultivated for food and medicinal purposes. It is cultivated on various lignocellulosic substrates such as wheat straw, sawdust, corncobs or cotton waste (Sánchez, 2010). The *Pleurotus* species is able to colonise and degrade a large variety of lignocellulosic residues due to the production of ligninolytic enzymes (Patrabansh and Madan, 1997). The production of 1 kg of mushrooms generates about 5 kg of spent mushroom substrate (SMS) (Tu and Huang, 2005), so in 2017 there was about 20.5 million tonnes of SMS generated from *P. ostreatus* cultivation around the world. This substrate must be removed somehow. There are many methods for this removal, including combustion, landfill, using it for soil and water remediation, using it as a soilless growing medium and using it for composting or as a cover soil for further mushroom cultivation (González-Marcos et al., 2015; Huan-Na et al., 2017; Lou et al., 2017). However, a gentler and faster method is vermicomposting. Vermicomposting is the biological non-thermophilic degradation of organic matter by earthworms and microorganisms (Edwards et al., 2011). SMS cannot be used for a soil immediately, because of its salinity. Therefore, vermicomposting, due to the activity of earthworms together with microorganisms involved in decomposition processes, provides a way to decrease the salinity of the substrate and transform it into a beneficial and nutrient-rich fertilizer.

The earthworms used in the vermicomposting process require a stable temperature of between 21 °C and 25 °C and the ideal moisture level about 80% (Edwards et al., 2011). The product of vermicomposting is called vermicompost. It has more available nutrients than the organic waste from which it is generated. There are, of course, some existing studies concerning vermicomposting of SMS (Izyan Nic Nor et al., 2009; Song et al., 2014; Tajbakhsh et al., 2008a), however, many of these studies do not define the composition of the substrate or the species of mushrooms used and most of them work with a combination of SMS and other biowaste.

Earthworms, microorganisms but also mushrooms produce a number of enzymes during decomposition processes. The enzymes produced by the mushrooms are mainly chitinase, cellobiohydrolase, β-D-glucosidase and arylsulphatase (Gooday, 1994; Mertz et al., 2007; Tabatabai and Bremner, 1971), but the *Pleurotus* species also produce ligninolytic enzymes such as a Mn-peroxidase or laccase (Patrabansh and Madan, 1997). Mn-peroxidase is the enzyme of white-rot fungi (Basidiomycota) involved in the oxidative degradation of lignin. The enzyme oxidises the bound $Mn^{2+}$ ion to $Mn^{3+}$ in the presence of hydrogen peroxide. $Mn^{3+}$ is released in the presence of a chelator (usually oxalate and malate), which stabilises it. The complex $Mn^{3+}$ ion can be diffused into a lignified cell wall, where it oxidises the phenolic components of lignin and other organic constituents (Brenda, 2018). Laccase participates in the oxidative degradation of lignin. It acts on o- and p-quinols, on aminophenols and often also on phenylenediamines. Laccase has the ability to oxidise benzene nuclei with their subsequent cleavage (Bamforth and Singleton, 2005).

The aim and novelty of this study lies in its determination of a large number of agrochemical and biological parameters across all layers of a vermicomposter. The study deals with vermicomposting within a continuous feeding system. The experiment assesses the development of individual parameters throughout the layers of both vermicomposters and compares the differences between the variants (variant with earthworms and control variant without earthworms). The study specifies the type of SMS used and its preparation and cultivation. The study, also, determines the enzymatic activity of ten enzymes and the content of microorganisms rather than just the basic agrochemical parameters.

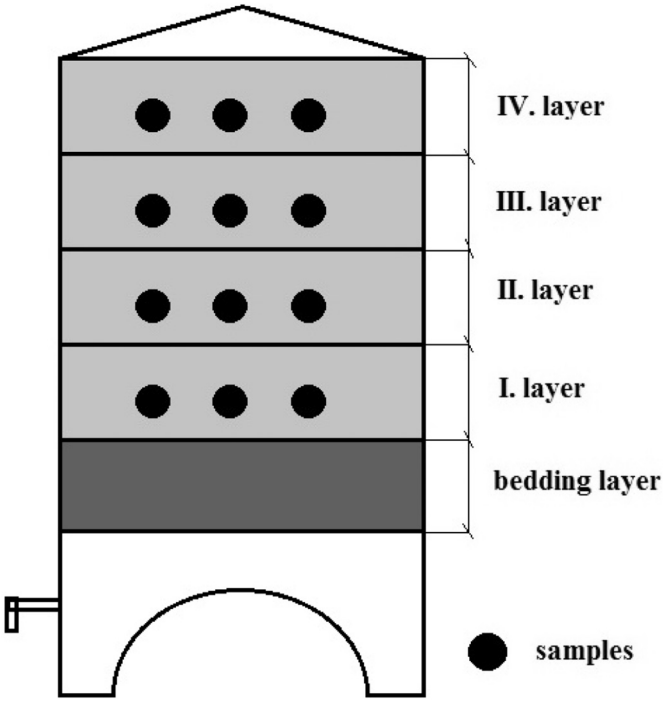


**Fig. 1.** Diagram of the vermicomposter.

## 2. Material and methods

### 2.1. Experimental design

The SMS was obtained from the mushroom growing room of the Department of Horticulture at the Czech University of Life Sciences in Prague. The substrate consisted of wheat straw and was fully colonised by the mycelium of *P. ostreatus*. The moisture content of the substrate ranged from 60 to 75% water. The substrate was thermally treated by pasteurisation at 90 °C for 24 h and then inoculated by the spawn of *P. ostreatus*. The culture was cultivated for 35 days. For the first 21 days the culture was in an incubation room at 24 °C, followed by 14 days in a growing room at 12 °C. After the first flush of fruiting bodies the substrate was vermicomposted.

The vermicomposting experiment was carried out in laboratory conditions at a vermicomposting laboratory at Červený Újezd in the Czech Republic. The vermicomposting process used a vertical continuous feeder (worm factory vermicomposter). The experiment was carried out in two variants, one with earthworms (*Eisenia andrei*, density about 50 pcs/kg) and the other without earthworms. Vermicomposting took place at a constant temperature of 22 °C. Vermicomposters were manually irrigated every 14 days to maintain ideal humidity (about 80%).

The bedding layer consisted of 10 L of substrate with earthworms, followed by 10 L of SMS as a new layer. Every one and a half months, a new layer of SMS was applied. The experiment ran from May till December 2017. At the end of the experiment both vermicomposters had four layers. Three samples were collected from each layer, as shown in Fig. 1.

### 2.2. Agrochemical and biochemical analysis

The pH/$H_2O$ and the electric conductivity (EC) were tested using a WTW pH 340i and WTW cond 730 (1:5 w/v), according to BSI EN 15933 (2012). The dry matter content was determined after drying the samples to a constant weight in a dryer at 35 °C. To determine the C/N ratio, a CHNS Vario MACRO cube (Elementar Analysensysteme GmbH, Germany) analyser was applied in accordance with Hanč et al. (2017). The total P, K, and Mg levels were determined by decomposition, utilising the wet method in a closed system with microwave

heating using an Ethos 1 system (MLS GmbH, Germany). The contents of N-$NH_4^+$, N-$NO_3^-$, dissolved organic carbon (DOC), and the available portions of P, K, and Mg were determined using the 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 the International BSI Standard EN 13651 (2001). The total and available element concentrations were determined using inductively coupled plasma optical emission spectrometry (ICP-OES, VARIAN VistaPro, Varian, Australia) with axial plasma configuration. The N-$NH_4^+$, N-$NO_3^-$ and DOC contents were measured colourimetrically using the SKALAR SANPLUS SYSTEM®.

The earthworms were separated from the vermicompost and counted manually. They were washed and weighed in order to calculate biomass. Groups of microorganisms were detected using phospholipid fatty acid (PLFA) analysis, in accordance with Hanč et al. (2017).

The enzymatic activity of hydrolytic enzymes (β–D–glucosidase, acid phosphatase, arylsulphatase, lipase, chitinase, cellobiohydrolase, alanine and leucine aminopeptidase) and ligninolytic enzymes (Mn-peroxidase and laccase) were measured in 96-well microplates. There was 0.2 g of lyophilised vermicompost extracted with 20 mL of acetate buffer (pH 5.0, c = 50 mmol.$L^{-1}$) in Erlenmayer flasks. The mixture was homogenised using the Ultra-Turrax (IKA Labortechnik, Germany) for 30s at 8000 rev/min. The substrates used for the enzymes were: MUFG (4-methylumbellyferyl-β-D-glucopyranoside, c = 2.75 mmol.$L^{-1}$) for β–D–glucosidase; MUFP (4-methylumbellyferyl-phosphate, c = 2.75 mmol.$L^{-1}$) for acid phosphatase; MUFS (4-methylumbellyferyl sulphate potassium salt, c = 2.50 mmol.$L^{-1}$) for arylsulphatase; MUFY (4-methylumbellyferyl-caprylate, c = 2.50 mmol.$L^{-1}$) for lipase; MUFN (4-methylumbellyferyl-*N*-acetylglucosaminide, c = 1.00 mmol.$L^{-1}$) for chitinase; MUFC (4-methylumbellyferyl-N-cellobiopyranoside, c = 2.50 mmol.$L^{-1}$) for cellobiohydrolase; AMCA (L-alanine-7-amido-4-methylcoumarin, c = 2.50 mmol.$L^{-1}$) for alanine aminopeptidase; and AMCL (L-leucine-7-amido-4-methylcoumarin, c = 2.50 mmol.$L^{-1}$) for leucine aminopeptidase. Mn-peroxidase activity was measured using a solution of DMAB (25 mmol.$L^{-1}$ 3,3-dimethylamino-benzoic acid), MBTH (1 mmol.$L^{-1}$ 3-methyl-2-benzothiazolinonehydrazone), $MnSO_4$ (2 mmol.$L^{-1}$), EDTA (2 mmol.$L^{-1}$ ethylenediaminetetraacetic acid), peroxide solution (0.08 mmol.$L^{-1}$) and succinate-lactate buffer (100 mmol.$L^{-1}$; pH 4,5), in accordance with Baldrian (2009). Laccase activity was measured using a solution of 150 μL citrate–phosphate buffer (2.1 g citric acid monohydrate $+3.56g Na_2HPO_4.2H_2O$) and 50 μL 0.08% ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid). Hydrolytic enzymes were measured for a change of fluorescence after 5 min and 125 min of incubating the microplates in the incubator (40 °C), with an excitation wavelength of 355 nm and an emission wave-length of 460 nm in accordance with Košnář et al. (2019a). Ligninolytic enzymes were measured spectrophotometrically (at 590 nm for Mn-peroxidase and 420 nm for laccase) for a change in absorbance every 2 min for 12 min (7 × 2 min). Individual enzyme activity was measured in four replications using the Tecan Infinite® M200 (Austria) in accordance with Baldrian (2009) and Štursová and Baldrian (2011).

### 2.3. Statistical analysis

All results are the means of the three replicates. An analysis of variance was performed using the non-parametric Kruskal-Wallis test ($P \leq 0.05$) using STATISTICA 12 software (StatSoft, Tulsa, USA).

## 3. Results and discussion

The dry matter of the SMS of *P. ostreatus* was 48.2%, pH value was 5.0, EC was measured at 2163.3 μS/cm. A high proportion of C/N (48.0) was found, which some scientific literature points to as inappropriate (Edwards et al., 2011). The $NH_4^+$-N/$NO_3^-$-N ratio was measured at 1.4 and the dissolved organic carbon (DOC) was about 49,234 mg/kg.

The dry matter in the vermicomposter with earthworms differed to the dry matter in the vermicomposter without earthworms. In the vermicomposter without earthworms the lowest amount of dry matter was found in layer I (the oldest layer) 15.3% (Table 1). This confirms that the highest humidity was in the bottom layer (Částková and Hanč, 2019). In the case of the vermicomposter with earthworms, the highest humidity was found in the layer IV (the youngest layer). This could be due to the recent watering of the substrate or the activity of the earthworms. The statistically significant difference was found only in the variant without e., between the layer I and the layer IV. In both vermicomposters the pH value ranged from 6.6 to 7.8. Statistically significant differences were found only between the layers of the vermicomposter with earthworms. The pH values in all layers of the vermicomposters were higher than in the SMS. The highest pH values were found in the layer I, which confirms the observation of Edwards et al. (2011), that the vermicomposting process increases the pH. In the vermicomposter without the earthworms the EC decreased with the age of the layers. The statistically significant differences were found between the layer I and the layer IV. However, in the case of the vermicomposter with earthworms the highest EC was in the layer IV (the youngest layer) (2228.3 μS/cm), while the lowest EC was in the second layer (2053.3 μS/cm). It decreased about 8%, but there were no significant differences between the layers. In the case of the variant without earthworms, it decreased about 33%, however, in the layer IV it was up to 48% higher than in the SMS. The EC values in the variant without earthworms were up to 1.9 times higher than the values in the variant with earthworms. This could be due to the activity of the fungi. SMS cannot be used as fertilizer due to high EC. However, earthworms feed on fungal cells (Schönholzer et al., 1999) and so the content of fungi in the variant with earthworms was considerably lower, and thus the EC value. The reduction of EC during vermicomposting confirms the experiment done by Tajbakhsh et al. (2008b), which vermicomposted spent mushroom compost (SMC) for 12 weeks using earthworms *Eisenia andrei* and *Eisenia fetida*. The EC of the vermicompost (8580 μS/cm) reduced about 40% when compared to week zero (14,650 μS/cm). All values of EC in the present study were higher than the EC of the SMC used by Bakar et al. (2011) (SMC 1560 μS/cm). After the cultivation of *Pleurotus sajor-caju*, they vermicomposted the SMC in plastic bags for 70 days using *Lumbricus rubellus*. The C/N ratio in the vermicomposter with earthworms was highest in the layer IV (the youngest layer) (16.9) and lowest in the layer I (the oldest layer) (14.1) (Table 1). In the vermicomposter without earthworms the results were the exact opposite; the highest value was found in layer I (24.7) and the lowest in layer IV (16.6). The values of the C/N ratio were higher in the vermicomposter without earthworms. It is possible to say that the vermicomposting process using *E. andrei* decreased the C/N ratio because the C/N (14.1) in layer I (vermicomposter with e.), where there was already matured vermicompost, was more than three times lower than the C/N of the SMS. Statistically significant differences were found only in the variant without e., as the C: N ratio was very variable between the layers. The reduction of C/N during the vermicomposting is in agreement with the findings of Tajbakhsh et al. (2008b), where the C/N ratio reduced by about 56%. A significant decrease in the C: N ratio can be caused by a reduction in the content of organic substances and the release of nitrogen during the mineralization process, or by washing the nutrients from the profile. Another possible reason is the higher content of nitrogen-binding microorganisms, as the anaerobic process (which occurs in the lower layers, where earthworms are no longer active) promotes nitrogen fixation (Ferrer et al., 2001). The low value of C/N confirms Senesi's (1989) findings, that a C/N ratio of <20 indicates an advanced degree of organic matter stabilisation and reflects a satisfactory degree of maturity of organic waste. The values of the N-$NH_4^+$/N-$NO_3^-$ ratio were really different in both vermicomposters, ranging from 0.95 (layer III, vermicomposter without e.) to 232.0 (layer II, vermicomposter with e.) (Table 1). Due to the high standard deviation,

**Table 1**
Agrochemical parameters of layers I-IV of each vermicomposter.

| Variant | Layer | Dry matter [%] | pH | EC [μS/cm] | C/N | $N\text{-}NH_4^+/N\text{-}NO_3^-$ | DOC [mg/kg] |
|---|---|---|---|---|---|---|---|
| With earthworms | IV | 17.3 ± 0.2 a | 7.8 ± 0.1 ab | 2228.3 ± 43.5 a | 16.9 ± 0.1 a | 180.7 ± 15.8 a | 19,360 ± 215 a |
| | III | 18.3 ± 0 a | 7.5 ± 0 ab | 2154.7 ± 15.0 a | 15.6 ± 0.1 a | 232.0 ± 34.6 a | 17,773 ± 214 ab |
| | II | 18.4 ± 0.1 a | 7.6 ± 0.1 a | 2053.3 ± 41.6 a | 14.2 ± 0.2 a | 52.1 ± 39.0 a | 14,927 ± 1946 ab |
| | I | 18.4 ± 0.2 a | 7.6 ± 0.1 b | 2143.3 ± 127.4 a | 14.1 ± 0.4 a | 50.7 ± 27.9 a | 13,375 ± 508 b |
| Without earthworms | IV | 19.9 ± 1.0 a | 6.6 ± 0.8 a | 4203.3 ± 289.4 a | 16.6 ± 1.1 a | 30.8 ± 26.3 a | 27,071 ± 232 a |
| | III | 15.7 ± 0.1 ab | 7.1 ± 0.1 a | 3492.7 ± 50.2 ab | 17.5 ± 0.1 b | 0.95 ± 0.7 a | 22,620 ± 1742 a |
| | II | 15.4 ± 0.1 ab | 7.0 ± 0.7 a | 3055.7 ± 25.0 ab | 16.8 ± 0.1 ab | 28.5 ± 0 a | 18,513 ± 281 a |
| | I | 15.3 ± 0.2 b | 7.1 ± 0.5 a | 2813.3 ± 73.7 b | 24.7 ± 2.0 b | 27.1 ± 3.5 a | 27,053 ± 1547 a |

Values are the means ± SD (n = 3). Different lowercase letters (a, b) in a column indicate statistically significant differences between layers (Kruskal-Wallis test, P ≤ 0.05).

especially for the vermicomposter with earthworms, no statistically significant differences were found between the layers of the individual vermicomposters. All values were multiple times higher than the value for the SMS, except for layer III of the vermicomposter without earthworms, where the value was only 0.95. However, this value was also higher than the values of the $N\text{-}NH_4^+/N\text{-}NO_3^-$ ratio in a 12-month-long vermicomposting experiment using grape marc and *Eisenia andrei* (Částková and Hanč, 2019), where the highest value was 0.45 in a three-month-old layer. While the values of DOC were higher in the vermicomposter without earthworms, where the DOC decreased with age up to layer II, and then increased in the layer I. No statistically significant differences were found. The DOC in the vermicomposter with earthworms decreased with the age of the layers and no statistically significant differences were found.

The number and biomass of the earthworms were determined (in vermicomposter with e.) and the values in the individual layers were as follows. The highest number, and also the largest biomass of earthworms, were found in the layer IV (the youngest layer), which was also the youngest layer. The number and biomass of the earthworms decreased with the age of the layers: layer IV- 929 pcs/kg, 146.3 g/kg; layer III- 465 pcs/kg, 70.6 g/kg; layer II- 405 pcs/kg, 46.6 g/kg; and layer I- 363 pcs/kg, 28.3 g/kg.

The values of total and available macronutrient content (P, K, Mg) for the SMS were as follows: total *P* value was about 725 mg/kg, from which about 307 mg/kg was the available P; total potassium content was measured at about 13,772 mg/kg and from that about 11,772 mg/kg was available K; total Mg content was about 1147 mg/kg and about 1032 mg/kg was available. González-Marcos et al. (2015) composted a combination of SMS after cultivating *Agaricus bisporus*, SMS after cultivating *P. ostreatus* and winery sludge. They tested raw materials and also measured the total P, K and Mg content. The values of all nutrients in the SMS after cultivation of *P. ostreatus* were higher than in our SMS (P 2600 mg/kg, K- 20000 mg/kg and Mg 3300 mg/kg).

The highest values of all macronutrients (P, K, Mg) in the vermicomposter with earthworms were found in the layer I (bottom layer), but there were no significant differences between any layers in total nutrient content (Fig. 2A). All content totals for P, K and Mg increased with the age of the layers. The highest available P content was also in layer I (849 mg/kg) and there was no statistically significant difference found between layer I and layer IV. The highest available K and also Mg levels were measured in the layer IV (K 19153 mg/kg; Mg 1044 mg/kg) (Fig. 2A). The minimum values of total and available nutrients were higher than the values in the SMS, except for the values of available Mg in layers I, II and III. The total values of P, K and Mg were higher in the vermicomposter without earthworms than in the vermicomposter with earthworms, but the highest values were found in different layers. Layer I of the vermicomposter with earthworms had the highest total values. The lowest content totals of P, K and Mg in the vermicomposter without earthworms were found in the layer I, while the highest content totals of P and Mg were measured in layer II (Fig. 2B). The highest content total of K (32,506.3 mg/kg) was found in the layer IV. The highest percentages of available P (38% of total content) and K (95.9% of total content) were found in layer III. The available content of Mg ranged from 1256.5 mg/kg (in layer III) to 1346.9 mg/kg (in layer I) (vermicomposter without e.). All values of total and available nutrient content were higher than those found in the SMS. Tajbakhsh et al. (2008a) also measured the total content of P, K and Mg in their experiment that recycled SMC for three months, with six variants using different agro-residues and *Eisenia andrei* and *Eisenia fetida*. In their experiment, the total K content decreased from 10% to 77% depending on the variant. According to them, a decrease in K could be caused by high water solubility and windrow leaching, but in our experiment, K increased with the age of the vermicompost. In their experiment, the total Mg content significantly increased from 1.29 to 2.67-fold. In our case it also increased, but not significantly. Total P content also increased in their experiment, as it did in our experiment. However, in their case it increased significantly—about 25% during the vermicomposting period—while in our case (variant with e.) there was an increase of 46%, but it was not statistically significant. Edwards and Lofty (1972) claim that an increase in P can be caused by mineralization resulting from the bacterial and faecal phosphate activity of earthworms, and also because of earthworm gut enzymes.

The SMS contained 54.5 ± 8.7 $\mu g.g^{-1}$dw PLFA of fungi and 15.3 ± 3.3 $\mu g.g^{-1}$dw PLFA of bacteria. The activity levels of G+ or G- bacteria were very low (G+ 0.6 ± 0.6 $\mu g.g^{-1}$dw PLFA; G- 6.0 ± 1.8 $\mu g.g^{-1}$dw PLFA) and the PLFAs for actinobacteria were zero. The total microbial biomass was about 103.6 ± 16.9 $\mu g.g^{-1}$dw PLFA. Gómez-Brandón et al. (2013) vermicomposted rabbit manure and also measured PLFAs, specifically fungal, bacterial and total PLFAs. Fungal PLFAs were many times lower in their rabbit manure than in our SMS (about 3 $\mu g.g^{-1}$dw PLFA), while their bacterial PLFAs (about 700 $\mu g.g^{-1}$dw PLFA) and total PLFAs (about 850 $\mu g.g^{-1}$dw PLFA) were many times higher.

The total microbial biomass content ranged from 599.6 $\mu g.g^{-1}$dw PLFA (layer I) to 971.2 $\mu g.g^{-1}$dw PLFA (layer IV) in the vermicomposter with earthworms (Fig. 3A), while a statistically significant difference was also found between these two layers in all microorganism activity, with the exception of the actinobacteria. The actinobacteria were almost suppressed—as with the fungi—but in the case of fungi, there was some statistical difference. This finding is in agreement with Hanč et al. (2017), who vermicomposted household biowaste for 12 months in outdoor conditions using a continuous feeding system and *Eisenia andrei*. The highest microorganism activity of all was found in the bacteria (771.9 $\mu g.g^{-1}$dw PLFA in layer IV), primarily G- bacteria, which ranged from 250.7 $\mu g.g^{-1}$dw PLFA (layer I) to 474.9 $\mu g.g^{-1}$dw PLFA (layer IV). This is similar to the findings of an experiment conducted by Částková and Hanč (2019), who vermicomposted grape marc in outdoor conditions in a continuous feeding system for 12 months using *Eisenia andrei*. However, their values of PLFAs (all values) were many times lower than in our experiment, where G+ bacteria showed the highest activity in the youngest layer, as did most of the microorganisms. The lowest activity levels of all microorganisms were measured in the layer I (the oldest layer). Activity of microorganisms in the vermicomposter without earthworms was lower than in the vermicomposter with earthworms, although the fungal PLFAs were two times higher (layer I- 34.4 $\mu g.g^{-1}$dw – layer IV- 49.1 $\mu g.g^{-1}$dw) (Fig. 2B). Epigeic earthworms possess a diverse pool of digestive

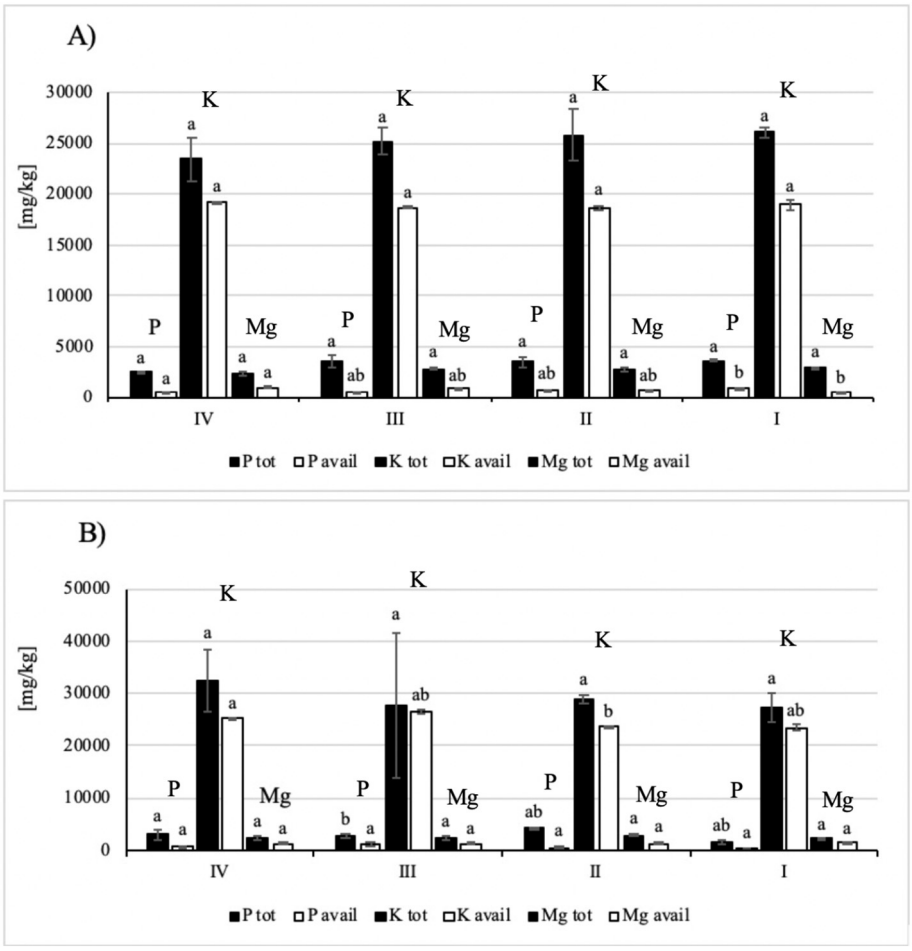


**Fig. 2.** Changes in total and available P, K and Mg in layers of vermicomposter with earthworms- A) and without earthworms- B). Values are the means ± SD ($n = 3$). Different lowercase letters (a, b) indicate statistically significant differences between layers (Kruskal-Wallis test, $P \leq 0.05$).

enzymes that enable them to digest fungi (Zhang et al., 2000), so this can cause lower fungal PLFA content. All of the highest activity levels of microorganisms in the vermicomposter without earthworms were found in the layer IV (the youngest layer), while the lowest activity levels were found in layer I. The total microbial biomass ranged from 445.3 μg.g$^{-1}$dw PLFA to 804.3 μg.g$^{-1}$dw PLFA. The bacteria represented the highest value of all the microorganisms. All activity in both vermicomposters, except fungal activity, was higher than in the SMS. Gómez-Brandón et al. (2013) vermicomposted rabbit manure for 250 days in polyethylene reactors using *Eisenia fetida* and a continuous feeding system. They also measured PLFA, bacterial, fungal and total PLFAs. All these PLFAs increased in the first 50–100 days of vermicomposting and then decreased with the age of the vermicomposting process. The total PLFAs ranged from 350 μg.g$^{-1}$dw PLFA (in 250-day-old vermicompost) to 800 μg.g$^{-1}$dw PLFA (100 days), which is almost identical to the findings of our experiment. Their fungal PLFAs ranged from 1 μg.g$^{-1}$dw PLFA (200–250 days) to 3.5 μg.g$^{-1}$dw PLFA (50 days), which is many times lower than in our experiment. Bacterial PLFAs ranged from 250 μg.g$^{-1}$dw PLFA (250 days) to 650 μg.g$^{-1}$dw PLFA (100 days), which is also almost identical to our experiment.

The enzymatic activity of hydrolytic enzymes in the SMS is shown in Fig. 4. The highest activity was seen in lipase (6834 μmol MUFY.g$^{-1}$.h$^{-1}$) and β-D-glucosidase (4037 μmol MUFG.g$^{-1}$.h$^{-1}$). On the other hand, the lowest values were seen in arylsulphatase (61 μmol MUFS.g$^{-1}$.h$^{-1}$) and alanine aminopeptidase (9 μmol AMCA.g$^{-1}$.h$^{-1}$). The activity of Mn peroxidase was 2.6 mU.g$^{-1}$ and of laccase was zero.

The activity of β-D-glucosidase was higher in all layers of the vermicomposter without earthworms. In the case of the vermicomposter without earthworms, activity decreased with the age of the layers. The highest β-D-glucosidase activity was measured in layer IV (3121 μmol MUFG.g$^{-1}$.h$^{-1}$). In the case of the vermicomposter with earthworms, β-D-glucosidase activity increased with the age of the layer and with the content of microorganisms, so the highest activity level was measured in the layer I (980 μmol MUFG.g$^{-1}$.h$^{-1}$) (Fig. 5A), where was the lowest number and the biomass of earthworms. However, the values of β-D-glucosidase activity were statistically significantly lower, than in the variant without e. This values are in agreement with Mondini et al. (2004), who composted cotton waste with yard waste for 180 days and also measured the increase alongside the age of the compost. Acid phosphatase activity was higher in layer I than in layer III in the vermicomposter with earthworms, but the activity was higher in the vermicomposter without earthworms, where the highest value was 7886 μmol MUFP.g$^{-1}$.h$^{-1}$ (layer IV) The value of acid phosphatase was lowest (6043 μmol MUFP.g$^{-1}$.h$^{-1}$) in the layer IV of the vermicomposter with earthworms (Fig. 5B), where was the highest number and the biomass of earthworms. All values were higher than in the SMS. In this case only one statistically significant difference between the vermicomposters was found, this was in the layer II. The arylsulphatase activity was higher in the vermicomposter with earthworms, where the highest value was measured in layer II (190 μmol MUFS.g$^{-1}$.h$^{-1}$) and the lowest value in layer III (171 μmol MUFS.g$^{-1}$.h$^{-1}$). The values of arylsulphatase activity in the vermicomposter without earthworms ranged from 92 μmol MUFS.g$^{-1}$.h$^{-1}$ to 176 μmol MUFS.g$^{-1}$.h$^{-1}$ (Fig. 5C). There were statistically significant differences found between the two vermicomposters, except for in layer I. All values were higher than in the SMS. Mondini et al. (2004), who composted

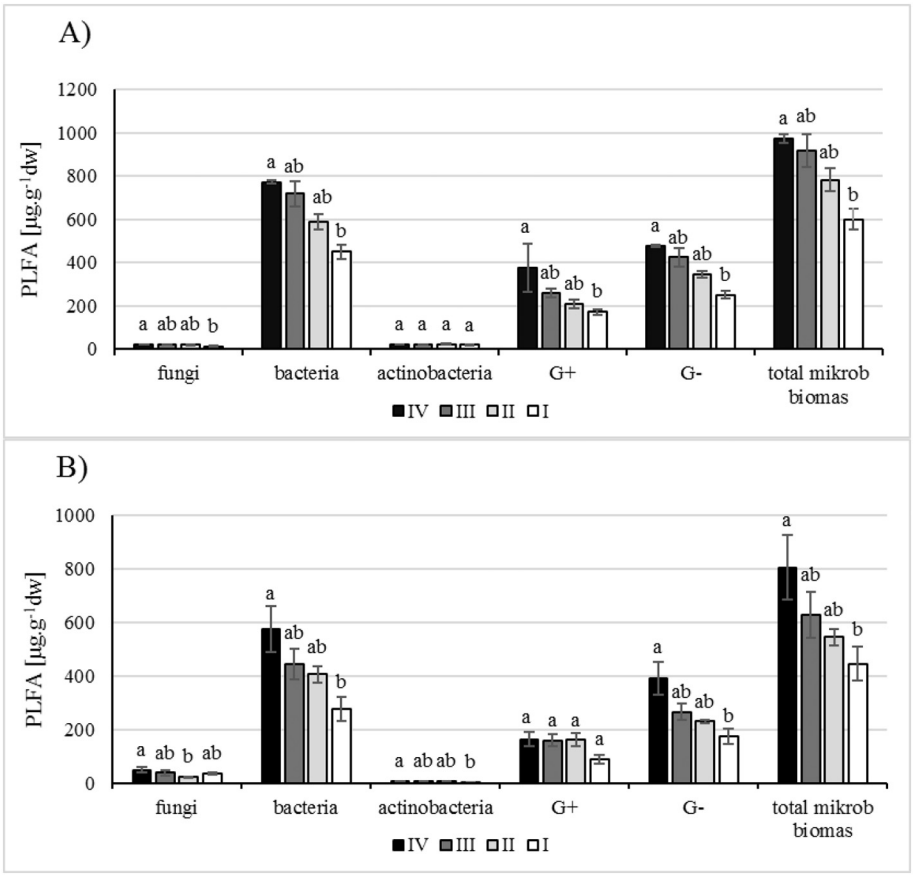


**Fig. 3.** Changes in microbial activity in layers of vermicomposter with earthworms- A) and without earthworms- B). Values are the means ± SD (n = 3). Different lowercase letters (a, b) indicate statistically significant differences between layers (Kruskal-Wallis test, P ≤ 0.05).

cotton waste and yard waste for 180 days, measured arylsulphatase activity and found several times lower arylsulphatase activity than β-glucosidase and acid phosphatase, as we did in our experiment. The lipase activity was 1.5–3 times higher in the vermicomposter without earthworms than in the vermicomposter with earthworms (Fig. 5D). According to Hřebečková et al. (2019), lower lipase activity may be due to higher pH values. The pH values in the vermicomposter with earthworms were up to 1.2 times higher than in the vermicomposter without earthworms. The highest values were found in the younger layers (without e.- layer IV 8249 µmol MUFY.$g^{-1}$.$h^{-1}$; with e.- layer III

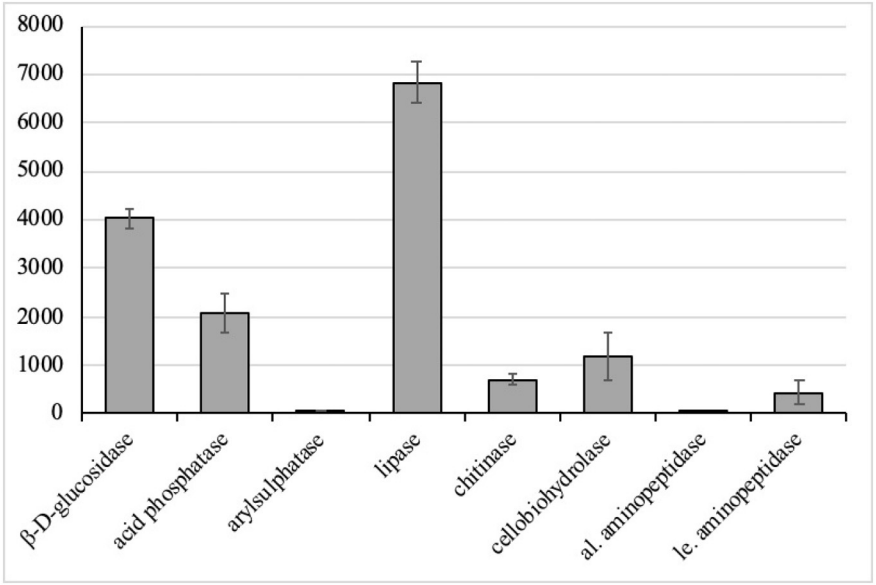


**Fig. 4.** The activity of hydrolytic enzymes in spent mushroom substrate after cultivation *Pleurotus ostreatus*. Values are the means ± SD (n = 3). Values are in µmol of substrate (MUF/AMC). $g^{-1}$.$h^{-1}$.

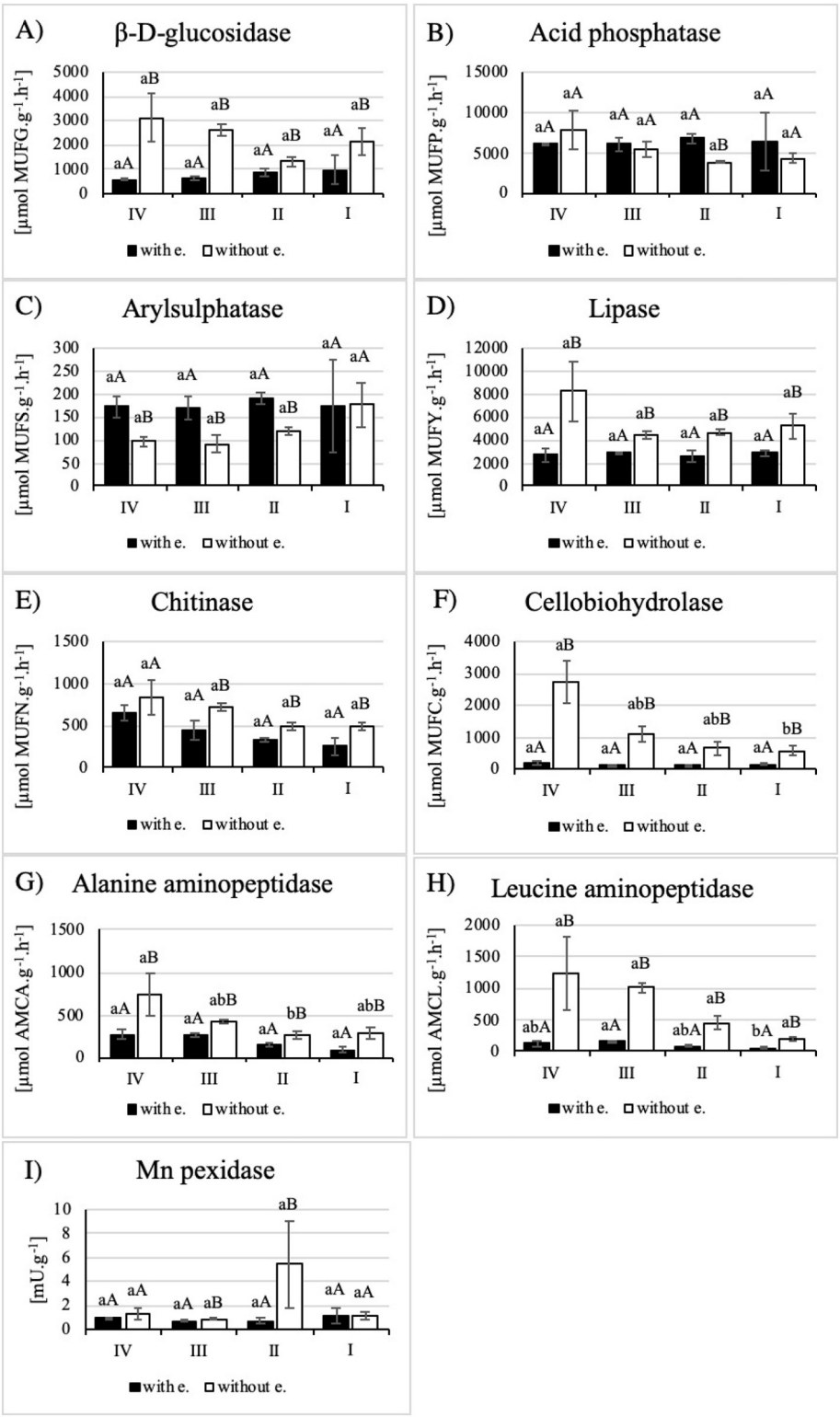


**Fig. 5.** Changes in enzymatic activity of chosen enzymes. Values are the means ± SD (n = 3). Lowercase letters (a, b) indicates statistically significant differences (Kruskal-Wallis test, $P \leq 0.05$) between layers, uppercase letters (A, B) indicate statistically significant differences between variants (vermicomposters) (Kruskal-Wallis test, $P \leq 0.05$).

2881 µmol MUFY.$g^{-1}$.$h^{-1}$). Statistically significant differences were found between vermicomposters. The highest chitinase activity in both vermicomposters was found in the layer I (with e.- 646 µmol MUFN.$g^{-1}$.$h^{-1}$; without e.- 835 µmol MUFN.$g^{-1}$.$h^{-1}$). In both cases chitinase activity decreased with the age of the layers (Fig. 5E). A decrease in chitinase activity as the process continues is consistent with an experiment done by Lee et al. (2016), which involved composting a mixture of pig and chicken manure, SMS, sawdust, rice hull and some fertilisers containing micronutrients. In layer I to layer III statistically significant differences were found between both vermicomposters. Chitinase is primarily produced by fungi, which were more active in the variant without earthworms; this is the main reason for the higher activity of chitinase in this vermicomposter. There were statistically significant differences between the variants – in layers I, II and III. The

activity of cellobiohydrolase was multiple times higher in the vermicomposter without earthworms than in the vermicomposter with earthworms (Fig. 5F). Cellobiohydrolase is also mainly produced by fungi, however, the fungi serve as a food for earthworms (Schönholzer et al., 1999) so in the vermicomposter without earthworms the fungi could be more active. The values of alanine aminopeptidase in all layers were multiple times higher than the value in the SMS. The highest values were found in the layer IV (with e.- 273 μmol AMCA. $g^{-1}.h^{-1}$; without e.- 746 μmol AMCA.$g^{-1}.h^{-1}$) (Fig. 5G) and they decreased with the age of the layers, as with the leucine aminopeptidase activity (Fig. 5H). In both cases, statistically significant differences were found between the vermicomposters. Activity of alanine aminopeptidase and also leucine aminopeptidase were higher in the vermicomposter without earthworms, which, according to Hřebečková et al. (2019), may be due to a higher value of EC. The EC was almost two times higher in this vermicomposter than in the vermicomposter with earthworms. Mn peroxidase activity was higher in the vermicomposter without earthworms, especially in layer II, where it was 5.4 mU.$g^{-1}$ (Fig. 5I). In layers II and III, statistically significant differences were found between the vermicomposters. All values of Mn peroxidase were higher than the values of Mn-peroxidase measured by Košnář et al. (2019b) (about 0.5 mU.$g^{-1}$), who used vermicomposting as a type of bioremediation of polycyclic aromatic hydrocarbons (PAHs). They also used a method of composting and found a statistically significant increase after 240 days. In our case, there was also some increase found (in layer II of the vermicomposter without e.), but it was not significant. Mn peroxidase was also measured by Luz et al. (2012), who cultivated *P. ostreatus* on different materials. The activity of Mn peroxidase on *P. ostreatus* (strains PLO 2 and PLO 6) growth in substrate based on sawdust increased between the fifth and tenth days (PLO 2 from 0.5 μM.$min^{-1}.kg^{-1}$ to about 0.9 μM. $min^{-1}.kg^{-1}$, PLO 6 from 1 μM.$min^{-1}.kg^{-1}$ to 2.5 μM.$min^{-1}.kg^{-1}$) and then decreased until the fifteenth day of cultivation (PLO 2 to 0.7 μM. $min^{-1}.kg^{-1}$, PLO 6 to 1.5 μM.$min^{-1}.kg^{-1}$). The activity of laccase was below the detection limit in all layers of both vermicomposters, as it was in the SMS. This finding is in the agreement with an experiment done by Košnář et al. (2019b), who used vermicomposting as a type of bioremediation of PAHs. Hřebečková et al. (2019) measured enzymatic activity during the vermicomposting of three different heaps. They vermicomposted household biowaste, malt house sludge with agricultural waste and grape marc using a continuous feeding system and *Eisenia andrei* in outdoor conditions for 12 months. The activity of β-D-glucosidase in the vermicomposter with earthworms was almost the same as the activity in all these vermicomposting heaps, but the activity in the vermicomposter without earthworms was higher than in the experiment done by Hřebečková et al. (2019). The activity levels of acid phosphatase and arylsulphatase were multiple times higher in both vermicomposters than the activity in their vermicomposting heaps. On the other hand, the activity of lipase was multiple times higher in their experiment than in ours, especially when compared to the vermicomposter with earthworms. In our experiment, the activity of chitinase was higher than the activity measured in their vermicomposting heaps that used malt sludge and grape marc. The activity in their vermicomposting heap of household biowaste was higher in layers I, III and IV than the activity of chitinase in both of our vermicomposters. The cellobiohydrolase activity in the vermicomposter with earthworms was almost comparable to their experiment, but the activity in the vermicomposter without earthworms was up to 36 times higher than the activity in their vermicomposting heaps. The activity of alanine aminopeptidase was higher in both vermicomposters than in their vermicomposting heaps. Leucine aminopeptidase activity in the vermicomposter with earthworms was comparable to the activity in their vermicomposting heaps, but the activity in the vermicomposter without earthworms was almost ten times higher. In their experiment, statistically significant differences were found between the individual layers for all enzymes except acid phosphatase and arylsulphatase. No statistically significant differences between layers were found in our experiment, except for the activity of cellobiohydrolase, alanine and leucine aminopeptidase.

## 4. Conclusion

It has been confirmed that the vermicomposting process increases pH value. Thanks to the increase in pH, vermicompost met the requirements of ČSN 46 5736 (2018) for the pH value for vermicomposts (6–9) and can thus be used as a fertilizer. The lowest value of EC was found in layer II of the vermicomposter with earthworms and the EC decreased about 8%. In the variant without earthworms the EC decreased about 33%, but the value in the layer IV was up to 48% higher than the value in the SMS. It is possible to say that the vermicomposting process using *E. andrei* decreased the C/N ratio. In this vermicomposter a higher $N\text{-}NH_4^+/N\text{-}NO_3^-$ ratio was found than in the variant without earthworms, but the DOC values were higher in the variant without earthworms. The number and biomass of the earthworms decreased with the age of the layers. All the content totals of P, K and Mg increased with the age of the layers in the vermicomposter with earthworms, but the highest were measured in the variant without earthworms. In the variant without earthworms, lower content of microbial PLFAs was found than in the vermicomposter with earthworms, although the fungal PLFAs were two times higher. Most hydrolytic enzymes and also Mn-peroxidase were more active in the variant without earthworms, due to the higher content of fungi. For most enzymes, statistically significant differences were found between vermicomposters. The laccase activity was below the detection limit in all layers of both vermicomposters, as it was in the SMS. Based on the measured parameters, it can be said that this method of vermicomposting appears to be suitable for processing the spent mushroom substrate.

## 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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