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The effect of different copper doses and organic fertilisation on soil's enzymatic activity

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Abstract: A three-year pot experiment carried out in the vegetation hall in 2014–2016 included studying the enzymatic activity of soil, into which various amounts of copper: (100, 200 and 300 mg Cu/kg soil) and organic materials (cattle manure, chicken manure, post-mushroom substrate) were introduced, used separately, at a soil-introduction dose of 2 g C_{org}/kg. Copper and organic materials were used once, only in the first year of the study, before sowing test plant orchard grass. In soil collected after the last (fourth) swath of grass in each year of the study, the activity of urease, dehydrogenases, acid, and alkaline phosphatase was determined. Applications of copper to the soil, regardless of its dose, resulted in a decrease in urease, dehydrogenases and alkaline phosphatase and an increase in acid phosphatase activity. The inactivating effect of this metal on the activity of urease, dehydrogenases and alkaline phosphatase increased with the increase of its dose. Organic fertilisation generally increased the enzymatic activity of the analysed soil. In subsequent years of the study, urease and alkaline phosphatase activity decreased, while acid phosphatase activity increased. Dehydrogenase activity did not change significantly in subsequent years of the study.

Keywords: enzymes; micronutrient; microorganism; toxic element; heavy metal

Enzymes are natural mediators and catalysts for many important processes in soils. They take part in the formation and decomposition of humus, biological reduction of molecular nitrogen from the air, nitrification and denitrification (Dick 1992, Acosta-Martínez 2007, Kalembasa and Kuziemska 2011, Olszowska 2016, Błońska et al. 2017, Bhavya et al. 2018). Their activity is influenced, among others, by temperature, pH value, humus content, and inhibitors, which include copper. According to Wang et al. (2009), the process of reducing the enzymatic activity of soil by copper occurs in several ways, including a direct inhibitory effect on the development of microorganisms, and the enzymes they produce, binding active protein groups of enzymes, forming complexes with substrates. The effect of this metal on soil enzymes may result from the penetration of Cu^{2+} ions into the cells of microorganisms or their adsorption on the surface of cells (Fernández-Calviño et al. 2010). The enzymes most sensitive to elevated copper levels in soil include urease and dehydrogenases, whereas phosphatases (acid and alkaline) are classified as medium-sensitive enzymes (Wyszkowska et al. 2005, 2013, 2017, Angelovičová et al. 2014).

The aim of the study was to determine the impact of various doses of copper and the protective organic fertilisation with cattle manure, chicken manure and mushroom substrate on the activity of urease, dehydrogenases, and acid and alkaline phosphatase.

MATERIAL AND METHODS

The pot experiment was carried out in the vegetation hall in 2014–2016. Pods with a capacity of 10 L were filled with 12 kg of soil Luvisols with the participation of the main granulometric fractions: sand 71%; silty 24%; clay 5%. The soil used in the study showed: pH in 1 mol/L KCl – 6.7; total nitrogen

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content 1.48 g N/kg; carbon in organic compounds 16.10 g C_{org}/kg; available phosphorus 189 mg P/kg, available potassium 110 mg K/kg, total copper 12.93 mg Cu/kg.

The following factors were taken into account in the scheme of the experiment carried out in a completely random system, in triplicate: I – copper dose: control object – without copper (0) and 100, 200, and 300 mg Cu/kg soil. This element was introduced into the soil once, before sowing the test plant, in the first decade of May, in the year of the beginning of the research, in the form of an aqueous solution of $CuSO_4 \cdot 5\ H_2O$; II – organic fertilisation: control object – without the use of organic materials (CO) and cattle manure (CM), chicken manure (ChM) and mushroom substrate (MS) used separately, once, in the year of starting the research, two weeks before sowing the test plant at a dose of 2 g of carbon in organic compounds – C_{org}/kg soil. Selected properties of organic materials are given in Table 1. In each organic materials samples, the following were determined: dry matter (DM in 105 °C), organic carbon by Tiurin method, total nitrogen content by CHNS method (Autoanalyser CHN with detector IDC, Series II 2400, Perkin-Elmer, California, USA), and the content of P, K, C, Mg, S and Cu by the ICP-AES method (Optima 3200 RL, Perkin-Elmer, Waltham, USA) after dry mineralisation of samples at 500 °C. In soil in each year of the experiment were determined: pH value (potentiometrically in 1 mol/L KCl), organic carbon by Tiurin method, Cu available for plants (by the ICP-AES method in extract 0.11 mol/L CH_3COOH).

In each year of research, orchard grass (*Dactylis glomerata* L.), cv. Amera was grown on all sites; four swaths (regrowth) were collected, with thirty days apart. In soil collected after the fourth and last swath of grass, in each (1, 2 and 3) year of the experiment: urease activity was determined using the Hoffman and Teicher method (1961), dehydrogenase activity was measured using the method of Casida et al. (1964), acid and alkaline phosphatase activity was measured using the Tabatabai and Bremner method (1969).

The results of the experiments were analysed by ANOVA. The significance of sources of variation was checked with the Fisher-Snedecor test, and mean values were separated with the Tukey's test at the significance level of $P < 0.05$. For these calculations, the Statistica 13 PL (StatSoft, Tulsa, USA) was used. In addition, Pearson's linear correlation coefficient was calculated for some of the examined features.

RESULTS AND DISCUSSION

The conducted studies showed a significant impact of differential copper doses and the application of organic materials – cattle manure, litter from laying hens, and post-mushroom substrate on the activity of urease, dehydrogenases, as well as acid and alkaline phosphatase in the analysed soil (Tables 2–5).

All copper doses reduced urease and dehydrogenase activity. The inhibitory effect of this metal intensified with the increase of its quantity in soil. Copper application at doses of 100, 200 and 300 mg Cu/kg resulted in a decrease in urease activity by 11.7, 44.5, and 55.8% (Table 2), while dehydrogenases by 10.3, 32.8 and 48.3% (Table 3), respectively in relation to the control object. Urease activity in soil fertilised with organic materials, regardless of their origin, was similar, but significantly higher compared to soil activity from the control object. The activity of this enzyme decreased in subsequent years of research. In the second and third years, it was 7.2% and 13.4% lower, respectively, than in the first year. All the organic materials used reduced the copper negative for dehydrogenase activity. Their activity determined in soil fertilised with cattle manure, mushroom substrate and chicken manure was 25.6, 20.5 and 7.7% higher, respectively than without their application. Interaction analysis indicated that the lowest copper dose (100 mg Cu/kg) used in combination with cattle manure and chicken manure did not significantly reduce dehydrogenase activity. Such protective effects of cattle manure and chicken manure were not obtained after using higher doses of copper (200 and 300 mg Cu/kg) and mushroom substrate after

Table 1. Chemical composition of organic materials used in a pot experiment

Organic material	Dry matter (DM, %)	C_{org}	N_{total}	C:N	P	K	Ca	Mg	S	Cu
		(g/kg DM)			(g/kg DM)					(mg/kg DM)
Cattle manure	20.0	394.5	23.70	16.6:1	6.50	17.02	11.28	3.24	3.68	5.97
Chicken manure	29.0	160.3	14.10	11.4:1	8.74	9.10	13.59	2.43	3.07	42.98
Mushroom substrate	31.0	315.7	24.50	12.9:1	6.14	17.20	45.18	3.12	26.20	15.61

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Table 2. Urease activity in the soil – averages for investigated factors (mg NH_4^+/kg/1 h)

Specification		Cu dose (mg/kg of soil)				Mean
		0	100	200	300	
Organic fertilisation	CO	8.98	7.79	3.65	2.80	5.81[a]
	CM	9.24	8.47	5.79	4.87	7.09[b]
	ChM	9.25	8.22	5.78	4.62	6.97[b]
	MS	9.73	8.38	5.40	4.14	6.91[b]
Year	I	9.84	8.87	5.72	4.32	7.19[c]
	II	9.25	8.07	5.19	4.18	6.67[b]
	III	8.82	7.70	4.57	3.83	6.23[a]
Mean		9.30[d]	8.21[c]	5.16[b]	4.11[a]	6.70
		Organic fertilisation				
		CO	CM	ChM	MS	
Year	I	6.22	7.69	7.40	7.43	
	II	5.77	6.99	7.00	6.93	
	III	5.43	6.60	6.51	6.39	

CO – control objects; CM – cattle manure; ChM – chicken manure; MS – mushrooms substrate. [a,b,c]Means for investigated factors with different letters (in the columns for organics fertilisation and ears but the row for cooper doses) are significantly different. All interactions (cooper dose/year, cooper dose/organic fertilisation and organic fertilisation/year) are not important

all doses of Cu was applied. The conducted studies showed no changes in dehydrogenase activity in subsequent years of the experiment.

The soil in which various doses of copper were introduced had similar acid phosphatase activity, but significantly higher its activity compared to the control object (Table 4). The application of cattle manure and manure from laying hens increased the activity of this enzyme in relation to its activity in soil without organic fertilisation, while fertilisation with post-mushroom substrate did not significantly affect its activity. In the case of alkaline phosphatase,

Table 3. Dehydrogenase activity in the soil – averages for investigated factors (mmol triphenyl formazan/kg soil/24 h)

Specification		Cu dose (mg/kg of soil)				Mean
		0	100	200	300	
Organic fertilisation	CO	0.54[C]	0.49[C]	0.32[B]	0.22[A]	0.39[a]
	CM	0.60[B]	0.56[B]	0.43[A]	0.38[A]	0.49[c]
	ChM	0.53[C]	0.48[C]	0.38[B]	0.29[A]	0.42[b]
	MS	0.64[D]	0.53[C]	0.42[B]	0.31[A]	0.47[c]
Year	I	0.58	0.53	0.39	0.32	0.45[a]
	II	0.59	0.52	0.38	0.28	0.44[a]
	III	0.56	0.51	0.40	0.30	0.44[a]
Mean		0.58[d]	0.52[c]	0.39[b]	0.30[a]	0.44
		Organic fertilisation				
		CO	CM	ChM	MS	
Year	I	0.38	0.50	0.43	0.51	
	II	0.40	0.49	0.42	0.46	
	III	0.41	0.49	0.41	0.46	

CO – control objects; CM – cattle manure; ChM – chicken manure; MS – mushrooms substrate. [A,B,C,D]Means for the interaction of the studied factors with different letters in the rows of the table are significantly different. Interactions: cooper dose/year and organic fertilisation/year are not important

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Table 4. Acid phosphatase activity in the soil – averages for investigated factors (mmol p-nitrophenol/kg soil/1 h)

Specification		Cu dose (mg/kg of soil)				Mean
		0	100	200	300	
Organic fertilisation	CO	0.45	0.48	0.49	0.46	0.47[a]
	CM	0.48	0.53	0.53	0.51	0.51[c]
	ChM	0.46	0.52	0.53	0.49	0.50[bc]
	MS	0.45	0.46	0.48	0.51	0.48[ab]
Year	I	0.44	0.46	0.49	0.46	0.46[a]
	II	0.46	0.51	0.51	0.49	0.49[b]
	III	0.48	0.53	0.53	0.53	0.52[c]
Mean		0.46[a]	0.50[b]	0.51[b]	0.49[b]	0.49
		Organic fertilisation				
		CO	CM	ChM	MS	
Year	I	0.44	0.49	0.47	0.45	
	II	0.46	0.51	0.51	0.48	
	III	0.51	0.54	0.52	0.50	

CO – control objects; CM – cattle manure; ChM – chicken manure; MS – mushrooms substrate. [a,b,c]Means for investigated factors with different letters (in the columns for organics fertilisation and ears but the row for cooper doses) are significantly different. All interactions (cooper dose/year, cooper dose/organic fertilisation and organic fertilisation/year) are not important

only the doses of 200 and 300 mg Cu/kg soil reduced its activity (Table 5). The lowest dose of 100 mg Cu/kg soil did not significantly affect the activity of this enzyme. According to research conducted by Nowak et al. (2003), in small amounts, heavy metals can stimulate phosphatase activity, while in large quantities; they reduce the number of microorganisms secreting this enzyme. No effects of cattle manure and chicken manure on alkaline phosphatase activity were found, whereas the introduction of

Table 5. Alkaline phosphatase activity in the soil – averages for investigated factors (mmol p-nitrophenol/kg soil/1 h)

Specification		Cu dose (mg/kg of soil)				Mean
		0	100	200	300	
Organic fertilisation	CO	0.80	0.75	0.72	0.66	0.73[a]
	CM	0.76	0.76	0.72	0.67	0.73[a]
	ChM	0.78	0.75	0.74	0.70	0.74[a]
	MS	0.84	0.80	0.80	0.78	0.81[b]
Year	I	0.85	0.83	0.82	0.76	0.81[c]
	II	0.80	0.77	0.72	0.67	0.74[b]
	III	0.74	0.70	0.69	0.68	0.70[a]
Mean		0.80[c]	0.77[bc]	0.74[b]	0.70[a]	0.75
		organic fertilisation				
		CO	CM	ChM	MS	
Year	I	0.78	0.81	0.81	0.86	
	II	0.73	0.70	0.73	0.80	
	III	0.69	0.66	0.69	0.76	

CO – control objects; CM – cattle manure; ChM – chicken manure; MS – mushrooms substrate. [a,b,c]Means for investigated factors with different letters (in the columns for organics fertilisation and ears but the row for cooper doses) are significantly different. All interactions (cooper dose/year, cooper dose/organic fertilisation and organic fertilisation/year) are not important

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Table 6. Soil's pH value and content of organic carbon (g C_{org}/kg) and copper is available for plant form (mg Cu_{av}/kg) – averages for investigated factors

Soil parameter	Organic fertilisation				Cu dose (mg/kg of soil)				Year		
	CO	CM	ChM	MS	0	100	200	300	I	II	III
pH	6.66	6.52	6.72	6.76	6.76	6.69	6.63	6.57	6.73	6.66	6.60
C_{org}	15.8^a	17.6^b	17.1^b	17.3^b	16.9^a	17.0^a	16.8^a	16.9^a	17.4^c	16.9^b	16.5^a
Cu_{av}	134.6^b	121.2^a	113.5^a	115.6^a	0.8^a	80.4^b	146.7^c	257.0^d	136.9^c	122.3^b	104.5^a

CO – control objects; CM – cattle manure; ChM – chicken manure; MS – mushrooms substrate. ^{a,b,c}Means for investigated factors with different letters in the rows are significantly different

the post-mushroom substrate caused its increase. In subsequent years of research, acid phosphatase activity in the analysed soil increased, while alkaline phosphatase decreased.

Increasing doses of copper introduced into the soil increased the amount of this metal in forms available to plants (Table 6). All organic materials increased the organic carbon content in the soil, and reduced the amount of copper in the form available to plants. In subsequent years of research, the content of organic carbon, available copper, and soil pH value was reduced.

Correlation analysis confirmed the negative effect of increasing copper doses and its amount of available forms to plants on the activity of urease, dehydrogenases, and alkaline phosphatase (Table 7).

The conducted study showed that the application of high doses of copper reduces the enzymatic activity of the soil, which indirectly reduces its fertility and capability, which is its measure according to Russel (2005). The negative effect of soil contamination with copper on its enzymatic activity is highlighted in studies by Chaperon and Sauvé (2007), Wang et al. (2007), Fernandéz-Calvińo et al. (2010), Zhan and Sun (2014), Wyszkowska et al. (2017), and Li et al. (2018). The inactivating effect of this metal on the activity of urease, dehydrogenases and alkaline phosphatase increased with the increase of its dose. This proves that copper gradually blocks active centres of enzymes, modifies their molecular structure and reduces the amount of soil microorganisms (Becker et al. 2006, Oliveira and Pampulha 2006). In the case of acid phosphatase, it was activated by copper, but its activity in soil fertilised with various doses of this metal was similar.

The effect of organic materials (cattle manure, chicken manure, and post-mushroom substrate) on the enzymatic activity of the studied soil was diverse but generally stimulating. This diversity could have been due to slightly different chemical composition, C:N ratio, which could differentiate their soil mineralisation rate. The activating effect of organic matter on the activity of soil enzymes was demonstrated by Chang et al. (2007) and Kotroczó et al. (2014). Cattle manure and chicken manure significantly limited the inactivating effect of 100 mg copper on dehydrogenase activity, which is associated with the activity of many enzyme systems commonly found in aerobic and anaerobic soil microorganisms. The organic substance introduced with them limited the mobility of copper as a result of the immobilisation process that occurs due to the mechanism of chemical complexation and sorption (Cuske and Karczewska 2016).

In the summary of the conducted study, it should be stated that the application to copper soil reduced

Table 7. Linear correlation coefficients between copper doses, soil's pH and enzymatic activity in the soil

Specification	Urease	Dehydrogenase	Acid phosphatase	Alkaline phosphatase
Cu dose	–0.938*	–0.916*	0.427	–0.697*
Cu available for plants	–0.897*	–0.921*	0.361	–0.736*
pH	0.543*	0.446	–0.558*	0.798*
Carbon organic	0.380	0.334	0.408	0.187
Urease	–	0.964*	–0.290	0.695*
Dehydrogenase	–	–	–0.250	0.721*
Acid phosphatase	–	–	–	–0.376

*$P \leq 0.05$

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the activity of urease, dehydrogenases, and alkaline phosphatase, and increased the activity of acid phosphatase. The inactivating effect of this metal on the activity of urease, dehydrogenases and alkaline phosphatase increased with the increase of its dose. Soil fertilised with cattle manure, chicken manure and mushroom substrate generally showed greater enzymatic activity compared to the control object, which indicates the possibility of their use for the recultivation of soils contaminated with copper, using the *in situ* method. Cattle manure had a particularly beneficial effect on this soil characteristic.

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