J. Elementol. 2010, 15(1): 213–226

ACTIVITY OF β-GLUCOSIDASE, ARYLSULFATASE AND PHOSPHATASES IN SOIL CONTAMINATED WITH COPPER

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Abstract

A pot experiment was carried out to determine the effect of soil (loamy sand and sandy loam) contamination with copper doses of 0, 150, 450 mg $Cu \cdot kg^{-1}$ d.m. soil on the activity of β-glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1) and arylsulfatase (EC 3.1.6.1) in soil. The resistance of these enzymes to copper pollution was also estimated. Soil samples were contaminated with copper chloride. The experiment was carried out in five replications, in two series. The first series was performed on uncropped soil and the second one – on cropped soil. The experimental plants were oat, spring rape and yellow lupine. The activity of soil enzymes was determined in the analyzed samples on the 25^{th} and the 50^{th} day of the experiment.

The results of the experiment showed that copper contamination in doses of 150 mg to 450 $mg \cdot kg^{-1}$ soil significantly inhibits soil's biochemical activity. The sensitivity of the tested enzymes to copper was determined in the following order: alkaline phosphatase > arylsulfatase > acid phosphatase > β-glucosidase.

The resistance of the above enzymes to copper depended on the cultivated plant species, soil type and the type of soil use and management. In samples of sandy loam, copper induced the smallest change in the activity of acid phosphatase and alkaline phosphatase, and in loamy sand – in the activity of arylsulfatase and acid phosphatase. In uncropped soil, copper was the least effective in changing the activity of arylsulfatase and acid phosphatase. All of the tested enzymes were less resistant to copper contamination in cropped than in uncropped soil. In soil planted with oat, β-glucosidase was the most resistant and arylsulfatase was the least resistant enzyme to copper contamination. In samples sown with spring rape, the analogous enzymes were arylsulfatase and alkaline phosphatase. In yellow lupine treatments, alkaline phosphatase was the most and β-glucosidase was the least resistant enzyme.

Key words: copper, β-glucosidase, phosphatase, arylsulfatase, resistance rate, soil contamination with copper.

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AKTYWNOŚĆ β-GLUKOZYDAZY, ARYLOSULFATAZY I FOSFATAZ W GLEBACH ZANIECZYSZCZONYCH MIEDZIĄ

Abstrakt

W doświadczeniu wazonowym badano wpływ zanieczyszczenia gleby (piasku gliniastego oraz gliny piaszczystej) miedzią w dawkach: 0, 150, 450 mg Cu·kg^{-1} s.m. gleby na aktywność: β-glukozydazy (EC 3.2.1.21), fosfatazy kwaśnej (EC 3.1.3.2), fosfatazy alkalicznej (EC 3.1.3.1) i arylosulfatazy (EC 3.1.6.1) w glebie. Określono także odporność tych enzymów na zanieczyszczenie miedzią. Glebę zanieczyszczano chlorkiem miedzi. Badania prowadzono w 5 powtórzeniach, w dwóch seriach. W pierwszej serii doświadczenia gleba była nieobsiana roślinami, w drugiej – obsiana. Roślinami doświadczalnymi były: owies, rzepak jary i łubin żółty. W 25. i 50. dniu trwania eksperymentu oznaczono w próbkach glebowych aktywność enzymów glebowych.

Stwierdzono, że zanieczyszczenie gleby miedzią w zakresie od 150 mg do 450 mg·kg^{-1} gleby istotnie hamuje jej aktywność biochemiczną. Testowane enzymy, pod względem wrażliwości na miedź, można uszeregować następująco: fosfataza alkaliczna > arylosulfataza > fosfataza kwaśna > β-glukozydaza.

Odporność enzymów na działanie miedzi zależała od gatunku uprawianej rośliny, rodzaju gleby i sposobu jej użytkowania. W glinie piaszczystej miedź wywołała najmniejsze zmiany w aktywności fosfatazy kwaśnej i fosfatazy alkalicznej, natomiast w piasku gliniastym – β-glukozydazy oraz arylosulfatazy. W glebie nieobsianej roślinami miedź wywoływała najmniejsze zakłócenia w aktywności arylosulfatazy i fosfatazy kwaśnej. W glebie obsianej roślinami wszystkie testowane enzymy były mniej odporne na zanieczyszczenie miedzią niż w glebie obsianej. Najbardziej odpornym enzymem na działanie miedzi pod uprawą owsa była β-glukozydaza, a najmniej – arylosulfataza, pod uprawą rzepaku jarego, odpowiednio – arylosulfataza i fosfataza alkaliczna, natomiast pod uprawą łubinu żółtego najbardziej odporna była fosfataza alkaliczna, a najmniej β-glukozydaza.

Słowa kluczowe: miedź, β-glukozydazy, fosfataza, arylosulfataza, indeks odporności, zanieczyszczenie gleby miedzią.

INTRODUCTION

Copper is a biogenic element, whose small quantities are necessary for proper functions of live organisms, while high copper doses can be toxic. In Poland, soils containing excessive levels of this metal are rare and can be found mostly in southern parts of the country, especially in Silesia and Małopolska. In 2005, a monitoring study was completed, which revealed that the distribution of soil falling into different copper contamination categories had not changed significantly since the previous years, i.e. 0ş degree of contamination was determined in 95.9% soils, I^{o} – in 2.7%, II^{o} – in 0.5%, III^{o} – 0%, IV^{o} – 0.9% and V^{o} – 0% (Terelak et al. 2008). The highest level of copper pollution is noticed in the vicinity of copper plants, where soil is strongly degraded and difficult to reclaim. For this reason, the accumulation of heavy metals, including copper, in surface soil layers is highly dangerous because it disrupts the soil metabolism (De Brouwere et al. 2007, Mertens et al. 2007, Oliveira, Pampulha 2006, Wyszkowska et al. 2005b).

If excessive quantities of heavy metals reach the soil, they have a strongly toxic effect on soil microbes and inhibit the activity of soil enzymes (RENELLA et al. 2005, MIKANOVA et al. 2001, KUCHARSKI, WYSZKOWSKA 2004). Soil contamination with heavy metals slows down many biological processes. It affects populations and species diversity of macro- and microorganisms as well as soil's enzymatic activity (BIELIŃSKA 2005, WELP 1999, WYSZKOWSKA et al. 2005b, WYSZKOWSKA et al. 2005a, ZHENG et al. 1999). The destructive effect of heavy metals on the microbiological and biochemical properties of soil is modified by the soil's granulometric composition, pH, organic content and sorptive capacity (MORENO et al. 2001).

The objective of this study was to determine the effect of copper contamination of soil characterized by different grain size distribution on the activity of b-glucosidase, acid phosphatase, alkaline phosphatase and arylsulfatase, and to estimate the resistance of these enzymes to excessive copper concentrations in soil. The study was carried out as part of research project No N N305 2258 33 supported by the Ministry for Science and Higher Education.

MATERIALS AND METHODS

The experiment was conducted in polyethylene pots (in five replications) in a greenhouse of the University of Warmia and Mazury in Olsztyn, Soil samples collected from the humus horizon were analyzed. In the natural state, they consisted of:

1) typical brown soil developed from loamy sand (pH in 1 mol $KCl \cdot dm^{-3}$ – 6.70; hydrolytic acidity – 7.8 mmol(+) kg^{-1}; total exchangeable cations – 98 mmol(+)$\cdot kg^{-1}$; exchange capacity of adsorption complex – 105.8 mmol(+) kg^{-1}; base saturation – 92.6%; content of: C_{org} – 11.0 $g \cdot kg^{-1}$, K – 180 $mg \cdot kg^{-1}$, Mg – 80 $mg \cdot kg^{-1}$, Ca – 1.43 $g \cdot kg^{-1}$, Na – 28 $mg \cdot kg^{-1}$ and N – 0.97 $g \cdot kg^{-1}$);

2) typical brown soil developed from sandy loam (pH in 1 mol $KCl \cdot dm^{-3}$ – 6.80; hydrolytic acidity – 5.2 mmol(+) kg^{-1}; total exchangeable cations – 80.0 mmol(+)$\cdot kg^{-1}$; exchange capacity of adsorption complex – 85.2 mmol(+) kg^{-1}; base saturation – 93.9 %; content of: C_{org} – 9.9 $g \cdot kg^{-1}$, K – 168 $mg \cdot kg^{-1}$, Mg – 50 $mg \cdot kg^{-1}$, Ca – 2.21 $g \cdot kg^{-1}$, Na – 57 $mg \cdot kg^{-1}$ and N – 1.14 $g \cdot kg^{-1}$);

The grain size composition of the above soils is presented in Table 1. Soil was contaminated with copper in the form of $CuCl_2 \cdot 2H_2O$ in the amount of 0, 150, 450 mg $Cu \cdot kg^{-1}$ d.m. soil. The first dose (150 $mg \cdot kg^{-1}$) was equivalent to the maximum admissible copper dose stated in the Regulation of the Minister for the Environment of 9 September 2002 (*Regulation of the Minister for the Environment,* 2002, Journal of Laws 02.165.1359). Soil samples were passed through through a 1 cm mesh sieve, mixed with mineral fertilizer and, in selected treatments, with copper chloride; afterwards, they were

Table 1

Granulometric composition of soil

Type of soil	Percentage of fractions (d)		
	sand 2.00 ≥ d > 0.05 mm	dust 0.05 ≥ d > 0.002 mm	clay d ≤ 0.002 mm
Loamy sand	75.56	22.92	1.52
Sandy loam	47.92	48.71	3.37

placed in pots. Macronutrients were added to all pots in the following doses (as pure substance per $mg \cdot kg^{-1}$ soil): N – 100 (yellow lupine was not fertilized with nitrogen), P – 35, K – 100, Mg – 20. Nitrogen was applied in the form of $CO(NH_2)_2$, phosphorus – KH_2PO_4, potassium – KH_2PO_4 + KCl and magnesium – $MgSO_4 \cdot 7H_2O$. Soil samples weighing 3 kg each were placed in pots, and the moisture content of soil was brought to 60% capillary water capacity. The samples were planted with oat cv. Kasztan, spring rape cv. Huzar and yellow lupine cv. Mister. The plants were thinned after emergence, and the following number of plants were left in pots: oat – 12, spring rape – 8 and yellow lupine – 5. A control series of uncropped soil samples was established to support the determination of copper's effect on soil enzymes. The moisture content of soil was maintained at 60% capillary water capacity throughout the entire experiment (50 days).

The activity of the investigated enzymes: β-glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1) and arylsulfatase (EC 3.1.6.1), was determined twice during the experiment (on day 25 and 50) in three successive replications. The above enzymes were determined in line with the procedure described by Alef and Nannpieri (1998). Their resistance to soil contamination with copper was estimated by the method proposed by Orwin and Wardle (2004).

The results were processed statistically with the use of Duncan's multiple range test. Statistical analysis was performed with the Statistica application (StatSoft, Inc. 2006).

RESULTS AND DISCUSSION

The results of the study indicate that soil contamination with copper affected the soil's biological balance measured by the activity of β-glucosidase, acid phosphatase, alkaline phosphatase and arylsulfatase (Tables 2–9). Disturbances in the soil's homeostasis were dependent on several factors, including the applied metal dose and cultivated plant species (Table 2). In unpolluted soil samples, the highest levels of β-glucosidase activity were noted in treatments sown with oat, alkaline phosphatase – in treatments sown

Table 2

Effect of soil pollution with copper and crop species on activity of soil enzymes

Cu dose (mg kg^{-1} of soil)	Crop species		
	oats	spring oilseed rape	yellow lupine
β-glukosidase, mmol PNP kg^{-1} h^{-1}			
0	0.795 ± 0.127	0.739 ± 0.022	0.691 ± 0.037
150	0.624 ± 0.023	0.597 ± 0.035	0.609 ± 0.015
450	0.673 ± 0.029	0.509 ± 0.030	0.570 ± 0.016
Average	0.697	0.615	0.624
LSD	a – 0.016; b – 0.016; $a \cdot b$ – 0.028		
Acid phosphatase, mmol PNP kg^{-1} h^{-1}			
0	2.072 ± 0.068	2.515 ± 0.061	2.577 ± 0.113
150	1.520 ± 0.088	1.677 ± 0.063	1.913 ± 0.101
450	0.963 ± 0.078	1.496 ± 0.082	1.547 ± 0.089
Average	1.519	1.896	2.012
LSD	a – 0.030; b – 0.030; $a \cdot$ b – 0.051		
Alkaline phosphatase, mmol PNP kg^{-1} h^{-1}			
0	3.684 ± 0.241	4.871 ± 0.175	4.480 ± 0.114
150	3.197 ± 0.073	3.489 ± 0.141	3.410 ± 0.148
450	1.657 ± 0.124	2.229 ± 0.115	2.382 ± 0.115
Average	2.846	3.529	3.424
LSD	a – 0.050; b – 0.050; $a \cdot b$ – 0.087		
Arylosulphatase, mmol PNP kg^{-1} h^{-1}			
0	0.284 ± 0.013	0.398 ± 0.086	0.464 ± 0.029
150	0.186 ± 0.020	0.287 ± 0.074	0.283 ± 0.009
450	0.113 ± 0.015	0.188 ± 0.059	0.156 ± 0.015
Average	0.194	0.291	0.301
LSD	a – 0.044; b – 0.044; $a \cdot b$ – 0.077		

LSD for: a – copper rate, b – crop species

with spring rape, acid phosphatase and arylsulfatase – in pots cropped with yellow lupine. The lowest activity of β-glucosidase was observed in soil samples sown with yellow lupine, acid phosphatase, alkaline phosphatase and arylsulfatase – in treatments sown with oat. Although β-glucosidase, acid phosphatase, alkaline phosphatase and arylsulfatase are members of the same enzyme group, they responded differently to copper pollution even in soil samples contaminated with the copper doses which are admissible by

Table 3

Index of resistance of enzymes to soil pollution with copper depending on crop species*

Cu dose (mg kg^{-1} of soil)	Crop species		
	oats	spring oilseed rape	yellow lupine
β-glukosidase			
150	0.653 *b*	0.508 *c*	0.476 *d*
450	0.715 *a*	0.463 *d*	0.398 *e*
Average	0.684 *x*	0.486 *y*	0.437 *z*
Acid phosphatase			
150	0.586 *a*	0.521 *c*	0.538 *b*
450	0.302 *f*	0.422 *e*	0.476 *d*
Average	0.444 *x*	0.471 *y*	0.507 *z*
Alkaline phosphatase			
150	0.572 *b*	0.531 *c*	0.614 *a*
450	0.255 *e*	0.269 *e*	0.346 *d*
Average	0.414 *x*	0.400 *y*	0.480 *z*
Arylosulphatase			
150	0.511 *c*	0.660 *a*	0.610 *b*
450	0.248 *f*	0.414 *d*	0.277 *e*
Average	0.380 *x*	0.537 *y*	0.443 *z*

* homogenous groups for the activity of each enzyme are marked with the same letter

the Resolution of the Minister of the Environment. Copper contamination affected the activity of β-glucosidase, acid phosphatase, alkaline phosphatase and arylsulfatase. The sensitivity of the tested enzymes to the highest copper dose (450 mg kg^{-1} d.m. soil) was determined in the following order: arylsulfatase (decrease in activity by 66% in pots sown with yellow lupine) > alkaline phosphatase (decrease of 55% in treatments planted with oat) > acid phosphatase (decrease of 53% in pots cropped with oat) > β-glucosidase (decrease of 31% in treatments sown with spring rape). The above enzymes showed different resistance to the inhibitory effect of copper, subject to the crop species (Table 3). β-glucosidase was most resistant in soil sown with oat, acid phosphatase and alkaline phosphatase – in soil sown with yellow lupine, and arylsulfatase – in soil planted with spring rape.

The strength and direction of copper's adverse effect on enzymes was determined not only by the level of contamination and the cultivated plant species, but also by the type of soil use and management (Table 4). Regardless of the degree of soil's contamination with copper, on average, higher levels of β-glucosidase, alkaline phosphatase and arylsulfatase were deter-

Table 4

Effect of soil pollution with copper and land use on activity of soil enzymes

Cu dose (mg kg^{-1} of soil)	Land use	
	unseeded soil	seeded soil
β-glukosidase, mmol PNP kg^{-1} h^{-1}		
0	0.709 ± 0.028	0.742 ± 0.062
150	0.665 ± 0.022	0.610 ± 0.024
450	0.443 ± 0.012	0.584 ± 0.025
Average	0.605	0.645
LSD	a – 0.016; b — 0.013; $a \cdot b$ – 0.023	
Acid phosphatase, mmol PNP kg^{-1} h^{-1}		
0	1.975 ± 0.088	2.388 ± 0.081
150	1.914 ± 0.065	1.703 ± 0.084
450	1.562 ± 0.052	1.335 ± 0.103
Average	1.817	1.809
LSD	a – 0.030; b – 0.025; $a \cdot b$ – 0.043	
Alkaline phosphatase, mmol PNP kg^{-1} h^{-1}		
0	3.283 ± 0.112	4.345 ± 0.176
150	1.931 ± 0.116	3.365 ± 0.121
450	1.680 ± 0.061	2.089 ± 0.118
Average	2.298	3.267
LSD	a – 0.050; b – 0.041; $a \cdot b$ – 0.071	
Arylosulphatase, mmol PNP kg^{-1} h^{-1}		
0	0.258 ± 0.015	0.382 ± 0.130
150	0.198 ± 0.011	0.252 ± 0.034
450	0.137 ± 0.009	0.152 ± 0.030
Average	0.198	0.262
LSD	a – 0.044; b – 0.036; $a \cdot b$ – 0.062	

LSD for: a – copper rate, b – land use

mined in cropped than in uncropped soil. The average activity of acid phosphatase was comparable in both series. β-glucosidase, acid phosphatase and arylsulfatase were more resistant to copper's inhibitory effect in uncropped than in cropped soil (Table 5), whereas the average resistance of alkaline phosphatase to copper was similar in both series.

Soil type was an important factor which modified soil's enzymatic activity (Table 6). All of the tested enzymes were marked by higher levels

Table 5

Index of resistance of enzymes to soil pollution with copper depending on land use*

Cu dose (mg kg^{-1} of soil)	Land use	
	unseeded soil	unseeded soil
β-glukosidase		
150	0.853 *a*	0.546 *b*
450	0.465 *d*	0.525 *c*
Average	0.659 *x*	0.536 *y*
Acid phosphatase		
150	0.750 *a*	0.548 *c*
450	0.591 *b*	0.400 *d*
Average	0.671 *x*	0.474 *y*
Alkaline phosphatase		
150	0.487 *b*	0.573 *a*
450	0.355 *c*	0.290 *d*
Average	0.421 *x*	0.431 *y*
Arylosulphatase		
150	0.620 *a*	0.594 *b*
450	0.396 *c*	0.313 *d*
Average	0.508 *x*	0.453 *y*

* homogenous groups for the activity of each enzyme are marked with the same letter

of activity in sandy loam than in loamy sand, but the presence of copper was more likely to affect the reactions catalyzed by the tested enzymes in loamy sand. In loamy sand samples, a copper dose of 450 mg kg^{-1} lowered the activity of alkaline phosphatase by 68%, arylsulfatase – by 60%, acid phosphatase by 50% and β-glucosidase by 33%. In sandy loam, the investigated pollutant lowered the activity of alkaline phosphatase by 41%, arylsulfatase – by 56%, acid phosphatase by 29% and β-glucosidase by 19%. In sandy loam samples, acid phosphatase, alkaline phosphatase and arylsulfatase were more resistant to copper's inhibitory effect, whereas in loamy sand, the above was observed for β-glucosidase (Table 7).

Our analysis of another experimental variable, i.e. copper persistence, revealed that the average activity of acid phosphatase and arylsulfatase was higher on experimental day 25, and the activity of β-glucosidase and alkaline phosphatase on the day 50 (Table 8). The highest difference was noted in respect of β-glucosidase, whose activity on day 50 was 66% higher than on day 25, as well as acid phosphatase, whose activity was 35% higher on day 25. On the 25^{th} day of copper deposition in soil, the average resistance

Table 6

Effect of soil pollution with copper and soil type on soil enzymatic activity

Cu dose (mg kg^{-1} of soil)	Type of soil	
	loamy sand	loamy sand
β-glukosidase, mmol PNP kg^{-1} h^{-1}		
0	0.683 ± 0.027	0.784 ± 0.080
150	0.574 ± 0.024	0.673 ± 0.023
450	0.460 ± 0.014	0.637 ± 0.030
Average	0.573	0.698
LSD	a – 0.016; b – 0.013; $a \cdot b$ – 0.023	
Acid phosphatase, mmol PNP kg^{-1} h^{-1}		
0	2.267 ± 0.083	2.303 ± 0.081
150	1.494 ± 0.075	2.018 ± 0.083
450	1.143 ± 0.062	1.641 ± 0.117
Average	1.634	1.988
LSD	a – 0.030; b – 0.025; $a \cdot b$ – 0.043	
Alkaline phosphatase, mmol PNP kg^{-1} h^{-1}		
0	3.108 ± 0.105	5.051 ± 0.216
150	2.061 ± 0.057	3.953 ± 0.182
450	1.001 ± 0.067	2.973 ± 0.141
Average	2.056	3.993
LSD	a – 0.050; b – 0.041; $a \cdot b$ – 0.071	
Arylosulphatase, mmol PNP kg^{-1} h^{-1}		
0	0.317 ± 0.171	0.385 ± 0.031
150	0.178 ± 0.039	0.299 ± 0.018
450	0.127 ± 0.035	0.170 ± 0.014
Average	0.207	0.285
LSD	a – 0.044; b – 0.036; $a \cdot b$ – 0.062	

LSD for: a – copper rate, b – type of soil

rate of β-glucosidase was 0.440, acid phosphatase – 0.567, alkaline phosphatase – 0.405 and arylsulfatase – 0.501. On the 50^{th} day of the experiment, the following resistance rates were noted: 0.693, 0.480, 0.453 and 0.433, respectively (Table 9).

Table 7

Index of resistance of enzymes to soil pollution with copper depending on type of soil*

Cu dose (mg kg^{-1} of soil)	Type of soil	
	loamy sand	loamy sand
β-glukosidase		
150	0.729 *a*	0.516 *c*
450	0.543 *b*	0.477 *d*
Average	0.636 *x*	0.497 *y*
Acid phosphatase		
150	0.529 *c*	0.668 *a*
450	0.342 *d*	0.553 *b*
Average	0.436 *y*	0.611 *x*
Alkaline phosphatase		
150	0.509 *b*	0.593 *a*
450	0.194 *d*	0.419 *c*
Average	0.352 *y*	0.506 *x*
Arylosulphatase		
150	0.543 *b*	0.657 *a*
450	0.353 *c*	0.315 *d*
Average	0.448 *y*	0.486 *x*

* homogenous groups for the activity of each enzyme are marked with the same letter

Copper's inhibitory effect on the activity of all the analyzed soil enzymes was also noted in our previous study (Wyszkowska et al. 2005a,b) as well as in experiments performed by other authors (Giller et al. 1998, Gulser et al. 2008, Karlen et al. 2003, Schoenholtz et al. 2000). The inhibitory effect of soil contamination with copper on enzymatic activity could be due to copper's indirect toxic influence on microbial proliferation (Giller et al. 1998, Kucharski, Wyszkowska 2004, Oliveira, Pampulha 2006) as well as its direct, destructive impact on enzymes (Karlen et al. 2003, Moreno et al. 2001, Wyszkowska et al. 2005b) and the processes catalyzed by those enzymes (Giller et al. 1998).

Table 8

Effect of soil pollution with copper and date of analysis on activity of soil enzymes

Cu dose (mg kg^{-1} of soil)	Time of analysis, days	
	25	50
β-glukosidase, mmol PNP kg^{-1} h^{-1}		
0	0.682 ± 0.077	0.785 ± 0.031
150	0.428 ± 0.022	0.819 ± 0.025
450	0.320 ± 0.021	0.777 ± 0.023
Average	0.477	0.794
LSD	a – 0.016; b – 0.013; $a \cdot b$ – 0.023	
Acid phosphatase, mmol PNP kg^{-1} h^{-1}		
0	2.688 ± 0.091	1.882 ± 0.074
150	2.121 ± 0.079	1.392 ± 0.079
450	1.804 ± 0.106	0.980 ± 0.074
Average	2.204	1.418
LSD	a – 0.030; b – 0.025; $a \cdot b$ – 0.043	
Alkaline phosphatase, mmol PNP kg^{-1} h^{-1}		
0	4.013 ± 0.180	4.146 ± 0.141
150	2.659 ± 0.111	3.355 ± 0.128
450	1.742 ± 0.096	2.232 ± 0.112
Average	2.804	3.244
LSD	a – 0.050; b – 0.041; $a \cdot b$ – 0.071	
Arylosulphatase, mmol PNP kg^{-1} h^{-1}		
0	0.372 ± 0.058	0.330 ± 0.144
150	0.273 ± 0.046	0.204 ± 0.010
450	0.172 ± 0.041	0.125 ± 0.009
Average	0.272	0.220
LSD	a – 0.044; b – 0.036; $a \cdot b$ – 0.062	

LSD for: a – copper rate, b – date of analysis

Table 9

Index of resistance of enzymes to copper pollution depending on the date of analysis*

Cu dose (mg kg^{-1} of soil)	Time of analysis, days	
	25	50
β-glukosidase		
150	0.524 *c*	0.722 *a*
450	0.356 *d*	0.664 *b*
Average	0.440 *y*	0.693 *x*
Acid phosphatase		
150	0.627 *a*	0.570 *b*
450	0.507 *c*	0.389 *d*
Average	0.567 *x*	0.480 *y*
Alkaline phosphatase		
150	0.529 *a*	0.574 *b*
450	0.281 *d*	0.332 *c*
Average	0.405 *y*	0.453 *x*
Arylosulphatase		
150	0.644 *a*	0.556 *b*
450	0.358 *c*	0.310 *d*
Average	0.501 *x*	0.433 *y*

* homogenous groups for the activity of each enzyme are marked with the same letter

CONCLUSIONS

1. Copper contamination in doses of 150 mg to 450 mg·kg^{-1} soil significantly inhibits soil's biochemical activity. The sensitivity of the tested enzymes to copper was determined in the following order: alkaline phosphatase > arylsulfatase > acid phosphatase > β-glucosidase.

2. The resistance of the enzymes to copper depended on the cultivated plant species, soil type and the type of soil use and management. In samples of sandy loam, copper induced the smallest change in the activity of acid phosphatase and alkaline phosphatase, and in loamy sand – β-glucosidase and arylsulfatase proved to be most resistant to the tested pollutant. In uncropped soil, copper least altered the activity of arylsulfatase and acid phosphatase. All the tested enzymes were less resistant to copper contamination in cropped than in uncropped soil. In soil planted with oat, the enzyme most resistant to copper contamination was β-glucosidase, while aryl-

sulfatase was the least resistant. In samples sown with spring rape, the analogous enzymes were arylsulfatase and alkaline phosphatase, and in yellow lupine treatments, alkaline phosphatase was the most resistant and β-glucosidase was the least resistant enzyme.

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