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# **Organic Matter Properties of Spent Button Mushroom Substrate in the Context of Soil Organic Matter Reproduction**

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**Abstract:** The objective of the work was to evaluate selected properties of spent substrates used for growing button mushrooms (SMSs) and the content and quality of the organic matter in this material in the context of rational use for fertilisation purposes and potential impact on the soil environment. The materials were sampled at production facilities located in the east of Mazovia. The density and amount of spent substrate on shelves where mushrooms were cultivated were determined. The following were analysed in the laboratory: reaction, carbonate content, TC (total carbon) and TOC (total organic carbon) contents, total nitrogen, organic matter fraction composition, and humic acids properties. It was confirmed that this material had a marked potential to enrich soils in organic matter, nitrogen, and carbonates. The analysis revealed that the most important qualitative properties of the organic matter were related to the relatively high share of labile organic compounds (the fraction separated with 0.05 M  $H_2SO_4$  and the fraction of fulvic acids). The humic acids had similar properties regardless of their origins. The humic acids (HAs) molecules displayed a substantial share of aliphatic structures which are typical of these materials at their initial decomposition stage. It can be assumed that, due to such properties, spent mushroom substrates are materials which can be directly introduced into the soil to improve their quality and prevent degradation.

Keywords: waste; spent mushroom substrates; carbon fractions; humic acids

# 1. Introduction

Poland has recently become a leading European producer of button mushrooms. The production cycle of these mushrooms in dedicated facilities lasts approximately six weeks. After the cycle is finished, it leaves spent substrate which, as organic waste, needs to be disposed of. The yearly output of this waste in Poland is estimated to approximate 1.5 million onnes. The technologies used at present result in this waste consisting of the following components:

- spent substrate for growing mushrooms—quantitatively, the most important component of the waste produced from compost based on cereal straw with an addition of chicken manure, peat, soy protein, urea, dolomite, and gypsum;
- a surface covering which is the top layer, several centimetres deep, from which button mushrooms grow out; it is produced from low peat with an addition of a component which contains CaCO<sub>3</sub> to neutralise acidity;
- mushroom mycelium which abundantly grows through the substrate used for cultivation;
- non-harvested mushrooms which are most frequently large or damaged [1,2].

In Poland, a popular method used to dispose of this waste material is to apply it for fertilisation purposes and introduce it to the soil. There are numerous published works on the reasonability of such a solution; the major focus of these papers is on the concentration



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). of nutrients in the waste which are likely to increase soil fertility and crop production profitability [3–5]. Spent substrate contains low amounts of heavy metals whose forms are unavailable for plants and safe for the environment [6]. Rational and systematic utilisation of organic waste in crop production reduces the amount of stored waste and increases soil production potential [7,8].

There is a relative paucity of scientific works on the issue of organic matter quality of spent mushroom substrate. So far, there has been no research conducted to determine physical parameters of the waste left after production on shelves of growing chambers, such as density. Such knowledge will make it possible to rationally utilise spent substrate left after growing button mushrooms, and more thoroughly estimate its effect on the amount and quality of soil organic matter.

Organic matter quality is largely determined by the quality of humic acids which constitute one of its major fractions and so participate in all the processes occurring in soil [9–11]. Formation of the humic substances results from transformation and decomposition processes which are collectively called 'humification'. The term 'humic substances' is used to scientifically describe specific components formed during the humification process and which may be isolated and fractionated in many ways [12]. Research on the properties of humic substances, which are characterised by a complex structure, therefore requires several instrumental methods [11].

The nature and origin of humic acids are reflected by such parameters as elemental composition [10,13–15] absorbance values of their solutions in the range UV-VIS (ultraviolet-visible)— $A_{280}$ ,  $A_{465}$ ,  $A_{665}$ , coefficients of absorbance  $A_{2/4}$ ,  $A_{2/6}$ ,  $A_{4/6}$ ,  $\Delta \log K$  [16–19], as well as hydrophilic and hydrophobic properties [20–22].

Due to increasing soil degradation and total organic C (TOC) losses following, e.g., changes in soil use and intensification of agricultural production, soil protection on a local, regional, and global scale has become one of the most important aims of the European Commission politics [23].

The research reported here was undertaken to check the hypothesis assuming that an application of spent mushroom substrate may add organic matter, nitrogen, and carbonates to soils, thus improving their quality and prevent their degradation. The aim of the research was to evaluate selected properties of spent substrates left after button mushroom growing, and the quality of their organic matter in the content of soil organic matter reproduction.

### 2. Materials and Methods

# 2.1. Research Site, Physical Properties, and pH

Waste material left after growing of common mushroom (*Agaricus bisporus*) was examined. The materials were sampled at production facilities located in the east of Mazovia. The area is well-known in Poland as a mushroom production cluster and a region of concentrated large-sized production facilities. Spent mushroom substrate (SMS) samples were collected in eight growing chambers of different production facilities, after button mushroom harvest and steam sterilisation of the chambers. Sampling took place from September 2019 to November 2019. The samples were taken at five points in each chamber in such a way as to calculate SMS density and reserves on growing shelves. The thickness (h) of SMS present on the shelves was measured. Next, SMS patches were cut out of an area of  $0.01 \text{ m}^2$  (using a  $0.1 \text{ m} \times 0.1 \text{ m}$  frame) and their weight (w) was recorded.

SMS density ( $D_{SMS}$ , kg m<sup>-3</sup>) was calculated according to the following formula:

$$D_{SMS} = w/v,$$

where:

w—SMS weight (kg),

*v*—volume (m<sup>3</sup>),  $v = h \cdot 0.01$ .

SMS reserves on the growing shelf (STOCK<sub>SMS</sub>, kg  $m^{-2}$ ) were calculated as follows:

The following parameters were determined in fresh SMS samples:

- dry matter (d.m.) content by weighing after a representative sample was dried at 105 °C;
- pH by the potentiometric method following pouring deionised water over the sample, (v/v = 1/5).

2.2. Chemical Properties

A representative part of an aggregate sample was dried at 40  $^{\circ}$ C and grounded (ø < 0.25 mm) in a ball mill. Next, laboratory analyses were conducted to determine:

- total carbon and nitrogen contents (TC and TN, respectively) by means of an elemental analyser Series II 2400, Perkin Elmer (TCD), Norwalk, CT USA and by using acetanilide as reference material to calibrate the device;
- carbon content in total organic compounds (TOC) by means of an elemental analyser following carbonate decomposition in a sample weighed out with H<sub>3</sub>PO<sub>4</sub> solution;
- carbon content in mineral compounds (IC) calculated using the formula

IC = TC - TOC;

- (approximate) CaCO<sub>3</sub> content estimated based on IC content

 $CaCO_3 = IC \cdot 8.33;$ 

- organic matter content (OM) calculated using the formula

 $OM = TC \cdot 1.724.$ 

2.3. Fractioning of Carbon Compounds

Sequential fractioning of carbon compounds was conducted using the Schnitzer method [24,25] (Scheme 1). The name, symbol, and procedure of obtaining carbon fractions are shown in Table 1.



Scheme 1. Diagram of sequential fractioning of the organic matter under study.

<b>Operational Carbon Fractions</b>	Procedure
Fraction after calcification Lab-C	Extraction with 0.05 M H <sub>2</sub> SO <sub>4</sub> ; extraction time 24 h; $m/v = 1/50$ ; centrifugation ( $g = 4000$ rpm) and filtration through a cellulose filter. Carbon in the solution was determined by the oxidative and titration method [26].
Lipid fraction Lip-C	Extraction (ethanol + n-hexane, $v/v = 1/1$ ) in an automatic solvent extractor SOXTHERM <sup>®</sup> (Gerhardt), Königswinter, Germany. The weight of bitumens was determined by weighing. Carbon was determined with an elemental analyser.
Humus substance fractions HS-C	Extraction with 0.1 M NaOH; extraction time = 24 h; $m/v = 1/50$ ; centrifugation ( $g = 4000$ rpm) and filtration through a cellulose filter. Carbon in the solution was determined by the oxidative and titration method.
Fulvic acid fractions FAs-C	Acidification (2.5 M $H_2SO_4$ , pH = 1.80) of the measured extraction part 0.1M NaOH. After humic acid precipitation and sedimentation (24 h) in a solution of fulvic acids, carbon was determined by the oxidative and titration method.
Humic acid fractions HAs-C	Calculation: HAs-C = HS-C $-$ FAs-C
Residual fraction (post-extraction residue) Res-C	Calculation: Res-C = $TC - (Lab-C + Lip-C + HS-C)$

Table 1. Procedure for obtaining carbon fractions.

# 2.4. Analyses of Humic Acids (HAs)

Samples for the examination of humic acids (HAs) were prepared following the Schnitzer procedure. The following characteristics were calculated:

- elemental composition: C, H, and N contents which were determined with an elemental analyser; O content (in %): O = 100 (C + H + N); atomic ratios and internal oxidation degree of HAs particles according to the formula  $\omega = [(2O+3N) H]/C$  [27]; ash content which averaged 0.98% (SD = 0.17) and was determined following incineration in a muffle furnace (at 600 °C); elemental composition results are expressed as the ash-free HAs weight;
- spectrophotometric properties of HAs solutions: Analysis of 0.02% HAs solution (in 0.05 M NaHCO<sub>3</sub>) was conducted using a spectrophotometer Lambda 25 (Perkin Elmer), Ueberlingen, Germany. Absorbance was measured at wavelengths of 280 nm (A<sub>280</sub>), 400 nm (A<sub>400</sub>), 465 nm (A<sub>465</sub>), 600 nm (A<sub>600</sub>), and 665 nm (A<sub>665</sub>). Also, spectrometric coefficients were calculated,
- $A_{2/4} = A_{280} / A_{465}$
- $A_{2/6} = A_{280} / A_{665}$
- $A_{4/6} = A_{465} / A_{665} [28]$
- $\Delta \log K = \log A_{400} \log A_{600}$  [16];
- chromatographic testing was performed with a liquid chromatograph HPLC Series 200 with a DAD by Perkin-Elmer, Shelton, CT, USA. Separation of humic acids into hydrophilic and hydrophobic fractions was obtained with a column Spheri-10 RP-18, 10  $\mu$ m, 220 mm × 4.6 mm. The solutions of humic acids were prepared in 0.01 M NaOH at the concentration of 2 mg mL<sup>-1</sup>; injection of the sample was 100  $\mu$ L; solvent—acetonitrile–water; solvents flow in the gradient (ratio H<sub>2</sub>O:ACN (v/v) over 0 min–6 min, 99.5:0.5; 7 min–13 min, 70:30; 13 min–20 min, 10:90); detection, at the 254 nm. Based on the areas determined under peaks, the share of hydrophilic (HIL) and hydrophobic ( $\Sigma$ HOB = HOB-1 + HOB-2) fractions in humic acid molecules and the parameter HIL/ $\Sigma$ HOB were determined [21,29].

## 2.5. Statistical Analysis

STATISTICA 13 PL (TIBCO Software, PaloAlto, CA, USA) was used to statistically analyse the results. The following descriptive statistics were used: arithmetic mean, minimum value, maximum value, standard deviation (SD), and coefficient of variation (CV).

# 3. Results and Discussion

# 3.1. SMS Properties

The experimental material had varied dry matter contents which averaged 35% (Table 2). It is higher compared with cattle or swine manure [30] but lower than values determined in composts produced from municipal waste [24,31].

Table 2. Physical properties of the tested spent mushroom substrates (SMSs).

Parameter	Unit of Measure	Mean	Range	SD	CV (%)
Den re	$\rm kg~m^{-3}$ (fresh matter)	549	499–592	34.1	6.21
DSMS	kg m $^{-3}$ (dry matter)	192	120-230	12.0	6.25
STOCK	$\rm kg~m^{-2}$ (fresh matter)	109	97.1-120	6.99	6.41
STOCKSMS	$kg m^{-2}$ (dry matter)	38.1	23.3-48.0	2.54	6.70
Dry matter	%	35.0	24.5-40.5	5.88	16.8

For explanations, see Materials and Methods.

Data on SMS density and reserves on growing chamber shelves revealed a slight variation of these characteristics in the tested wastes obtained from eight growing chambers. Primarily, it demonstrated the degradation scale of this waste during mushroom growing. Based on the data, it can be claimed that on a standard-sized mushroom farm (the area of growing shelves is 10 th m<sup>2</sup>), completing seven growing cycles per year, the potential amount of produced waste may approximate 7.5 th tonnes year<sup>-1</sup>.

The tested SMSs displayed a relatively wide pH range (6.29–7.72). The obtained values (Table 3) indicate that the wastes were slightly acidic to slightly alkaline. The pH values are similar to those recorded for composts obtained from municipal waste. The distinguishing property of the tested wastes was a substantial carbonate content (62.9 g kg<sup>-1</sup> –133 g kg<sup>-1</sup> dry matter) which indicates they might have a deacidifying effect after their application as fertiliser. This influence was confirmed in a study by Becher [7]. It is a very positive characteristic in the context of applying the tested wastes as fertilisers to acidified soils which are a dominant type of ploughed land in Poland [32,33]. CaCO<sub>3</sub> presence in the experimental material is due to the necessity of applying large amounts of alcalifying products (in the form of carbonates) during substrate and cover production so as to assure mushroom bed has an optimum reaction [5].

Table 3. Selected chemical properties of spent mushroom substrates.

Parameter (Unit of Measure)	Mean	Range	SD	CV (%)
pH <sub>KCl</sub>	-	6.29-7.72	-	-
$CaCO_3$ (g kg <sup>-1</sup> d.m.)	107	62.9–133	27.0	25.2
TC (g kg <sup><math>-1</math></sup> d.m.)	279	234-312	34.1	12.2
TOC (g kg <sup>-1</sup> d.m.)	266	219-301	36.2	13.6
IC (g kg <sup><math>-1</math></sup> d.m.)	12.9	7.55-16.0	3.23	25.0
TN (g kg <sup><math>-1</math></sup> d.m.)	22.6	19.6-25.9	2.23	9.87
TOC/TN	11.8	11.1-12.7	0.611	5.18
Organic matter (g kg <sup><math>-1</math></sup> d.m.)	458	378-378	61.4	13.4

For explanations, see Materials and Methods.

Carbon and nitrogen contents recorded in the present study confirm claims of other researchers that the material displays a marked potential when applied to enrich soils in biogenic elements [3,4]. Nitrogen contents in the tested substrates were similar to those in cattle manure [2] and about twice as high as the contents in municipal waste-based composts [24,31].

The tested material had low values of the ratio reflecting quantitative relationships between organic carbon and total nitrogen content (TOC/TN). The values may be indicative of a high degree of transformation of organic matter present in the tested material, and

prevalence of mineralisation over the synthesis of organic nitrogen compounds. Values of this parameter are similar to those recorded for humus accumulation layers of cultivated soils [34,35]. Based on this observation, it can be inferred that an introduction of this material into cultivated soils will not significantly change the quantitative relationships of carbon and nitrogen in the fertilised soil but will facilitate the prevalence of organic matter mineralisation processes and nutrient release.

Coefficients of variation for organic carbon and nitrogen, and TOC/TN ratio in particular, suggest that the chemical composition of the tested SMSs barely changed.

Organic matter content in the tested SMSs (378–519 g kg<sup>-1</sup>) was higher than the values recorded in Polish composts produced from municipal waste whose organic matter content averaged 282 (n = 6) g kg<sup>-1</sup> [24]. The obtained results confirm a marked role of SMS in the enrichment of the fertilised soils with organic matter.

# 3.2. Carbon Fractions

The fractional composition of organic matter is presented as carbon content and share (in TOC) in the operationally separated fractions (Table 4).

Exection of Organic Motton	Mean	Range	SD	OV(0/)
Fraction of Organic Matter	% TOC			- CV (%)
Lab-C	7.53	4.75-9.37	1.78	23.7
Lip-C	1.97	1.44-2.59	0.356	18.1
HŜ-C	20.8	16.8-24.5	2.94	14.2
FAs-C	8.58	6.47-9.86	1.08	12.6
HAs-C	12.2	9.82-14.6	1.98	16.2
Res-C	69.8	66.0-76.6	3.30	4.73
HAs-C/FAs-C	1.42	1.25-1.60	0.135	9.54
HAs-C/(Lab-C + FAs-C)	0.767	0.571-0.925	0.146	19.02

Table 4. Carbon share in organic matter fractions of spent mushroom substrates.

For explanations, see Materials and Methods.

A substantial amount of carbon was found which was separated at the stage of sample decalcification in the solution of  $0.05 \text{ M H}_2\text{SO}_4$  (Lab-C). The fraction is represented mainly by simple low-molecular-weight organic compounds which are loose and poorly bound to mineral components of the tested material [34]. The amounts are distinctly higher than those recorded in mature composts whose share of carbon of this fraction ranged from 1.01 to 2.05 [24]. The fraction may likely reflect the amount of non-stable organic compounds present in the tested material which are labile and susceptible to microbiological decomposition during storage and application to the soil.

In the tested material, a small amount of carbon was found which represented the lipid fraction (Lip-C)—a wide group of organic compounds which are extractable with organic solvents. The fraction includes waxes, tars, resins, fatty acids, and their esters as well as many other compounds which are aliphatic in character and of high energy value [36,37]. A similar low share of this fraction is present in mineral soils which are agriculturally utilised and in eutrophic organic soils [32]. Research indicates that biological activity and intensive organic matter mineralisation are not conducive to an accumulation of organic compounds which are extractable with organic solvents. It is possible that a low amount of bitumen carbon is also due to the reactions of fatty acid saponification with alkaline ions whose large amounts occur in the tested material [38].

During extraction with 0.1M NaOH, an average of 20% organic carbon reserves were separated from the tested material. The organic compounds included in this fraction conventionally represent its part which has been transformed due to the humification process (HS-C). In the alkaline extract, the more stable humic acid fraction was found to predominate as indicated by values of the humic acids/fulvic acids ratio. The values are universally accepted as an important qualitative parameter of humus substances—high

values are typical of the best-cultivated soils, indicate humus stability and its positive effect on physical and chemical soil properties [32,39].

In the sequential extraction used in the present study, fulvic acid, as a humus fraction soluble within the whole pH range, was probably separated during the first stage of extraction (using  $0.05M H_2SO_4$ ). Taking this into account, additionally, the ratio of humic acid carbon to the sum of fulvic acid carbon and carbon of the fraction after decalcification (HAs-C/(Lab-C + FAs-C) was calculated. The value of this ratio (less than one) indicates the material is at the initial stage of humification when, according to many authors [40,41], the fraction of fulvic acid prevails. A substantial amount of the fractions Lab-C and FAs-C (on average about 16% TOC) suggests that it is quite possible that complexation of metals by organic compounds representing these fractions occurs, as well as increased lability in the environment of the fertilised soil [6,34].

The residual fraction (Res-C) constituted the most important quantitative component in the tested organic material. The compounds representing this fraction should rather be classified as non-humified organic matter than humins. The residual fraction of the examined SMSs probably consists of relatively hard-to-decompose plant material (mainly cellulose, hemicelulose, and lignin) which are genetically associated with straw and peat used during the process of substrate and surface covering production.

#### 3.3. Properties of Humic Acids (HAs)

As shown in Table 4, an average of 12.2% of all the carbon reserves of the tested SMS was classified as the humic acid fraction. The estimated average share of humic acids in the part of organic matter transformed by humification (extract of 0.1M NaOH) was 58.6% (HAs-C/HS-C  $\cdot$  100%).

Basic indicators used in an assessment of humic acid properties include elemental composition and values of atomic ratios calculated on the basis of this composition. It is widely acknowledged that in order to determine the make-up of large-particle compounds of, e.g., natural origin, it is necessary to apply many instrumental methods, particularly when the complex make-up of humic acids is concerned, whose structure is not explicitly defined. However, the abundant experimental material collected so far based on an application of various instrumental techniques allows evaluation of humic acids properties, and so indirectly making inferences about the transformation degree.

Properties of HAs isolated from SMSs indicate there was chemical similarity regardless of the origins of the samples. This is confirmed by the elemental composition (Table 5) and, determined with it, values of atomic ratios H/C, O/C, and N/C (Table 6) which are some of the basic indicators applied to evaluate the properties of humic acids [13–15]. The obtained H/C values (1.13–1.20) indicate that the structure of the examined humic acids corresponds to the aromatic systems coupled with an aliphatic chain containing up to 10 carbon atoms [42]. H/C values higher than 1 may be indicative of the prevalence of aliphatic structures in molecules of the examined humic acids [43]. With the values of atomic ratios H/C, one can determine the degree of condensation of aromatic rings (domination of groups of aromatic rings compared to aliphatic chains) and, as a result, the 'degree of maturity' of the molecules of humic acids (the term 'degree of maturity' provides information about, e.g., the share of aliphatic structures which are typical of materials at the initial stage of decomposition process). Dergacheva et al. [44] stressed that the value of ratio H/C depends on the conditions under which the humic acids originated. Of course, one has to bear in mind that the properties, degree, and direction of changes occurring in humic acid molecules of a given soil type predominantly depend on the chemical composition of plant remains. Also, conditions under which the humification process takes place have to be accounted for. Moreover, the dynamics of transformation of a given type of plant remains in various soil types will display different tendencies. Changes in the aromaticity of humic acids, as well as environmental perturbations such as changes in soil use (reforestation, clearing, and cultivation, or bush encroachment) and wildfires, were demonstrated by Tinoco et al. [45]. The parameter used to describe the

advancement of the humification process is also the degree of internal oxidation of the HAs molecules and the ratios O/C, O/H, and N/C. Higher  $\omega$ , O/C, and O/H values and lower H/C values correspond to the humic acids with a higher 'degree of maturity' [46,47]. It should be noted that the examined HAs particles had lower O/C and O/H values and internal oxidation degree compared with those found by Debska et al. [41] in the humic acids of forest and cultivated soils, and by Becher et al. [24] in humic acids separated from municipal waste-based composts.

Element	Unit of Measure	Mean	Range	SD	CV (%)
С		53.6	53.1-54.9	0.703	1.31
Н	weight	5.24	5.12-5.34	0.080	1.53
Ν	%	4.30	3.99-4.59	0.223	5.17
О		36.8	35.9–37.4	0.573	1.56
С		36.3	35.8–37.2	0.525	1.45
Н	atomic %	42.5	41.9-43.1	0.410	0.96
Ν		2.49	2.30-2.68	0.137	5.50
О		18.7	18.1–19.0	0.297	1.59

Table 5. Elemental composition of humic acids (HAs) isolated from spent mushroom substrates.

For explanations, see Materials and Methods.

**Table 6.** Atomic ratio, degree of internal oxidation, and spectrometric coefficients of the HAs molecules isolated from spent mushroom substrates.

Parameter	Mean	Range	SD	CV (%)
H/C	1.17	1.13-1.20	0.025	2.13
O/C	0.515	0.490-0.530	0.016	3.11
O/H	0.439	0.425-0.451	0.008	1.86
ω	0.063	0.014-0.108	0.029	45.6
A <sub>2/4</sub>	11.19	10.50-11.6	0.360	3.22
A <sub>2/6</sub>	111.2	82.10-129.3	13.3	11.98
A <sub>4/6</sub>	9.96	7.10-11.10	1.20	12.02
ΔlogK	1.10	1.01-1.13	0.038	3.49

For explanations, see Materials and Methods.

Values of internal oxidation degree of particles ( $\omega$ ) of the tested humic acids ranged from 0.014 to 0.108 (Table 6). This parameter as well as O/C and O/H values suggest that synthesis of the discussed humic acids occurred under conditions of good oxygenation [40,43].

Based on absorbance values of humic acids in the UV-VIS range A<sub>280</sub>, A<sub>400</sub>, A<sub>465</sub>,  $A_{465}$ ,  $A_{600}$ , and  $A_{665}$ , and the coefficients of absorbance  $A_{2/4}$ ,  $A_{2/6}$ ,  $A_{4/6}$ , and  $\Delta logK$ , the following properties can be determined: the degree of advancement of organic material humification as well as changes in the properties of the humic acids which occur due to various anthropogenic factors [16–19,48,49]. In the study reported here, quite high coefficients of absorbance A<sub>2/4</sub>, A<sub>2/6</sub>, and A<sub>4/6</sub> (11.19, 111.2, and 9.96, respectively) were obtained. They are indicative of low humic acid 'maturity' thus suggesting their molecular mass and aromatic centre condensation were low [50,51]. Corresponding to values of absorbance coefficients, values of the coefficient  $\Delta \log K$ , which were on average 1.10, also indicate a low level of their humification [16]. Compared with ploughed soil humic acids, the examined HAs molecules had a similar elemental composition and values of atomic ratios, their spectrophotometric parameters having higher values [32,41,52,53]. Similarly, the examined humic acids displayed higher spectrophotometric parameters (A4/6 and  $\Delta \log K$ ) than humic acids separated from waste-based composts [24]. Of interest is also the comparison of the obtained results to those reported by Dores-Silva et al. [54] They found lower  $A_{4/6}$  and  $\Delta \log K$  values for humic acids of fresh and composted cattle manure and rice straw. According to Susic [55], fluorescence spectrophotometry is an innovative technique of humic acid analysis. When applied to HAs molecules extracted from soil, kelp, and commercial humic acid (sourced from Leonardite coal), it demonstrated that they were almost identical, which the author believed may indicate that humic acids are formed in aging plants and algae matter before they reach the soil.

The degree of organic matter humification is also connected with hydrophilic and hydrophobic properties of humic acids which were determined by means of chromatographic analysis. Similar to Preube et al. [21] and Debska et al. [20,29,56], in this research was separated HAs molecules into hydrophilic (HIL) and hydrophobic (HOB-1 and HOB-2) fractions. Mutual proportions of both the fractions determine the solubility of humic acids and, as a result, their migration deep down the soil profile. The share of hydrophilic fractions in humic acids isolated from spent mushroom substrates was lower than the share of hydrophobic fractions and averaged 35.3% (Table 7). It should be noted that the share of HOB-1 fraction, compared with HOB-2 fraction, was higher and ranged from 49.0% to 52.1%. The changes in the share of the respective fractions resulted in the values of HIL/ $\Sigma$ HOB ratio averaging 0.54. The shares of hydrophilic and hydrophobic fractions and the parameter HIL/ $\Sigma$ HOB are connected with the degree of organic matter humification. According to Preube et al. [21] and Debska et al. [20,29,56], the values HIL/ $\Sigma$ HOB of the parameter increase with an increase in the degree of maturity of humic acid molecules. The observed similarities for HA molecules of spent mushroom substrates indicate a lower degree of HA maturity compared with the humic acids of the forest, meadow, and agricultural ecosystems.

**Table 7.** Share of hydrophilic and hydrophobic fractions of humic acids isolated from spent mushroom substrates.

Parameter	Mean	Range	SD	CV (%)
HIL	35.3	33.0-37.6	1.65	4.69
HOB-1	50.4	49.0-52.1	1.23	2.44
HOB-2	14.3	13.0-16.1	0.97	6.78
∑HOB	64.5	62.4-67.0	1.69	2.63
$HIL/\Sigma HOB$	0.543	0.446-0.602	0.052	9.50

For explanations, see Materials and Methods.

### 4. Conclusions

The tested spent mushroom substrates obtained from eight different growing chambers had similar organic matter properties, contents, and quality. It was demonstrated that there was an average of 109 kg·m<sup>-2</sup> fresh SMS on the shelves of a standard-sized chamber following button mushroom growing. It is characterised by a substantial potential of enriching soils with organic matter, nitrogen, and carbonates. A relatively high share of labile organic compounds (the fraction separated with  $0.05 \text{ M H}_2\text{SO}_4$  and the fulvic acid fraction) is the most important qualitative characteristic of the organic matter in the tested material. It should be assumed that this material, when introduced into the soil, will facilitate relatively rapid organic compound mineralisation and nutrient release. Regardless of their origins, the humic acids of the experimental material were chemically similar. The HAs molecules included a substantial share of aliphatic structures which typically occur in materials at the initial decomposition stage (as indicated by the obtained atomic ratios, spectrometric properties as well as hydrophilic and hydrophobic properties). The assumed hypothesis that spent substrate may enrich soils with organic matter, nitrogen, and carbonates, thus potentially improving their fertility, has been confirmed. According to the authors, spent mushroom substrates are a valuable organic matter source and may be used as a corrective or soil conditioner, aiming at a structural and chemical improvement of agricultural soil, for example, by preventing soil degradation and, thus, increasing carbon sequestration.

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