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ORIGINAL PAPER

EFFECT OF DIFFERENT ZINC DOSES AND ORGANIC FERTILIZATION ON SOIL'S ENZYMATIC ACTIVITY*

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Abstract

The enzymatic activity of soil was studied in a three-year pot experiment, where different amounts of zinc were introduced: 0, 200, 400, and 600 mg of Zn kg^{-1} of soil, as well as organic materials (control object, bovine manure, laying hens' litter, spent mushroom substrate), used separately with the soil application dose of 2 g C_{org} kg^{-1} soil. Zinc and organic materials were used once, only in the first year of research, before sowing the test plant. In each year of the experiment, the test plant was orchard grass (*Dactylis glomerata* L.), harvested after 4 cuts. The activity of urease, dehydrogenases, as well as acid and alkaline phosphatase were determined in the soil collected after the last grass cutting, in each year of the study. Application of zinc to soil, regardless of its dose, resulted in a decrease in the activity of urease, dehydrogenase and alkaline phosphatase, although the significance of differences in relation to the control object was demonstrated only at higher doses, such as 400 and 600 mg Zn kg^{-1} soil. The study showed no negative effect of zinc on acid phosphatase activity. Organic fertilizers generally resulted in an increase in the enzymatic activity of the analyzed soil, and simultaneously limited the negative effects of higher zinc doses (400 and 600 mg Zn kg^{-1} of soil) on urease and dehydrogenase activity. During the consecutive years, urease and alkaline phosphatase activity decreased, while acid phosphatase activity increased. Dehydrogenase activity did not change significantly in the subsequent years of the study.

Keywords: zinc, organic materials, urease, dehydrogenases, acid phosphatase, alkaline phosphatase.

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INTRODUCTION

Soil quality depends mainly on the transformation of organic matter, related to the activity of microorganisms and their secreted enzymes, as well as the rate of biochemical changes in cycles of elements (Schloter et al. 2003, Bielińska, Mocek-Płóciniak 2010, Olszowska 2014, Błońska et al. 2017). Soil enzymes are natural mediators and catalysts of important soil processes, such as soil humus formation and degradation, change in the amount of available plant nutrients, nitrogen fixation process, etc. (Błońska et al. 2017). The level of enzymatic activity also provides information about ecological changes in the soil environment, constituting a sensitive indicator of its fertility and capability (Zajączkowska, Kucharski 2018).

Assessment of soil's biological activity can be based on interactions of enzymes, such as urease, which is a specific enzyme catalyzing the hydrolysis of urea to carbon dioxide and ammonia, dehydrogenases catalyzing oxidoreductive processes, which are also enzymes that accelerate the rate of dehydrogenation of specific substrates in biochemical oxidation processes of organic compounds, and alkaline and acid phosphatases, which belong to a broad group of enzymes that catalyze the hydrolysis of organic phosphorus compounds, and are used to assess the potential mineralization rate of these compounds in soil (Bastida et al. 2008, Mocek-Płóciniak 2011). Their activity depends on many soil factors, among which the most important role is played by pH, temperature, organic matter content, as well as activators and inhibitors (Russel 2005, Nannipieri et al. 2018). Wyszkowska and Kucharski (2003), and Telesiński et al. (2015) report that heavy metals demonstrate a strong influence on enzymatic activity. At low concentrations, they stimulate, while in large quantities they are soil enzyme inhibitors. The effect of heavy metals on soil enzymes can be direct or indirect. The direct influence concerns the activity of free extracellular enzymes; the indirect effect appears via the biosynthesis of enzymes in microorganisms, and the composition of soil microbial communities, production of root secretions or liberation of enzymes from dead roots (Wyszkowska et al. 2006). Heavy metal toxicity results mainly from blocking the centers of active enzymes, as well as from the displacement of some cations important in the functioning of the cell and taking over their functions (Schmidt et al. 2005). Considering the metal toxicity to microorganisms, Wang et al. (2010) arranged metals in the following order: Cr>Pb>As>Co>Zn>Cd>Cu. One of the metals whose quantity in the environment changes dynamically is zinc. Its sources include industrial dust and waste materials used in agriculture. Zinc tends to accumulate in soil. The forms in which it occurs depend on many factors, among which soil organic substance has an important role (Cheng, Zhiping 2007, Vodyanitskii 2010, Wuana, Okieimen 2011).

The aim of the study was to determine the effect of varying amounts of zinc introduced into soil in combinations: without organic fertilization and after the application of bovine manure, laying hens' litter and spent mushroom substrate, on the activity of urease, dehydrogenases, as well as acid and alkaline phosphatase.

MATERIAL AND METHODS

The pot experiment was carried out in a greenhouse at the Siedlce University of Natural Sciences and Humanities, in 2014, 2015 and 2016. 10 dm^3 pots were filled with 12 kg of soil taken from the humic horizon of pseudogley soil, which belonged to the fallow brown soil order, lessive soil type (Systematics of Polish Soils 2011), with the following shares of the main grain-size fractions: sand 70%, silt 24%, clay 6%, sieved through a 2 cm mesh sieve. The following factors were considered when designing the experiment, carried out in triplicate in a completely random system:

I – zinc doses: control object – 0 and 200, 400 and 600 mg Zn kg^{-1} soil. This element was introduced into soil once, before sowing the test plant, in the first ten days of May 2014, in the form of an aqueous solution of $ZnSO_4$ $7H_2O$. In the second and third year, zinc was not introduced into the soil. The first dose of zinc was established corresponding to the amount of this metal indicating the first degree of soil pollution. Higher doses were multiplications of the base one.

II - organic materials: control object - without the use of organic compound (CO), bovine manure (BM), laying hens' litter ("chicken manure" – ChM) and spent mushroom substrate (MS) used separately. These fertilizers were introduced into the soil in 2014, two weeks before sowing the test plant. Bovine manure was used in a dose used for soil cultivation (75 Mg ha^{-1}), which corresponds to 2.0 g C_{org} per 1 kg of soil in the 0-0.2 m layer. For comparative purposes, the remaining organic materials were used at doses supplying the same amount of C_{org} to soil. The chemical composition of organic materials is provided in Table 1. No additional N, P, K fertilization was used in the study.

Table 1

Chemical composition of organic materials used in pot experiment

Organic materials	Dry matter (%)	C_{org}	N_{tot}	C:N	P	K	Ca	Mg	S	Zn
		(g kg^{-1} DM)			(g kg^{-1} DM)					(mg kg^{-1} DM)
Bovine manure	19.6	405.1	23.90	16.9:1	5.38	15.28	10.04	2.90	3.07	60.28
Laying hens' manure	27.8	167.3	13.50	12.4:1	8.44	9.32	13.72	2.68	3.12	190.8
Mushroom substrate	30.4	319.3	24.20	13.2:1	6.22	17.48	47.32	3.12	25.08	117.5

The soil used in the study had the following properties: pH at 1 mol dm^{-3} KCl – 6.65; total nitrogen content 1.52 g kg^{-1}; carbon in organic compounds 16.40 g kg^{-1}; available phosphorus 176 mg kg^{-1} of soil, available potassium 108 mg kg^{-1} soil, total zinc 56.60, mg kg^{-1} of soil. The activity of selected enzymes was typical for agricultural soils, namely urease: 10.00 mg NH_4^+ kg^{-1} 1h^{-1}, dehydrogenase: 0.60 mmol TPF kg^{-1} soil 24 h^{-1}, acid and alkaline phosphatase: 0.40 and 0.86 mmol PNP kg^{-1} soil 1 h^{-1}.

The test plant was orchard grass (*Dactylis glomerata* L.), Amera variety, which was sown in the amount of 1g of seeds per pot. It was harvested in four cuts each year (re-growths), at thirty-day intervals. The following determinations were made in samples of the soil collected after the fourth and last grass cut, in each (1,2 and 3) year of the experiment: urease activity was measured using the Hoffman and Teicher method (1961), dehydrogenase activity was measured using the Casida et al. (1964) method, acid and alkaline phosphatase activity was measured using the Tabatabai and Bremner method (1969). The test results were statistically evaluated by ANOVA using the Fisher-Snedecor *F*-distribution, and LSD values at significance level p=0.05 were calculated using the Tukey's test. Statistica 13 PL software package (StatSoft, Tulsa, USA) was used for the calculations. In addition, the Pearson's linear correlation coefficient was calculated for some of the examined traits.

RESULTS AND DISCUSSION

Changes in some soil properties and a decrease in the soil enzymatic activity may be caused by a high content of toxic substances, including heavy metals (Cheng, Zhiping 2007, Kucharski et al. 2011, Wang et al. 2013, Li et al. 2018, Kuziemska et al. 2020). This study showed a significant effect of different doses of zinc and the use of organic materials, i.e. bovine manure, laying hens' litter and spent mushroom substrate, on the activity of urease, dehydrogenases, as well as acid and alkaline phosphatase in the soil.

Urease activity (Table 2) depended on the factors studied in the experiment and their interactions, in addition to which it changed over the years of the experiment. In all the years, however, the application to zinc soil at doses of 400 and 600 mg Zn kg^{-1} of soil reduced the activity of this enzyme by 16.46% and 24.92% on average, respectively, compared to its activity in soil to which zinc was not introduced. Yang et al. (2006) also demonstrated the effect of zinc application on the inhibition of urease.

All organic materials increased the activity of urease. Its highest activity was determined in soil after the application of spent mushroom substrate, as it was by 12.29% higher than in soil from the control object. This relationship repeated in all the years. The stimulating effect of organic waste materials such as straw and lignite on urease activity has been demonstrated

Table 2

Urease activity in soil – averages for investigated factors (mg NH_4^+ kg^{-1} 1 h^{-1})

Specification		Zn dose (mg kg^{-1} of soil)				Mean
		0	200	400	600	
Organic fertilization	control object	8.61*D*	8.20*C*	6.68*B*	5.90*A*	7.35*a*
	bovine manure	8.88*C*	8.75*C*	7.69*B*	7.24*A*	8.14*b*
	laying hens' manure	8.99*C*	8.89*C*	7.95*B*	6.91*A*	8.18*bc*
	mushroom substrate	9.47*C*	9.35*C*	7.74*B*	6.97*A*	8.38*c*
Year	I	9.60	9.41	8.01	7.02	8.51*c*
	II	8.78	8.59	7.46	6.71	7.88*b*
	III	8.57	8.40	7.07	6.53	7.64*a*
Mean		8.99*c*	8.80*c*	7.51*b*	6.75*a*	8.01
		Organic fertilization				
		control object	bovine manure	laying hens' manure	mushroom substrate	
Year	I	7.96*A*	8.76*B*	8.62*B*	8.70*B*	
	II	6.94*A*	7.49*B*	8.19*C*	8.47*C*	
	III	7.15*A*	7.72*B*	7.74*B*	7.97*B*	

a,*b*,*c* – means with different letters (in the columns for organic fertilization and for years, but in the row for zinc doses) are significantly different, *A*,*B*,*C*,*D* – means with different letters in the rows of the table are significantly different
Interactions: zinc dose/year is not important

by others (Kalembasa, Kuziemska 2011). Sołek-Podwika and Ciarkowska (2008) associate this with an increase in the content of $C_{org.}$, which stimulates biochemical changes in soil and activates most soil enzymes. In this study, we were able to conclude that the application of any of the organic substances used in the experiment limited the inactivating effect of higher zinc doses (400 and 600 mg Zn kg^{-1}) on urease activity, which proves the stimulating effect of organic matter on the soil's detoxification process. Regarding the subsequent years of the experiment, it was found that the soil presented the highest urease activity in the first year.

Dehydrogenase activity in the analyzed soil (Table 3) was dependent on the amount of introduced zinc, organic fertilization, and interaction between these factors, but did not change significantly over the years of the study. The application of zinc in the amounts of 400 and 600 mg Zn kg^{-1} of soil caused a significant reduction in the activity of dehydrogenase, both in comparison with its activity in the control soil (by 42.10% and 56.14%, respectively) and with the treatment where 200 Zn mg kg^{-1} had been added to soil

Table 3

Dehydrogenase activity in soil – averages for investigated factors
(mmol TPF kg^{-1} soil 24 h^{-1})

Specification		Zn dose (mg kg^{-1} of soil)				Mean
		0	200	400	600	
Organic fertilization	control object	0.57*C*	0.50*B*	0.25*A*	0.19*A*	0.38*a*
	bovine manure	0.56*B*	0.55*B*	0.39*A*	0.33*A*	0.46*b*
	laying hens' manure	0.52*C*	0.52*C*	0.33*B*	0.25*A*	0.40*a*
	mushroom substrate	0.62*C*	0.59*C*	0.34*B*	0.23*A*	0.45*b*
Year	I	0.60	0.56	0.32	0.25	0.43*a*
	II	0.57	0.53	0.32	0.23	0.41*a*
	III	0.53	0.53	0.34	0.27	0.42*a*
Mean		0.57*c*	0.54*c*	0.33*b*	0.25*a*	0.42
		Organic fertilization				
		control object	bovine manure	laying hen's manure	mushroom substrate	
Year	I	0.40	0.45	0.40	0.48	
	II	0.37	0.46	0.40	0.43	
	III	0.37	0.47	0.41	0.43	

Determination of the significance of differences between means as in Table 2.
Interactions: zinc dose/year and organic fertilization/year are not important.

(by 38.89% and 53.70% respectively). Oliveira and Pampulha (2006), and Mikanova (2006) obtained similar results. Chaperon and Sauve (2007), and Wyszkowska et al. (2008) also found an inhibitory effect of zinc on the activity of dehydrogenases and urease. Soil amended with organic materials was characterized by greater activity of the said enzyme compared to soil without added organic materials, but the significance of differences appeared only in the case of soil fertilized with bovine manure and spent mushroom substrate. At the same time, all the organic materials restrained the adverse effects of higher zinc doses (400 and 600 mg of Zn kg^{-1} soil) on dehydrogenase activity.

We did not observe an effect of the different amounts of zinc added to soil on the activity of acid phosphatase (Table 4), which is inconsistent with the results obtained by Wyszkowska et al (2006), who demonstrated its inhibitory effect. Telesiński et al. (2015) also found a negative effect of zinc on acid phosphatase activity, but the doses of this metal used in their experiment were much higher than in ours. The application of bovine manure increased the activity of this enzyme by 8.33% compared to its activity in soil from the control object or the one fertilized with spent mushroom substrate.

Table 4

Acid phosphatase activity in soil – averages for investigated factors
(mmol PNP kg^{-1} soil $1h^{-1}$)

Specification		Zn dose (mg kg^{-1} of soil)				Mean
		0	200	400	600	
Organic fertilization	control object	0.43	0.44	0.44	0.45	0.44*a*
	bovine manure	0.47	0.47	0.49	0.47	0.48*b*
	laying hens' manure	0.45	0.46	0.47	0.42	0.45*ab*
	mushroom substrate	0.41	0.44	0.45	0.45	0.44*a*
Year	I	0.41	0.41	0.45	0.44	0.43*a*
	II	0.47	0.48	0.45	0.45	0.46*b*
	III	0.45	0.48	0.48	0.46	0.47*b*
Mean		0.44*a*	0.46*a*	0.46*a*	0.45*a*	0.45
		Organic fertilization				
		control object	bovine manure	laying hens' manure	mushroom substrate	
year	I	0.43	0.44	0.41	0.43	
	II	0.43	0.49	0.48	0.45	
	III	0.46	0.50	0.47	0.44	

Determination of the significance of differences between means as in Table 2.
All interactions (zinc dose/year, zinc dose/organic fertilization and organic fertilization/year) are not important.

In the second and third year of the research, there was a significant increase in acid phosphatase activity in the analyzed soil, compared to its activity in the soil collected after the first year of the experiment.

Alkaline phosphatase activity in the analyzed soil (Table 5) depended on organic fertilization, zinc doses and years of the research. Regardless of the amount, the application of zinc caused a decrease in the activity of the enzyme, although the significance of differences in relation to the control object was demonstrated only for doses of 400 and 600 mg Zn kg^{-1} of soil. The soil with spent mushroom substrate and laying hens' litter introduced was characterized by significantly higher alkaline phosphatase activity compared to the soil to which no organic fertilization was applied. Over the subsequent years, the activity of the enzyme in soil decreased significantly, which is associated with a decrease in the pH value.

The correlation analysis confirmed the negative effect of increasing doses of zinc on the activity of urease, dehydrogenases and alkaline phosphatase, as well as a decrease in the pH value of the analyzed soil, as evidenced by the values of the linear correlation coefficient (Table 6). It also showed

Table 5

Alkaline phosphatase activity in soil – averages for investigated factors (mmol PNP kg^{-1} soil 1 h^{-1})

Specification		Zn dose (mg kg^{-1} of soil)				Mean
		0	200	400	600	
Organic fertilization	control object	0.78	0.70	0.68	0.67	0.71*a*
	bovine manure	0.76	0.75	0.72	0.67	0.72*ab*
	laying hens' manure	0.79	0.80	0.74	0.70	0.76*bc*
	mushroom substrate	0.85	0.81	0.75	0.74	0.79*c*
Year	I	0.82	0.80	0.74	0.72	0.77*b*
	II	0.81	0.75	0.71	0.69	0.74*ab*
	III	0.76	0.74	0.71	0.69	0.72*a*
Mean		0.80c	0.76*bc*	0.72*ab*	0.70*a*	0.74
		Organic fertilization				
		control object	bovine manure	laying hens' manure	mushroom substrate	
Year	I	0.73	0.76	0.79	0.81	
	II	0.72	0.71	0.76	0.78	
	III	0.69	0.70	0.73	0.78	

Determination of the significance of differences between means as in Table 2.
All interactions (zinc dose/year, zinc dose/organic fertilization and organic fertilization/year) are not important.

Table 6

Linear correlation coefficients between Zn doses, soil pH and enzymatic activity in soil

Specification	Urease	Dehydrogenase	Acid phosphatase	Alkaline phosphatase
Zn dose	-0.875*	-0.914*	0.180	-0.747*
pH	0.695*	0.618*	-0.588*	0.830*
Urease	-	0.960*	-0.085	0.869*
Dehydrogenase	-	-	-0.111	0.779*
Acid phosphatase	-	-	-	-0.287

* the value of the correlation coefficient is important, $p<0.05$

significant positive relationships between the pH value and urease activity, dehydrogenases and alkaline phosphatase, as well as a negative one between the pH value and acid phosphatase activity. Koper et al. (2004) showed similar results.

The negative effect of applying increasing doses of zinc to soil on the activity of urease, dehydrogenases and acid phosphatase was demonstrated by Kucharski et al. (2011), who used this element in doses ranging from 70 mg Zn^{2+} to 10,000 mg Zn^{2+} kg^{-1} of soil, in a 120-day experiment conducted in laboratory conditions. According to these authors, the sensitivity of the studied enzymes to zinc can be presented in this order: dehydrogenase > acid phosphatase > urease.

Our study did not show a deactivating effect of zinc on acid phosphatase, yet this can be explained by the low dose effect (max. 600 mg Zn kg^{-1} of soil). The effect of the organic materials used on the enzymatic activity of the studied soil varied, but most of them showed an activating activity. The stimulating effect of organic fertilization on the enzymatic activity of soils has also been demonstrated by Cheng et al. (2007) and Piaszczyk et al. (2017). At the same time, organic fertilization, regardless of its type, hindered the inactivating effect of higher zinc doses (400 and 600 mg of Zn kg^{-1} of soil) on urease and dehydrogenase activity.

CONCLUSIONS

1. The activity of the tested enzymes was varied under the influence of increasing doses of zinc and fertilization with organic materials.

2. Significant reduction of urease, dehydrogenase and alkaline phosphatase activity in soil was obtained after the application of 400 and 600 mg Zn kg^{-1} of soil, and at the same time, no negative effect of any of the zinc doses on acid phosphatase activity was demonstrated.

3. Organic fertilization generally increased the enzymatic activity of the analyzed soil.

4. All the used organic substances limited the adverse effects of higher doses of zinc (400 and 600 mg of Zn kg^{-1} of soil) on urease and dehydrogenase activity.

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