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PROPERTIES OF ORGANIC MATTER OF SOIL FERTILISED WITH SPENT MUSHROOM (*AGARICUS* L.) SUBSTRATE

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Abstract. The objective of the study was the estimation of quality of organic matter of the humus horizon of soil fertilised every year, for ten years, with spent mushroom (*Agaricus* L.) substrate, as compared to a soil without such amendment. Fractionation of organic matter was performed, as well as quality analyses of humic acids and fractionation of organic nitrogen complexes. In the humus horizon of the soil fertilised with spent mushroom substrate lower acidity was noted, and higher levels of C, N, P and Ca. In the soil amended with the substrate greater accumulation of soil humus was noted (by nearly 50%), and especially of the fraction of humic acids, which had a positive impact on increasing the value of the ratio of humic to fulvic acids. The fertilisation with the substrate only slightly modified the quality parameters of humic acids. In the soil fertilised with the substrate a higher level of carbon and nitrogen was observed – related with the soluble forms of organic matter, as well as greater accumulation of nitrogen, mainly in a stable non-hydrolysing form.

Keywords: organic matter of soils, spent mushroom substrate, forms of nitrogen

INTRODUCTION

The high share of light soils in the structure of agricultural lands in Poland and their aerobic character, combined with unfavourable water balance and warming climate, are conducive to the mineralisation of organic carbon introduced into soils. Another contribution to the negative balance of organic matter in soils is the dominance of cereal plants in the sowing structure, as well as insufficient organic and mineral fertilisation (Stevenson 1985, Turski 1988, Dziadowiec 1993, Gonet and Markiewicz 2007). Therefore, searching for methods of improvement of organic matter balance and of humus reproduction in soils in an unavoidable necessity. One of such methods can be the agricultural utilisation of spent mushroom (*Agaricus* L.)

substrate. In the region of the central-eastern Mazowsze the concentration of mushroom growing farms creates the necessity of utilisation of large amounts of spent substrate. Agricultural application of that waste organic material may be the best way of its utilisation, enriching soil with necessary nutrients, and it may contribute to the sequestration of organic carbon (Kalembasa and Majchrowska-Safaryan 2009, Medina *et al.* 2009, Rutkowska *et al.* 2009, Kalembasa and Becher 2011, 2012, Kalembasa *et al.* 2012). Substrates for mushroom growing, together with mulches, are materials prepared from natural organic (straw, peat, chicken manure, plant proteins) and mineral components ($CaCO_3$). Those components are desirable for soils with low humus content, low levels of nutrients, acidified.

In the region of Siedlce, with the development of mushroom farming, spent mushroom substrate has been and is used for soil fertilisation on agricultural farms on a routine basis. Soil subjected to this treatment should be studied in the aspect of transformation of soil organic matter. The objective of the study presented herein was the estimation of the properties of soils, including the quality of organic matter of the humus horizon of soils fertilised with spent mushroom substrate for a period of ten years, in comparison to a soil in which that external organic matter was not applied.

MATERIAL AND METHOD

The pedological studies were conducted on arable lands in the locality of Stok Lacki (commune of Siedlce), in October 2011. An arable field was located that, for a period of ten years, was fertilised every year with spent mushroom substrate at the dose of 6-7 t ha^{-1} dry matter. Two soil pits were made, situated at a small distance from each other (50 m), on an arable field not fertilised and a field fertilised with spent substrate from the cultivation of white mushroom. Over the past ten years, on both fields no natural fertilisers were applied, and both were under cereal cultures. Both soils were classified as illuvial-gley grey-brow podzolic soils with highly similar lithology and morphology, with sandy loam particle size distribution in the humus horizon. Samples were taken from the humus horizons of the soils studied (in three replicates) and the following assays were performed: particle size distribution – with the areometric method; pH in 1M KCl solution – potentiometrically; organic carbon content (C_{org}) – with the oxidative-titration method (Kalembasa and Kalembasa 1992); total nitrogen content (N_t) – with the Kjeldahl method (Kalembasa 1995); total content of selected macroelements (P, K Ca, Mg and S) – with an inductively coupled plasma atomic emission spectroscope (ICP – AES), Optima 3200 RL, Perkin Elmer (after mineralisation of samples at

$500^{o}C$ and preparation of extracts of elements, at 20% HCl); dry soil density (bulk density), after taking soil samples in natural state into cylinders (V = 100 cm^3) and drying at temperature of $105^{o}C$.

The study of organic matter quality comprised the following: A – fractionation, according to the Shnitzer method (Dziadowiec and Gonet 1999); B – quality assays of humic acids; C – sequential fractionation of organic nitrogen complexes (Kalembasa 1995).

Ad. A. The extraction of soil organic matter consisted in sequential separation of the following fractions: the fraction after decalcification, in 0.05M H_2SO_4 (DEKA); the fraction of bitumens on a Soxtherm solvent extraction automatic extractor, with a mixture of hexane and ethanol (BITUM); the fractions of humus substances, in 0.1M NaOH, with separation into humic acids (HA) and fulvic acids (FA) through acidification; the post-extraction residue (HUMINS). In the isolated fraction assays were made of the content of organic carbon (with the oxidative-titration method) and of nitrogen (with the Kjeldahl method). Carbon and nitrogen contents in the fraction of humic and fulvic acids were determined from the difference. In the bitumens the content of nitrogen was not assayed.

Ad. B. In humic acids purified with the Schnitzer method (Dziadowiec and Gonet 1999) the following assays were made: contents of C, H and N – on the Perkin Elmer autoanalyser Series II 2400; light absorbance of humic acids solution (in 0.05 M $NaHCO_3$) at wavelengths of: 280 nm (A_{280}), 400 nm (A_{400}), 465 nm (A_{465}), 600 nm (A_{600}) and 665 nm (A_{465}) – on a Perkin Elmer UV-VIS spectrophotometer Lambda 25. The absorbance quotients were calculated – $A_{2/6}$ (A_{280} to A_{665}), $A_{4/6}$ (A_{465} to A_{665}), and $\Delta logK = logA_{400} – logA_{600}$.

Ad. C. The sequential extraction was made with the use of: 0.25M KCl, for the extraction of nitrogen in easily soluble mineral and organic forms, and 0.25 M H_2SO_4 and 2.5 M H_2SO_4 (3-hour hydrolysis at temperature equal to the boiling point of the solution) for the extraction of organic nitrogen complexes, easily and hard-hydrolysing. In the solutions obtained the content of nitrogen was assayed with the Kjeldahl method. The method applied permitted soil nitrogen to be defined in the following forms (fractions): N-NH_4 – nitrogen in mineral ammonium form, distilled out from the extract of 0.25 M KCl, after alkalisation with MgO; N-NO_3 and N-NO_2 – nitrogen in mineral ammonium form, distilled out from the extract of 0.25 M KCl, after distillation of N-NH_4 and reduction of nitrates with the Devard mixture; N_{MIN} – nitrogen in mineral compounds: N_{MIN} = N-NH_4 + N-NO_3 + N-NO_2; N_{ORG} – nitrogen in organic compounds: $N_{ORG} = N_t – N_{MIN}$; N_{KCl} – nitrogen after extraction in KCl solution and mineralisation of the solution; N_{OR} – easily soluble organic nitrogen: $N_{OR} = N_{KCl} – N_{MIN}$; N_{OLH} – easily hydrolysing organic nitrogen, after hydrolysis in 0.25 M H_2SO_4 and mineralisation of the solution; N_{OTH} – hard-hydrolysing organic nitrogen, after hydrolysis in 2.5 M H_2SO_4 and mineralisation of the solution; N_{ONH} – non-hydrolysing organic nitrogen, remaining in the soil after the extractions: $N_{ONH} = N_t - (N_{KCl} + N_{OŁH} + N_{OTH})$.

In the solutions obtained during the fractionation also the content of organic carbon was assayed, with the oxidative-titration method. In the calculation of the amount of easily soluble organic carbon account was taken of the amount of Cl ions (originating from KCl) that, during the mineralisation of organic compounds, cause the reduction of Cr^{6+} in $K_2Cr_2O_7$. Carbon fractions were defined in an analogous manner: C_{OR}, $C_{OŁH}$, C_{OTH}, C_{ONH}.

RESULTS AND DISCUSSION

The soils chosen for the study represent taxonomic units and properties characteristic for arable soils on the Siedlce Upland, characterised by coarse grained humus horizon (low content of clay fraction) and very low levels of carbonate minerals, which is not conducive to humus accumulation in the soil (Stevenson 1985, Kalembasa and Becher 2009, Becher and Kalembasa 2011, Pakuła and Kalembasa 2012).

The results (Tab. 1) indicate an enriching effect of the application of spent mushroom substrate on the accumulation of organic matter and of elements whose biogeochemistry is closely related with soil organic matter transformations (C, N, S and P). Among the elements related with the mineral solid phase, in the soil fertilised with spent mushroom substrate a higher level (about 3-fold) of calcium was found, and similar contents of magnesium and potassium. This results from the fact of calcium carbonate being added to the substrates as an alkalising agent (Kalembasa *et al*. 2012). In the soil amended with spent mushroom substrate lower acidification was observed (pH value higher by more than one unit). Also, a higher content of organic matter was noted, by nearly a half. In spite of the distinctly enriching effect of fertilisation with spent mushroom substrate on the content of carbon, nitrogen and phosphorus, there was no significant effect on the values of the C/N and C/P ratios. On the one hand, the values of those parameters (especially C/N) are relatively stable in the humus horizons of arable soils, and on the other the spent mushroom substrates from the region of Siedlce are characterised by values of those ratios that are similar to those of the humus horizons of arable soils (Kalembasa and Becher 2012, Kalembasa *et al.* 2012).

The fraction composition of organic matter of the soils studied in presented in the form of absolute amounts of carbon and nitrogen in the fractions isolated, and also in the form of percentage share (%) in the total content of those elements (Tab. 2 and 3). Organic matter fraction composition is related with the genesis and the kind of use of soils. In arable soils it may be significantly modified by the kind of fertilisation applied (Turski 1988, Kalembasa 2000, Dębska 2004, Kalembasa and Becher 2009). In the soil fertilised with spent mushroom substrate a higher content of carbon and nitrogen was noted in the fractions isolated, with the exception of the fraction of bitumens whose content was higher in the soil without fertilisation with the substrate. This may result from the favourable effect of the substrate on microbiological activity which is not conducive to the accumulation of organic compounds that can be extracted with organic solvents. In addition, the spent mushroom substrate itself is characterised by a low content of bitumens and it introduces to the soil a relatively large amount of alkaline cations, which in turn may initiate the reactions of saponification of fatty acids (Solinas *et al*. 1987, Becher and Kalembasa 2006, Kalembasa *et al*. 2012).

Table 1. Selected properties of the humus horizons of the soils studied

Parameter	Soil without fertilisation with spent mushroom substrate	Soil fertilised with spent mushroom substrate
Depth (cm)	25	30
Content of fractions (%):		
sand (2-0.5 mm)	67	65
silt (0.5-0.002 mm)	29	31
clay (<0.002 mm)	4	4
pH in KCl	4.52	5.56
Density of dry soil (g cm^{-3})	1.44	1.32
Content of elements (g kg^{-1}):		
S	0.105	0.208
P	0.575	0.844
Ca	0.623	1.87
Mg	0.620	0.638
K	0.692	0.632
Corg	9.58	13.0
Nt	0.930	1.19
C/N	10.3	10.9
C/P	16.6	15.4
Organic matter content (kg m^2) *	5.95	8.88

* %C x 1.724.

Table 2. Content and percentage share of carbon in organic matter fractions of the soils studied

Fraction	Soil without fertilisation with spent mushroom substrate		Soil fertilised with spent mushroom substrate	
	g kg^{-1}	% Corg	g kg^{-1}	% Corg
C_{DEKA}	0.20	2.09	0.22	1.69
C_{BITUM}	0.51	5.32	0.43	3.31
C_{KH+KF}	5.20	54.3	7.38	56.8
C_{KF}	2.46	25.7	2.82	21.7
C_{KH}	2.74	28.6	4.56	35.1
C_{HUMIN}	3.67	38.3	4.97	38.2
C_{KH}/C_{KF}	1.11		1.62	

Table 3. Content and percentage share of carbon in organic matter fractions of the soils studied

Fraction	Soil without fertilisation with spent mushroom substrate			Soil fertilised with spent mushroom substrate		
	g kg^{-1}	% Nt	C/N	g kg^{-1}	% Nt	C/N
N_{DEKA}	0.036	3.87	5.56	0.035	2.94	6.29
N_{KH+KF}	0.609	65.5	8.54	0.770	64.7	9.58
N_{KF}	0.287	30.9	8.57	0.322	27.1	8.76
N_{KH}	0.322	34.6	8.51	0.448	37.6	10.2
N_{HUMIN}	0.290	30.6	12.9	0.390	32.4	12.9
N_{KH}/N_{KF}	1.12		–	1.39		–

One possible modifying effect of fertilisation with spent mushroom substrate of the soil studied is an increase of the amount of organic matter transformed in the process of humification (KH+KF), and especially of the humic acids fraction, and an increase of the ratio expressing the quantitative relations between humic and fulvic acids (KH/KF) – an important parameter defining the quality of soil humus. This is a favourable effect on humus quality, as an advantage of the humic acids fraction in arable soils with high agricultural culture is a desirable feature of humus since it guarantees its stability in the soil environment and a more beneficial effect on the physical and physicochemical properties of soils (Stevenson 1985). The second most important quantitatively fraction of organic matter of the soils studied are humins. In the soil fertilised with spent mushroom substrate greater amounts of humins were noted, but without any modifying effect on the percentage share of carbon of that fraction in the total content of that element.

The applied analytical procedure of organic matter fractionation permitted the isolation of greater amounts of nitrogen than carbon (Tab. 3). The lowest values of C/N were noted in the fraction extracted with 0.05 M H_2SO_4, which confirms that the organic compounds representing it are susceptible to mineralisation and potentially the most labile in the soil environment. In the soil with the addition of spent mushroom substrate higher values of C/N were observed.

In the humus horizons of the soils studied approximately 1/3 of organic matter was accounted for by humic acids – in the opinion of many researchers the most interesting and at the same time the best studied group of humic substances (Turski 1988, Senesi *et al.* 2003). The presented results of elemental composition and spectrophotometric parameters (Tab. 4) indicate that in the soil fertilised with spent mushroom substrate no greater quality differences were noted for that important group of humic compounds (similar values of the parameters studied in both soils). It was observed that humic acids isolated from the soil fertilised with spent mushroom substrate were characterised by a somewhat lower content of atoms of carbon, higher content of hydrogen and nitrogen, a higher value of the H/C ratio, and a lower value of the degree of internal oxidation (ω). In accordance with the rules of formulation of conclusions about the chemical nature of humic acids on the basis of the enumerated features, encountered in the literature of the subject, it can be concluded that the acids from the soil with an admixture of the substrate, in relation to the soil without such fertilisation, displayed traits of lower "maturity". This indicates a greater share of aliphatic groups and a lower condensation of aromatic centres (Kalembasa 2000, Dębska 2004, Kalembasa and Becher 2009). It is also supported by the slightly higher values of absorbance indices for humic acids extracted from soil fertilised with spent mushroom substrate (Chen *et al.* 1977, Kumada 1987). Spent mushroom substrate applied to soil as external organic matter may cause the formation of new humic acids. Moreover, spent mushroom substrate itself contains humic acids which, as follows from a study by Kalembasa *et al.* (2012), have properties conforming to the direction of their transformation observed in the studied soil fertilised with the substrate. Therefore, they are rather "young" and characterised by high "aliphaticity" and a low molecular mass.

The sequential extraction applied permitted the isolation of soil nitrogen in various forms (Tab. 5), by means of reagents with increasing extraction power (0.25 M KCl < 0.5 M H_2SO_4 < 2.5 M H_2SO_4), and the extracted fractions of nitrogen presumably represent organic compounds with various potential stability in the soil environment ($N_{OR} < N_{OŁH} < N_{OTH} < N_{ONH}$) whish remain in an inverse relation of susceptibility to the processes of microbiological degradation.

Table 4. Elemental composition (atomic %) and spectrophotometric coefficients of humic acids of the soils studied

Parameter	Soil without fertilisation with spent mushroom substrate	Soil fertilised with spent mushroom substrate
C	37.0	35.9
H	40.9	42.3
N	2.56	2.58
O	19.6	19.2
H/C	1.11	1.18
ω	0.162	0.106
$A_{2/4}$	31.8	34.9
$A_{4/6}$	4.27	4.72
ΔlogK	0.709	0.733

$\omega = [(2O+3N)-H]/C$.

Table 5. Content and percentage share of nitrogen fractions in humus horizons of the soils studied

Fraction	Soil without fertilisation with spent mushroom substrate		Soil fertilised with spent mushroom substrate	
	$g\ kg^{-1}$	% Nt	$g\ kg^{-1}$	% Nt
N_{MIN}	0.020	2.16	0.026	2.21
N_{ORG}	0.910	97.84	1.164	97.79
N_{OR}	0.017	1.79	0.028	2.35
$N_{OŁH}$	0.315	33.87	0.354	29.71
N_{OTH}	0.296	31.81	0.361	30.29
N_{ONH}	0.282	30.37	0.422	35.44

In the humus horizons of the soils studied a similar percentage share of mineral forms of nitrogen was observed, characteristic of soils under tillage. In the soil fertilised with the substrate there was greater accumulation of each of the organic forms of nitrogen identified in the course of the fractionation. Analysing the shares of the particular fractions, in the soil fertilised with the substrate a greater share of the soluble forms (N_{OR}) was noted, a lower share of the easily hydrolysing forms ($N_{OŁH}$), a similar one of the hard-hydrolysing forms (N_{OTH}), and a greater share of forms not undergoing hydrolysis (N_{ONH}). In an earlier study on nitrogen forms in the humus horizons of 11 grey-brown podzolic soils, Becher and Kalembasa (2011) found the following average shares (w %) of the studied forms of soil nitrogen: N_{MIN} – 3.21; N_{OR} – 1.09; $N_{OŁH}$ – 28.0; N_{OTH} – 35.9 and N_{ONH} – 35.0. Studies on the shares of organic forms of nitrogen in spent mushroom substrate, with the application of the same principle of fractionation, permitted the formulation of the following increasing series of quantitative importance of the identified fractions: N_{OLH} (15.3%) < N_{OR} (18.5%) < N_{OTH} (18.9%) < N_{ONH} (47.3%) (Kalembasa and Becher 2012). As follows from the presented data, the substrate applied to soil probably enriches the soil to the greatest degree in stable non-hydrolysing forms, and in lower order in forms hard hydrolysing and soluble. The data obtained for the soils studied here support this observation.

In the case of carbon similar relations were observed, with varied percentage shares of the element in the fractions isolated (Tab. 6). In the soil fertilised with spent mushroom substrate higher levels of soluble forms of carbon and nitrogen were observed. Organic compounds extractable with a neutral solution (0.25 M KCl) represent the so-called soluble part of soil organic matter (Dębska and Gonet 2002, McDowell 2003, Paul and Williams 2005). This is the most mobile fraction, subject to seasonal fluctuations, the composition of which can include carbohydrates, amino acids, hydrocarbons and their derivatives, low-molecular fractions of humic acids, and other simple organic compounds (Dębska 2004). The content of soluble organic matter in soils under agricultural use depends primarily of the kind of organic material that gets to the soil (Dębska *et al.* 2002, Dębska and Gonet 2002). Under the effect of acidic hydrolysis, nitrogen bound by humic compounds and nitrogen included in the composition of microbial proteins migrate to the solution. That nitrogen partially hydrolyses to ammonia, amino acids, amino sugars, and other organic compounds (Kalembasa 1995). Other authors, in studies on various soils, observed percentage shares of hydrolysing nitrogen in the range of 84-89% of the total content of that element (after hydrolysis in 6 M HCl), which included: amino acid nitrogen 33-42%, amino sugars nitrogen 4.5-7.4%, ammonium nitrogen 18-32% (Sowden *et al.* 1977). Approximately 1/3 of the content of total nitrogen in the soils studied was accounted for by its forms that do not undergo hydrolysis. For that fraction decidedly the highest value of C/N were calculated, which probably confirms the greatest resistance of the organic compounds remaining in the material after the extractions (the residue) to microbial degradation. Among the fractions of non-hydrolysing soil nitrogen dominant are derivatives of aromatic and heterocyclic compounds (Schulten and Schnitzer 1998), and Knicker *et al.* (1995) maintain that soil organic matter contains peptide structures resistant to microbial degradation.

Table 6. Content and percentage share of carbon and C/N values in the isolated fractions

Fraction	Soil without fertilisation with spent mushroom substrate			Soil fertilised with spent mushroom substrate		
	g kg^{-1}	% Corg	C/N	g kg^{-1}	% Corg	C/N
C_{OR}	0.060	0.626	3.60	0.195	1.50	6.96
C_{OLH}	2.94	30.7	9.33	3.30	25.4	9.34
C_{OTH}	1.66	17.3	5.62	1.91	14.7	5.30
C_{ONH}	4.92	51.3	17.4	7.59	58.4	18.0

CONCLUSIONS

1. In the humus horizon of the soil fertilised for 10 years with spent mushroom substrate lower acidification was than in the soil without such fertilisation, and a higher content of biogenic elements – C, N, P and Ca. No changes were noted in the quantitative relations expressed by the ratios C/N and C/P.
2. In the soil fertilised with spent mushroom substrate greater accumulation of soil humus was noted (by nearly 50%), mainly of the fraction of humic acids.
3. Fertilisation with the substrate only slightly modified the quality parameters of humic acids – there was a tendency towards accumulation of younger acids, with a lower share of aromatic structures and a lower degree of humification.
4. In the fertilised soil higher shares of carbon and nitrogen bound with soluble forms of organic matter were found. Fertilisation with spent mushroom substrate enhanced nitrogen accumulation, mainly in the stable non-hydrolysing form.
5. Mushroom substrate is used for the production of high quality food (mushrooms). The production function of the substrate should not end with the moment of its removal from the mushroom farm – it can be continued, improving the fertility of soils and the profitability of plant production.

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WŁAŚCIWOŚCI MATERII ORGANICZNEJ GLEBY NAWOŻONEJ PODŁOŻEM PO UPRAWIE PIECZARKI (*AGARICUS* L.)

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Streszczenie. Celem badań była ocena jakości materii organicznej poziomu próchnicznego gleby corocznie nawożonej przez dziesięć lat podłożem po uprawie pieczarki (*Agaricus* L.), w porównaniu do gleby bez tego stosowania. Przeprowadzono frakcjonowanie materii organicznej, badania jakościowe kwasów huminowych oraz frakcjonowanie organicznych połączeń azotu. W poziomie próchnicznym gleby nawożonej podłożem popieczarkowym stwierdzono mniejsze zakwaszenie oraz większą zawartość C, N, P i Ca. W glebie nawożonej podłożem notowano większą akumulację próchnicy glebowej (o blisko 50%), a zwłaszcza frakcji kwasów huminowych, co wpłynęło pozytywnie na większą wartość stosunku kwasów huminowych do fulwowych. Nawożenie podłożem w niewielkim stopniu zmodyfikowało parametry jakościowe kwasów huminowych. W glebie nawożonej podłożem stwierdzono większy udział węgla i azotu – związanych z rozpuszczalnymi formami materii organicznej, a także większą akumulację azotu głównie w trwałej postaci niehydrolizującej.

Słowa kluczowe: materia organiczna gleb, podłoże popieczarkowe, formy azotu