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IMPACT OF A SPENT MUSHROOM SUBSTRATE, *AGARICUS BISPORUS* ON CHROMIUM AND COPPER SPECIATION IN THE HUMUS HORIZON

Anna Majchrowska-Safaryan, Dorota Kalembasa, Krzysztof Pakuła

Chair of Soil Science and Agricultural Chemistry
Siedlce University of Natural Sciences and Humanities

Abstract

Spent mushroom substrate used as fertilizing material provides nutrients for plants in forms with a different degree of availability. A two-year experiment was conducted in central eastern Poland (Siedlecka High Plain) to determine the fertilizing effect of substrate previously used to grow mushrooms *(Agaricus bisporus)* on the total content chromium and copper content and their quantitative content in the fraction in humus horizon pseudogley loessive soil. The experiment included the control (without fertilization), and several fertilized variants: NPK, swine manure, swine manure + NPK, spent mushroom substrate and spent mushroom substrate +NPK. The sequential fractionation of chromium and copper, carried out according to the BCR protocol, in the soil humus horizon under the above treatments demonstrated various concentration of these metals in the extracted fractions and their shares in the total content. Fertilization with spent mushroom substrate alone and with NPK contributed to a decrease in the Cr content in the F2 and F3 fractions, but resulted in an increase in the Cu content in the F1, F2 and F3 fractions after the second year of plant cultivation in comparison with the first year. The highest share of the tested metals in the total content was detected in the residual fraction F4: after the second year for chromium and after the first year of the experiment for copper.

Keywords: fractions of chromium and copper, BCR method, spent mushroom substrate, Luvisol.

WPŁYW PODŁOŻA PO UPRAWIE PIECZARKI *AGARICUS BISPORUS* NA SPECJACJĘ CHROMU I MIEDZI W POZIOMIE PRÓCHNICZNYM GLEBY

Abstrakt

Zużyte podłoże po uprawie pieczarki zastosowane jako nawóz dostarcza do gleby składniki pokarmowe dla roślin w formie różnie dostępnej. W dwuletnim doświadczeniu polowym, zlokalizowanym w środkowo-wschodniej Polsce (Wysoczyzna Siedlecka), określono wpływ nawożenia

prof. dr hab. Dorota Kalembasa, Chair of Soil Science and Agricultural Chemistry, B. Prusa 14 street, 08-110 Siedlce, Poland, e-mail: kalembasa@uph.edu.pl; brill73@wp.pl

podłożem po uprawie pieczarki białej *(Agaricus bisporus)* na zawartość ogólną chromu i miedzi oraz ich ilościowy udział w wydzielonych frakcjach ornego poziomu próchniczego gleby płowej opadowo-glejowej użytkowanej rolniczo. Obiektami doświadczalnymi były: obiekt kontrolny (bez nawożenia); nawożony mineralnie NPK; nawożony obornikiem trzody chlewnej; nawożony obornikiem trzody chlewnej + NPK; nawożony podłożem po uprawie pieczarki; nawożony podłożem po uprawie pieczarki + NPK. Frakcjonowanie sekwencyjne chromu i miedzi, wg procedury BCR, w poziomie próchnicznym gleby poszczególnych obiektów doświadczenia wykazało zróżnicowaną zawartość tych metali w wydzielonych frakcjach oraz ich udział w zawartości ogólnej. Wprowadzone podłoża: popieczarkowe oraz popieczarkowe z dodatkiem NPK wpłynęły na zmniejszenie udziału chromu we frakcji F2 i F3, zwiększenie zaś udziału miedzi we frakcji F1, F2 i F3 po 2. roku uprawy w stosunku do 1. roku. Największy udział badanych metali w zawartości ogólnej stwierdzono we frakcji rezydualnej F4 – chromu po 2. roku badań, a miedzi po 1. roku.

Słowa kluczowe: frakcje chromu i miedzi, metoda BCR, podłoże popieczarkowe, gleba płowa.

INTRODUCTION

In soil, metal elements such as chromium and copper are found in different chemical compounds and forms, mainly mineral or organic ones, as ions in the soil solution or bound to the solid phase. They have different bioavailability to plants and soil organisms, being most easily absorbed as simple ions from highly soluble compounds, but less readily as complex ions. The soil's supply of easily absorbable compounds determines the degree of plant nutrition and is used to assess fertilization requirements of cultivated soil. Fertilizers introduced to cropped soil increase the content of compounds, including metals, in their active forms mainly in the soil solution, while their excess is absorbed by the solid phase. The deficit of natural fertilization in Poland has encouraged the search for new technologies to increase the content of organic matter in cultivated soil. New developments include the use of waste organic materials such as spent mushroom substrate (Kalembasa, Wiśniewska 2004). Such substrate used as fertilizing material provides nutrients for plants in forms characterized by different degrees of availability (Herrero-Hernandez et al. 2011). The content of heavy metals in substrate mainly depends on the composition of components used during the manufacturing processes, but generally does not exceed the permissible limits specified in the Polish regulations (Kalembasa, Majchrowska-Safaryan 2009).

The goal is to simplify the methods for fractionating heavy metals. The BCR method, approved by the European Union, is the basic procedure used to fractionate metals with different bioavailability in soil, sewage sludge and marine deposits (Mosso, Davidson 2003).

The aim of this study was to determine the impact of fertilization with substrate previously used to grow mushrooms *(Agaricus bisporus)* on the total content of chromium and copper and their quantitative content in fractions. The concentrations of the metals were determined with the sequential

BCR method in tilled humus horizon of cultivated pseudogley loessive soil during a two-year field experiment located in central eastern Poland (Siedlecka High Plain).

MATERIAL AND METHODS

The plant growing experiment was carried out in 2008 and 2009, on a production field (52°20′00″N and 22°03′00″E). The duration of the experiment was set on the assumption that spent mushroom substrate used as a fertiliser had a beneficial impact on plant yield mainly during the first two years after its introduction into soil. The experiment was designed in a randomized block design with four replications on 7×7 m plots on pseudogley soil lessive with granule composition typical of sandy loam (74% sand, 19% silt, and 7% clay). The experiment included the following treatments: the control (without fertilization), fertilized with NPK, with swine manure – 25 t ha^{-1} (used routinely because of the hog fattening production on the farm), with swine manure (25 t ha^{-1}) + NPK, with spent mushroom substrate (20 t ha^{-1}), and with spent mushroom substrate (20 t ha^{-1}) +NPK.

The starch cultivar of potato (*Solanum tuberosum* L.) Pasat was the test plant in the first year, replaced by winter wheat (cv. Finezja) in the second year. Fertilization with organic materials was performed in spring. The doses of manure and spent mushroom substrate were established based on their nitrogen content. Mineral fertilization with NPK was carried out in spring before potato and in autumn before winter wheat. After harvest, samples of soil were collected from the ploughed humus horizon (0-25 cm) and sieved through 2 mm mesh. The following parameters were determined: pH in 1 mol KCl dm^{-3} with the potentiometric method; cation exchange capacity (CEC) calculated from hydrolytic acidity (Hh) and the sum of exchangeable base cations (S) determined with the Kappen's method; carbon in organic compounds (C_{org}) with the oxidation-titration method (Kalembasa, Kalembasa 1992); total content of chromium (Cr_t) and copper (Cu_t) after initial mineralization of the tested material in a mixture of concentrated acids HCl + HNO_3 at the 3:1 ratio; metals extracted in a fraction with the BCR procedure – Table 1 (Ure et al. 1993) determined with ICP – AES. The analyses were performed in three replications. The reference material WEPAL Soil Reference Material BCR 142R (light sandy soil) was used to verify the accuracy of measurements.

Spent substrate obtained after 6-week cultivation of common mushroom, *Agaricus bisporus,* originated from a mushroom farm where mushrooms were cultivated on phase III substrate (after fermentation and inoculation with mycelium). The cover consisted of raised peat, chalk, urea and protein supplement as well as swine manure obtained from deep litter pig pens. The

Table 1

Scheme of sequential extraction of heavy metals by the BCR method

Fraction	Name	Extraction reagent	Extraction time (h)	pH
F1	exchangeable/ acid extractable	0.11 mol dm^{-3} CH_3COOH	16	3.00
F2	reducible – bound to Fe and Mn oxyhydroxides	0.5 mol dm^{-3} NH_2OH HCl	16	2.00
F3	oxidizable - bond to organic matter	30% H_2O_2 (1 h, 85°C) + 1 mol dm^{-3} CH_3COONH_4	16	2.00
F4	residual	calculated as the difference between the total content of the metal and the sum of the above determined fractions	-	-

soil: solution ratio 1 g : 10 cm^3

content of dry matter was determined in the substrate and manure with the drying-weighing method (at 105°C) and the other parameters were tested as explained above.

The results were statistically analysed with a two-way analysis of variance (Anova). The F Fisher-Snedecor test was used to determine the significance of impact of the experimental factors on the tested parameters. $LSD_{0.05}$ was calculated with the Tukey's test. The calculations were performed with Analwar-5FR software. The coefficients of simple correlation were determined with the Pearson's method using a Statistica 9.1 software package.

RESULTS AND DISCUSSION

The chemical analysis of swine manure and spent mushroom substrate used as fertilizers revealed that they differed in the content of dry matter, carbon in organic compounds and pH (Table 2). In comparison with swine manure (assumed as 100%), spent mushroom substrate had a higher content of dry matter (by 23%), a slightly higher pH (by 2.6%), a lower content of carbon in organic compounds (by 27.4%), a higher total content of chromium (by 66%) and a four times lower amount of copper.

The extracted fractions with the BCR procedure revealed various percentages (%) of Cr and Cu in the total content (Figure 1). The highest content of the metals was detected in manure in the residual fraction F4, i.e. 52.5% Cr and 76.8% Cu, respectively, and in substrate in the oxidative fraction F3 (42.6% Cr) and for Cu (50.7%) in F4 fraction. The content of these metals in the fraction F2 bound to iron and manganese oxides and

Table 2

The properties of swine manure and spent mushroom substrate used to fertilize the soil

Organic material	DM in 105°C	OrgC	Cr_t	Cu_t	pH_{KCl}
	(g kg^{-1})		(mg kg^{-1})		
Swine manure	250	383	2.82	41.3	6.97
SMS	309	278	4.20	11.6	7.15

SMS – spent mushroom substrate

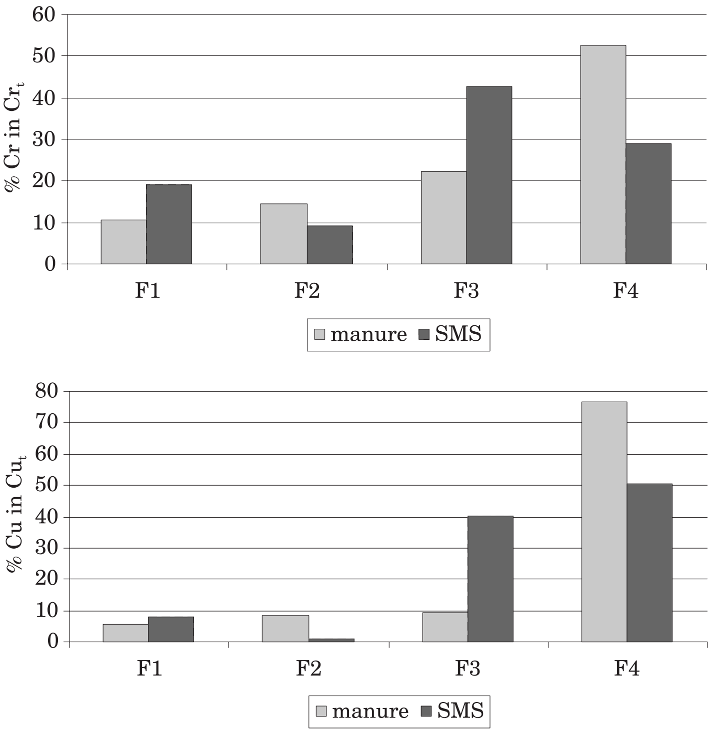

Fig 1. The percentages of chromium and copper in the total content in the separated fractions of organic materials used for fertilizing

manure – swine manure; SMS – spent mushroom substrate; Fractions: F1 – exchangeable; F2 – reducible; F3 – oxidizable; F4 – residual

hydroxides, exposed to transformations under reductive conditions, was considerably diverse: 9.2% of chromium and 1.04% of copper were bound to this fraction. The metals constituted from 8.04% for Cu to 19.1% for Cr of the total content of the fraction easily available to plants (F1). Similar results of fractionating heavy metals in spent mushroom substrate which originated from three different farms were reported by KALEMBASA, MAJCHROWSKA-SAFARYAN (2009). HERRERO-HERNANDEZ et al. (2011) fractionated spent mushroom substrate in accordance with the BCR procedure and detected approx. 5% of copper in the F1 fraction, 2% in the F2 fraction, 60% in the F3 fraction and 38% in the F4 fraction.

The content of chromium and copper in the humus horizon under the individual treatments was low and within the natural range, never exceeding the permissible limits set for cultivated soil in Poland (*The Regulation*... 2002). After two seasons of plant growing, the soil contained between 4.72 and 4.85 mg kg^{-1} of Cr and between 2.03 and 1.85 mg kg^{-1} of Cu (Table 3). The analysis of variance revealed that the total content of chromium did not depend on the experimental factors, unlike total chromium, which was significantly differentiated by the applied fertilization ($LSD_{0.05}$ = 0.627). KALEMBASA and WIŚNIEWSKA (2004) and HERRERO-HERNANDEZ et al. (2011) claim that the application of spent mushroom substrate increases the content of

Table 3

The properties of soil humus horizon of individual fertilizer objects after first and second year of cultivation in a field experiment

Experimental objects	pH	OrgC	CEC	Cr_t	Cu_t
	KCl	(g kg^{-1})	(mmol(+) kg^{-1})	(mg kg^{-1})	
First year of cultivation (after the potato cultivation)					
Control objects	4.79	6.02	67.6	4.770	1.745
NPK	5.18	7.00	76.9	4.897	1.838
Swine manure	5.07	7.40	71.4	4.832	2.687
Swine manure + NPK	4.44	7.72	68.4	4.618	1.872
SMS	5.11	7.65	90.3	4.702	2.189
SMS + NPK	4.92	7.67	87.8	4.498	1.825
Mean	-	7.24	77.1	4.719	2.026
Second year of cultivation (after the wheat cultivation)					
Control objects	4.61	6.09	64.5	4.715	1.511
NPK	4.58	6.40	67.6	5.130	2.061
Swine manure	4.69	8.30	77.1	4.979	2.256
Swine manure + NPK	4.90	6.75	75.7	5.099	2.127
SMS	4.59	7.65	79.2	4.323	1.682
SMS + NPK	4.27	8.00	81.3	4.856	1.484
Mean	-	7.20	74.2	4.850	1.853
$LSD_{0.05}$ for:					
A(years)	-	n.s.	-	n.s.	n.s.
B(fertilization)	-	0.353	-	n.s.	0.612
B/A interaction	-	0.499	-	n.s.	n.s.
A/B interaction	-	0.333	-	n.s.	n.s.

OrgC – carbon in organic compounds; CEC – cation exchanage capacity; Cr_t – total content of chromium; Cu_t – total content of copper; SMS – spent mushroom substrate; n.s. – not significant difference

Table 4

The chromium content (mg kg-1) in separated fractions and the percentage (%) of the total content in the humus horizon of individual objects soil of field experiment

Experimental objects	F1		F2		F3		F4	
	(mg kg-1)	(%)	(mg kg-1)	(%)	(mg kg-1)	(%)	(mg kg-1)	(%)
First year of cultivation (after the potato cultivation)								
Control objects	0.134	2.80	0.652	13.7	1.476	30.9	2.508	52.6
NPK	0.137	2.79	0.668	13.6	1.515	30.9	2.577	52.6
Swine manure	0.154	3.18	0.660	13.7	1.671	34.6	2.347	48.6
Swine manure + NPK	0.172	3.72	0.715	15.5	1.551	33.5	2.180	47.2
SMS	0.143	3.04	0.735	15.6	1.644	35.0	2.180	46.4
SMS + NPK	0.170	3.78	0.677	15.1	1.584	35.3	2.058	45.8
Mean	0.152	3.22	0.684	14.5	1.574	33.4	2.308	48.9
Second year of cultivation (after the wheat cultivation)								
Control objects	0.150	3.18	0.521	11.0	1.048	22.2	2.996	63.5
NPK	0.148	2.88	0.593	11.5	1.038	20.2	3.351	65.3
Swine manure	0.200	4.01	0.558	11.2	0.930	18.7	3.291	66.1
Swine manure + NPK	0.167	3.27	0.510	10.0	1.031	20.2	3.391	66.5
SMS	0.166	3.83	0.549	12.6	0.906	20.9	2.702	62.5
SMS + NPK	0.159	3.27	0.543	11.2	1.148	23.6	3.006	61.9
Mean	0.165	3.41	0.546	11.3	1.017	21.0	3.123	64.3
$LSD_{0.05}$ for: A(years) B(fertilization) B/A interaction A/B interaction	 0.008 0.020 0.029 0.019		 0.015 0.038 0.054 0.036		 0.008 0.019 0.028 0.018		 0.011 0.025 0.036 0.030	

SMS – spent mushroom substrate; Fractions: F1 – exchangeable; F2 – reducible; F3 – oxidizable; F4 – residual

copper, which is strongly bound by organic matter and clay minerals in humus horizons. The use of substrate did not increase the content of chromium in soil, the finding which was also reported by KALEMBASA and WIŚNIEWSKA (2004). The import of elements (particularly from atmospheric deposition and waste organic matter) and their loss (mainly by leaching into the depth of the soil profile and being absorbed by plants) are the essential factors that explain fluctuations of heavy metals in soil and their final balance (KARCZEWSKA 2002).

The sequential analysis of chromium and copper in the soil from the treatments after the first and second year of cultivation revealed their different content in the extracted fractions (Tables 4, 5). In the bioavailable fraction F1, the amount of Cr was lower than in the other fractions and its concentration in the total content was slightly higher (on average) after the second

Table 5

The copper content (mg kg^{-1}) in separated fractions and the percentage (%) of the total content in the humus horizon of individual objects soil of field experiment

Experimental objects	F1		F2		F3		F4	
	(mg kg^{-1})	(%)	(mg kg^{-1})	(%)	(mg kg^{-1})	(%)	(mg kg^{-1})	(%)
First year of cultivation (after the potato cultivation)								
Control objects	0.066	3.78	0.056	3.21	0.536	30.7	1.087	62.3
NPK	0.098	5.33	0.065	3.54	0.566	30.8	1.109	60.3
Swine manure	0.149	5.54	0.166	6.18	0.884	32.9	1.488	55.4
Swine manure + NPK	0.066	3.52	0.084	4.48	0.585	31.2	1.137	60.7
SMS	0.074	3.38	0.097	4.43	0.635	29.0	1.383	63.2
SMS + NPK	0.085	4.65	0.106	5.80	0.564	30.9	1.070	58.6
Mean	0.089	4.36	0.096	4.60	0.629	31.0	1.212	60.1
Second year of cultivation (after the wheat cultivation)								
Control objects	0.066	4.36	0.047	3.11	0.484	32.0	0.914	60.5
NPK	0.083	4.03	0.059	2.86	0.670	32.5	1.249	60.6
Swine manure	0.085	3.77	0.065	2.88	0.833	36.9	1.273	56.4
Swine manure + NPK	0.069	3.24	0.093	4.37	0.808	38.0	1.157	54.4
SMS	0.081	4.82	0.093	5.52	0.600	36.7	0.908	53.9
SMS + NPK	0.090	6.06	0.105	7.08	0.565	38.1	0.724	48.8
Mean	0.079	4.38	0.077	4.30	0.660	35.5	1.037	55.8
$LSD_{0.05}$ for:								
A(years)	n.s.		0.011		0.059		0.054	
B(fertilization)	0.043		0.028		0.152		0.137	
B/A interaction	n.s.		0.040		n.s.		0.193	
A/B interaction	n.s.		0.026		n.s.		0.133	

Explanations see Table 4; n.s. - not significant difference

year than after the first year of cultivation; it was also higher in the soil of fertilized treatments than in the non-fertilized control.

After the first year, the highest content of Cr was detected in the object with spent mushroom substrate + NPK (3.72%), whereas after the second year it was the most elevated in the variants with manure (4.01%) and substrate (3.83%). Kalembasa and Pakuła (2009*a*) determined from 1.59 to 3.58% of chromium in the exchangeable fraction F1 in the surface layers of cultivated brown forest soil in central eastern Poland. The Cu content in the F1 and F2 fractions was similar (on average) after the first and the second year of cultivation. Fertilization significantly differentiated this content. The concentration of this metal in soil after the first year was the highest in the object fertilized with manure (5.54%) and after the second year – with spent mushroom substrate + NPK (6.06%). These results indicate that the soil processes during the two years of the experiment, which involved the cultivation of two different plants, enhanced the copper solubility in some objects, especially with spent mushroom substrate + NPK. HERRERO-HERNANDEZ et al. (2011) reports that one year after the application of spent mushroom substrate at 40 t ha^{-1}, the percentage of copper in the F1 fraction was 4.22%. KALEMBASA and MAJCHROWSKA-SAFARYAN (2011) detected, on average, 4.06% of copper in the bioavailable fractions (F1 and F2), extracted with the Zeien -Brummen method, of the humus horizons of different types of soil. This proves that only a small amount of copper appeared in easily soluble fractions, indicating copper mobility in soil environment, which has also been shown by DOMAŃSKA and FILIPEK (2011).

The content of chromium in the reductive fraction F2 (in total content) was higher than in the F1 fraction; it was higher after the first year of cultivation (14.5%) than after the second year (11.3%). The application of spent mushroom substrate alone and with NPK in the first year of cultivation contributed to the increase in Cr content in this fraction to a higher degree than after the second year. The introduction of organic matter into soil has an impact on proportions of chromium in individual fractions, indicating that the amount of this metal increases significantly in complexes with iron, manganese and organic compounds (KALEMBASA, PAKUŁA 2009*a*). The sequential analysis revealed three-fold less copper than chromium in the F2 fraction. The proportion of F2 Cu in the total content was comparable in the objects (4.60% after the first year of cultivation and 4.30% after the second year of cultivation). The highest content of Cu after the first year of cultivation was recorded in the object fertilized with manure (5.80%) and after the second year in the object fertilized with spent mushroom substrate + NPK (7.08%). The concentration of Cu in the F2 fraction after the first year of cultivation was higher in the treatments fertilized with organic materials, whereas after the second year it went up in the treatments fertilized with spent mushroom substrate, both compared with the controls. The analysis of variance revealed that the content of chromium and copper in the F2 fraction significantly depended on the year of study, fertilization and interaction between these factors.

In the organic fraction F3, the content of chromium was much higher than in the reductive fraction F2, i.e. 33.4% on average after the first year and 21.0% after the second year. The use of spent mushroom substrate alone (35.0%) and with NPK (35.3%), particularly in the first year of cultivation, contributed to the increase in Cr content in relation to the control object. KALEMBASA, PAKUŁA (2009*a*,*b*) detected a comparable proportion of Cr in the organic fraction in the humus horizons of brown forest soil in the Central-East Poland (18.5-41.4%). The content of copper in the F3 fraction was on average higher after the second year of cultivation (35.5%) than after the first year (31.0%). After the first year, the highest content of Cu was detected in the objects fertilized with manure alone and with NPK, whereas after the second year it was with manure and substrate + NPK. After the second year of cultivation, an increase in Cu content was recorded in all fertilized objects in comparison with the first year with the highest gain in the object fertilized with spent mushroom substrate alone (by 7.7%) and with NPK (by 7.2%). An increase in copper in the organic fraction by 30% in soil supplemented with a spent mushroom substrate in relation to a control object has been also reported by HERRERO-HERNANDEZ et al. (2011). Copper is found in considerable amounts in organic complexes (the oxidative fraction F3), which confirms its high affinity to the formation of complexes with functional groups of humic and fulvic acids (KARCZEWSKA 2002). The content of chromium and copper in the organic fraction F3 significantly depended on the year of study and type of fertilization.

The highest content of chromium and copper in the tested objects was detected in the residual fraction F4 after the first and the second year of cultivation. A higher proportion of Cr in this fraction was determined after the second year of cultivation (64.3%) than in the first year (48.9%). The lowest content of Cr in this fraction was recorded in the object fertilized with spent mushroom substrate alone and with NPK. The easy reduction of soluble Cr^{6+} to Cr^{3+} of low solubility in soil environment results in strong binding of this metal to soil solid phase (KALEMBASA, PAKUŁA 2009*a*). The content of copper in the F4 fraction was on average higher after the first year of cultivation than after the second year (60.1% and 55.8% respectively). After the first year of cultivation, the highest content of Cu of this fraction was detected in the object fertilized with spent mushroom substrate alone (63.2%), whereas after the second year in the object fertilized with NPK (60.6%). After the second year of cultivation in the objects supplemented with spent mushroom substrate alone and with NPK, the content of Cu in this fraction decreased in comparison with the first year. KALEMBASA and PAKUŁA (2009*b*), WÓJCIKOWSKA-KAPUSTA and NIEMCZUK (2009), GUAN et al. (2011) and HERRERO-HERNANDEZ et al. (2011) fractionated copper with the BCR method and found that this metal accumulated mainly in two frac-

Table 6

The correlation coefficients between the fractions of chromium and copper (mg kg^{-1}) and some properties after two years of field experiment

Parametr	Element	F1	F2	F3	F4
Total content	Cr Cu	-0.05 0.61*	-0.28 0.54	-0.22 0.88*	0.67* 0.93*
pH_{KCl}	Cr Cu	-0.34 0.37	0.46 0.25	0.55 0.21	-0.36 0.63*
OrgC	Cr Cu	0.66* 0.26	0.16 0.46	0.05 0.68*	-0.22 -0.14
CEC	Cr Cu	0.17 0.06	0.26 0.36	0.22 -0.03	-0.33 0.05

* significant α =0.05.

tions: residual F4 and oxidative F3. An analysis of variance showed that the content of both tested metals in F4 fraction depended on the year of study, type of fertilization and interaction between these factors. The statistical calculations (Table 6) revealed that the total content of copper was significantly correlated with the concentration of this metal in the easily soluble and exchangeable fraction F1 and the organic fraction F3, whereas for both these metals with the residual fraction F4. The pH value of the tested soil had a significant impact on the content of chromium in the F4 fraction and the content of carbon in organic compounds significantly correlated with the amount of chromium in the F1 fraction and copper in the F3 fraction.

CONCLUSIONS

1. Spent mushroom substrate, used for growing *Agaricus bisporus* and afterwards applied to soil significantly increased the total content of copper in the humus horizon. The content of chromium and copper in the soil under a two-year field experiment did not exceed the permissible limits specified in the Regulation of the Minister of the Environment and stayed with the natural value range.

2. The sequential fractionation of chromium and copper with the BCR procedure in the humus horizon of soil in the individual experimental objects revealed diversified concentration of these metals in the extracted fractions and their share in the total content. The spent mushroom substrate alone and with NPK contributed to a decrease in Cr content in the F2 and F3 fractions and to an increase in Cu content in the F1, F2 and F3 fractions after the second year of the cultivation in comparison with the first year. The highest concentration of tested metals in the total content was detected in the residual fraction F4: for chromium after the second year and for copper after the first year of the experiment.

3. The concentration of tested metals in the total content, in the extracted fractions and in the soil of experimental objects after two years of cultivation was arranged in the following order of decreasing values: for chromium F4>F3>F2>F1; and for copper F4>F3>F2⇔F1.

4. The analysis of variance showed that the content of chromium and copper in the extracted fractions depended significantly on the year of study and type of fertilization.

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