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ACTIVITY OF ENZYMES IN ZINC CONTAMINATED SOIL

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ABSTRACT

The aim of the study has been to determine the effect of soil contamination with zinc on the activity of soil enzymes. The study consisted of two laboratory experiments. Same, light loamy soil of pH 7.1, was used in both experiments. The variables in the first experiment were: a degree of soil contamination with zinc in mg Zn kg^{-1} d.m. of soil: 0, 5, 500, 1000, 1500 and 2000; dose of cellulose in g kg^{-1} d.m. of soil: 0, 15, and time of soil incubation (15 – 120 days). In the second experiment the following variables were tested: the degree of soil contamination with zinc in mg Zn kg^{-1} d.m. of soil: 0, 1000 and 2000; soil pH: 7.1, 6.4 and 5.5, and the time of soil incubation (15 – 120 days).

The results of the experiments demonstrated that contamination of soil with zinc led to depressed activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase. Dehydrogenases and urease appeared to be more vulnerable to zinc contamination than phosphatases. The soil enzymes were adversely affected not only by zinc contamination but also by increasing soil acidity. According to their vulnerability to soil acidity the soil enzymes can be ordered as follows: dehydrogenases > urease > alkaline phosphatase > acid phosphatase. Cellulose added to soil (15 g kg^{-1}) proved to be a good factor in the improvement of soil biochemical properties, although it did not limit the effects produced by zinc.

Key words: zinc; contamination; dehydrogenases; urease; acid phosphatase; alkaline phosphatase; enzymes.

INTRODUCTION

Zinc is an element essential for many organisms, including microorganisms. Many enzymes present inside cells could not function properly in the absence of zinc. Zinc is present in over 300 enzymes, which belong to six classes [8]. Among the enzymes which contain zinc are carbon anhydrase, acid phosphatase, carboxypeptidases, dehydrogenases (3-phosphoglycerol aldehyde, alcohol and glutamine dehydrogenases), fructose diphosphate aldolase, peroxide dismutase, DNA and RNA polymerases, tRNA transferase [14].

According to Cordovy and Alvarez-Mona [3], the role of zinc as a component of metaloenzymes should be considered in three aspects: catalytic, structural and regulatory. This means that zinc can be substantial for the activity of some enzymes, e.g. carbon anhydrase, carboxypeptidase, thermolysine and aldolase. It can stabilise their protein structure and either activate or inhibit these enzymes [4]. Such natural functions of zinc can be disrupted when this element is present in excessive amounts, which can be the case in heavily industrialised regions. Obviously, the negative influence of zinc contamination on soil environment depends on a number of factors, including soil type [19] and reaction [7]. Typically, zinc is responsible for smaller modifications in more fertile and non-acidic soils.

The effect of heavy metals, including zinc, on soil enzymes can be direct or indirect. The direct influence concerns the activity of free, extracellular enzymes; the indirect effect appears in terms of the biosynthesis of enzymes in microorganisms, and the composition of soil microbial communities [9], production of root secretions, or liberation of enzymes from dead roots [5].

Determination of the soil enzymatic activity can serve as a basis for an evaluation of soil quality, as the enzymes are particularly susceptible to changes in the environment [16]. Heavy metals present in soil in small amounts have a stimulating effect on the activity of enzymes. However, having surpassed certain threshold limits, they contribute to the inhibition of microbial activity and extracellular enzymes [17].

The purpose of the study has been to determine the role of zinc present in excessive quantities in soil on the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase. In addition, the authors have undertaken to verify the hypothesis whether cellulose added to soil can mollify the effects produced by zinc contamination as well as to determine to what extent the influence of zinc on soil enzymes is correlated with the soil pH. Cellulose has been chosen for the study in view of the fact that this carbohydrate is supplied to soil in large amounts as harvest residues or in organic manure.

MATERIAL AND METHODS

The study was conducted as two laboratory experiments. For both experiments the same light loamy soil was used (1.0-0.1 mm – 61%, 0.1-0.02 mm – 12% < 0.02 mm – 27%), sampled from the humus arable horizon of typical brown soil. The soil had the following characteristics: pH in 1 mol KCl dm^{-3} was 7.10, cation exchange capacity (CEC) of sorptive complex was 126.5 mmol(+) kg^{-1}, base saturation (BS) was 89.2% and organic carbon ($C_{organic}$) content was 6.0 g kg^{-1}.

In the first experiment the following factors were tested as variables: a degree of soil contamination with zinc in mg Zn kg^{-1} d.m. of soil: 0, 5, 500, 1000, 1500 and 2000; dose of cellulose in g kg^{-1} d.m. of soil: 0, 15, time of soil incubation: 15, 30, 60, 90 and 120 days. In the second experiment the variables comprised degree of soil contamination with zinc in mg Zn kg^{-1} d.m. of soil: 0, 1000 and 2000; soil pH: 7.1, 6.4 and 5.5, and time of soil incubation in days: 15, 30, 60, 90 and 120.

The experiments were conducted in three replications. Portions of 50 g of air-dry soil mass were placed in 50 cm^3 beakers and afterwards contaminated with an appropriate dose of $ZnSO_4 \cdot 7H_2O$. In the first experiment the proper doses of cellulose were added; the soil was mixed carefully and its moisture content was brought up to 60% capillary water capacity by pouring in distilled water. This moisture content was maintained through the whole experiment.

The reaction of soil in the second experiment was regulated using 5% aqueous solution of HCl, added to soil prior to the establishment of the experiment. Once the soil pH was stabilised, soil was weighted out into beakers and contaminated with zinc sulphate. The beakers with soil (from both experiments) were incubated in an incubator at 25°C. On the scheduled dates, soil was analysed in six replications, and the activity of the following enzymes was determined: dehydrogenases (Deh) – with a TTC substrate [13], urease (Ure) – according to Alef and Nannpieri [1], as well as acid phosphatase (Pac) and alkaline phosphatase (Pal) – according to the method described by Alef *et al*. [2].

The substrate of dehydrogenases was 3% aqueous TTC solution (2,3,5-triphenyltetrazolium chloride). Soil incubation was carried out for 24 h at the temperature of 37°C. Extinction of the TPF produced was measured on a spectrophotometer at wavelength of 485 nm. The results were converted into cm^3 H_2 kg^{-1} d.m. of soil d^{-1}. The substrate of urease was 10% aqueous urea solution. The soil was incubated for 24 hours at 37°C. The amount of N-NH_4 produced was determined with Nessler's reagent. Extinction of the amidomercury iodide was measured spectrophotometrically at wavelength of 410 nm and converted into the amount of N-NH_4 kg^{-1} d.m. of soil h^{-1} produced (mg). The substrate of phosphatases was sodium 4-nitrophenylophosphate (PNPP). The soil was incubated at 37°C for 1 h (acid phosphatase – pH 6.5; alkaline phosphatase – pH 11). After the incubation, the extinction of p-nitrophenol (PNP) produced was determined spectrophotometrically at wavelength of 410 nm. The results were recalculated into mmols of PNP kg^{-1} d.m. of soil h^{-1} produced.

The results of the experiments were elaborated statistically using three-factor analysis of variance ANOVA. The Pearson's simple correlation coefficients between the degree of soil contamination with zinc and activity of soil enzymes were also computed. The computations were aided by the software package Statistica [12].

RESULTS AND DISCUSSION

The enzymatic activity of soil is a function of soil biological, physical and chemical properties [8,11]. It is obvious that the role of zinc and other heavy metals in the enzymatic activity of soil depends on a degree of soil contamination with these elements. When heavy metals are present in soil in excessive amounts, they act as typical inhibitors of soil enzymes [16, 18]. This thesis has been confirmed in the present study as zinc turned out to be a strong inhibitor of dehydrogenases, urease, acid phosphatase and alkaline phosphatase.

A decline in the activity of dehydrogenases was greater at a higher degree of soil contamination with zinc (Table 1, Fig. 1). The dose of 5 mg Zn kg^{-1} of soil depressed the activity of dehydrogenases by 3.5%; a 100-fold higher dose caused a 37% decrease; the dose 200-fold higher was responsible for a 68% decrease; 300-fold more zinc resulted in an 81% decrease and a 400-fold larger dose of zinc depressed the analysed parameter by 89%. Under the influence of 200 mg Zn kg^{-1} of soil, the inhibition was 8.8-fold higher in unfertilised soil and 11-fold higher in the cellulose-fertilised soil (Table 1). This means than an addition of cellulose to soil did not mollify the negative effects of zinc on dehydrogenases, although their activity increased 2.5-fold as a result of the influence of cellulose alone. The same results analysed from another viewpoint would allow us to draw a contradictory conclusion, as it was in the soil containing additional cellulose that the activity of dehydrogenases remained on a higher level, which may suggest that by introducing cellulose to soil we reduce the inhibitory effects produced by zinc. However, when comparing the activity of these enzymes in the control object (not contaminated with zinc) versus those which contained elevated levels of the metal, it can be stated unambiguously that the inhibition of the activity of dehydrogenases occurred over a larger range in the cellulose-fertilised soil. In this soil the highest activity of dehydrogenases was determined on day 30 of the experiment, and the lowest – on days 15 and 120. In the soil without additional cellulose, dehydrogenases were the most active on day 30 of the experiment, and became the least active on day 120. Dehydrogenases were adversely affected not only by zinc but also by soil acidity. When soil pH changed from 7.1 to 6.4, the activity of these enzymes fell by 3.6-fold; and the modification in soil pH from 7.1 to 5.5 resulted in the inhibition of their activity higher by 9.5-fold (Table 2).

Table 1. Activity of dehydrogenases in relation to soil contamination with zinc and addition of cellulose (cm^3 H_2 kg^{-1} d.m. soil d^{-1})

Cellulose dose (g kg^{-1})	Zn dose (mg kg^{-1} d.m.)	Soil incubation time (in days)						
		15	30	60	90	120	Average	r
0	0	6.04	6.22	5.34	5.27	5.05	5.58	-0.92**
	5	6.15	6.40	5.20	5.01	4.13	5.38	-0.96**
	500	3.48	3.99	3.62	3.26	3.37	3.54	-0.61**
	1000	1.98	2.09	1.57	1.54	1.90	1.82	-0.44
	1500	1.24	1.28	0.80	0.99	1.02	1.07	-0.57**
	2000	0.80	0.77	0.48	0.55	0.59	0.64	-0.70**
	Average	3.28	3.46	2.84	2.77	2.68	3.00	-0.89**
	r	-0.96**	-0.97**	-0.97**	-0.96**	-0.97**	-0.97**	
15	0	12.88	14.78	13.83	14.05	13.90	13.89	0.21
	5	12.95	16.24	13.76	13.68	11.85	13.70	-0.55*
	500	8.12	10.02	9.66	9.51	8.93	9.25	0.13
	1000	3.66	4.68	4.54	4.39	4.90	4.43	0.67**
	1500	2.01	2.01	2.20	2.82	2.67	2.34	0.90**
	2000	1.24	1.32	1.35	1.21	1.21	1.27	-0.49*
	Average	6.81	8.18	7.56	7.61	7.24	7.48	-0.01
	r	-0.96**	-0.97**	-0.97**	-0.98**	-0.98**	-0.97**	
LSD*$_{p=0.01}$ a = 0.18; b = 0.16; c = 0.10; a x b = 0.40; a x c = 0.25; b x c = 0.23; a x b x c = 0.57								

*LSD (least statistical difference) for: a – zinc dose, b – soil incubation time, c – cellulose addition
r – correlation co-efficient significant at: **p<0.01; *p<0.05

Fig 1. Inter-relationship between soil contamination with zinc and soil enzymes activity and cellulose addition to soil

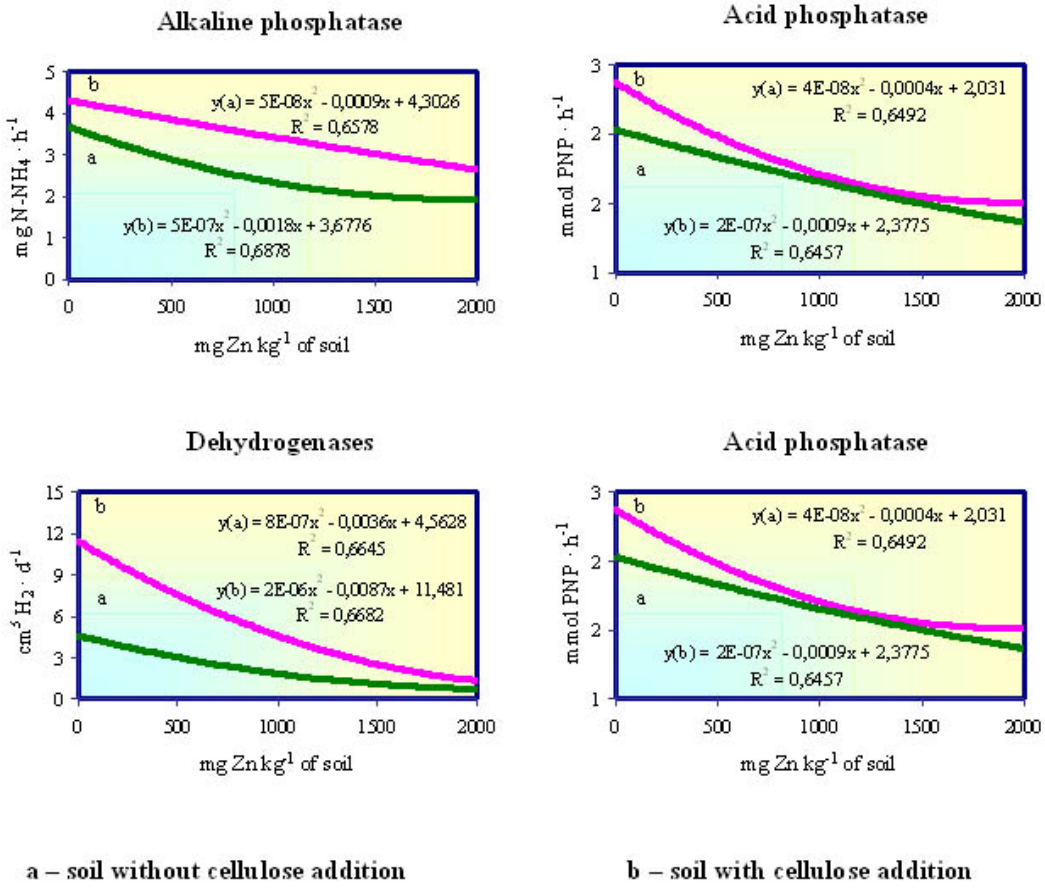

Table 2. Activity of dehydrogenases in relation to soil contamination with zinc and soil pH (cm^3 H_2 kg^{-1} d.m. soil d^{-1})

Soil pH	Zn dose (mg kg^{-1} d.m.)	Soil incubation time (in days)						
		15	30	60	90	120	Average	r
7.1	0	6.09	6.37	6.48	6.06	5.98	6.20	-0.47*
	1000	2.22	2.55	2.83	2.47	2.39	2.49	0.10
	2000	0.60	0.80	0.85	0.82	0.71	0.76	0.28
	Average	2.97	3.24	3.39	3.12	3.03	3.15	-0.10
	r	-0.97**	-0.98**	-0.99**	-0.98**	-0.98**	-0.98**	
6.4	0	2.25	2.33	2.36	2.17	2.00	2.22	-0.78**
	1000	0.33	0.33	0.44	0.38	0.44	0.38	0.77**
	2000	0.00	0.00	0.05	0.11	0.11	0.05	0.96**
	Average	0.86	0.89	0.95	0.89	0.85	0.89	-0.15
	r	-0.93**	-0.92**	-0.93**	-0.92**	-0.94**	-0.93**	
5.5	0	0.66	0.69	0.82	1.04	1.02	0.85	0.96**
	1000	0.11	0.11	0.16	0.11	0.11	0.12	-0.04
	2000	0.00	0.00	0.00	0.08	0.08	0.03	0.89**
	Average	0.26	0.27	0.33	0.41	0.41	0.33	0.96**
	r	-0.93**	-0.93**	-0.94**	-0.88**	-0.88**	-0.91**	
$LSD^*_{p=0.01}$ a = 0.03; b = 0.04; c = 0.03; a x b = 0.06; a x c = 0.05; b x c = 0.06; a x b x c = 0.11								

LSD for: a – zinc dose, b – soil incubation time, c – soil acidity
r – correlation co-efficient significant at: $^{**}p<0.01$; $^{*}p<0.05$

Excessive amounts of zinc in soil evidently inhibited the activity of urease, and the inhibition observed was greater at higher levels of zinc pollution (Table 3, Fig. 1). However, the inhibitory effect of zinc on this enzyme was much weaker than that on dehydrogenases. Under the influence of 2000 mg Zn kg^{-1} of soil unfertilised with cellulose, the

activity of urease fell by 4.3-fold; in the cellulose treated soil the respective decline was 5.1-fold. Also cellulose had a weaker influence on urease than on dehydrogenases. It increased the activity of urease by only 1.8-fold, which was much less than in the case of dehydrogenases. The activity of urease varied in time. In the cellulose untreated soil, urease was the most active on day 15, whereas in the cellulose fertilised soil, it reached the maximum activity on day 120. Urease turned out to be more resistant to soil acidity than dehydrogenases. The change of pH from 7.1 to 6.4 depressed urease activity by 2.4-fold, and when the soil reaction fell from 7.1 to 5.5, the activity of urease declined by 4-fold only (Table 4).

Table 3. Activity of urease in relation to soil contamination with zinc and addition of cellulose (mg N-NH_4 kg^{-1} d.m. soil h^{-1})

Cellulose dose (g kg^{-1})	Zn dose (mg kg^{-1} d.m.)	Soil incubation time (in days)						
		15	30	60	90	120	Average	r
0	0	39.27	36.9	30.23	24.11	29.59	32.02	-0.82**
	5	44.57	43.65	22.38	21.92	24.84	31.47	-0.82**
	500	27.76	24.66	21.83	15.53	22.10	22.38	-0.69**
	1000	20.82	18.81	13.24	6.03	19.18	15.62	-0.39
	1500	16.99	14.61	11.78	1.28	2.37	9.41	-0.94**
	2000	15.16	11.32	8.13	1.28	1.28	7.43	-0.96**
	Average	27.43	24.99	17.93	11.69	16.56	19.72	-0.85**
	r	-0.94**	-0.94**	-0.94**	-0.96**	-0.95**	-0.98**	
15	0	49.86	52.79	57.08	47.31	82.93	57.99	0.69**
	5	50.41	54.61	49.41	51.33	120.2	65.19	0.73**
	500	46.03	39.64	32.06	20.46	90.23	45.68	0.45
	1000	34.52	25.39	21.28	20.09	48.22	29.90	0.36
	1500	20.64	16.8	15.25	16.07	20.64	17.88	0.04
	2000	18.08	13.33	10.32	7.31	8.04	11.42	-0.90**
	Average	36.59	33.76	30.90	27.10	61.71	38.01	0.55*
	r	-0.98**	-0.98**	-0.95**	-0.91**	-0.95**	-0.98**	
LSD*$_{p=0.01}$ a = 0.18; b = 0.16; c = 0.10; a x b = 0.40; a x c = 0.25; b x c = 0.23; a x b x c = 0.57								

* – explanation disclose under the table 1

Table 4. Activity of urease in relation to soil contamination with zinc and soil pH (mg N-NH_4 kg^{-1} d.m. soil h^{-1})

Soil pH	Zn dose (mg kg^{-1} d.m.)	Soil incubation time (in days)						
		15	30	60	90	120	Average	r
7.1	0	29.73	29.59	42.33	50.69	53.5	41.10	0.97**
	1000	16.99	19.86	23.29	23.15	24.25	21.51	0.90**
	2000	7.40	7.95	7.95	7.67	8.90	7.97	0.75**
	Average	18.04	19.13	24.52	27.17	28.77	23.53	0.98**
	r	-0.99**	-0.99**	-0.99**	-0.99**	-0.98**	-0.99**	
6.4	0	12.06	11.37	12.88	27.95	35.34	19.92	0.94**
	1000	5.34	5.48	5.62	5.62	6.58	5.73	0.86**
	2000	2.33	3.70	4.66	4.93	5.62	4.25	0.94**
	Average	6.58	6.85	7.72	12.83	15.85	9.97	0.96**
	r	-0.98**	-0.96**	-0.91**	-0.88**	-0.88**	-0.91**	
5.5	0	6.99	9.59	11.64	8.36	9.04	9.12	0.18
	1000	4.66	4.79	4.79	5.07	5.21	4.90	0.97**
	2000	1.37	2.47	4.66	4.79	4.93	3.64	0.89**
	Average	4.34	5.62	7.03	6.07	6.39	5.89	0.66**
	r	-0.99**	-0.98**	-0.87**	-0.90**	-0.89**	-0.95**	
LSD*$_{p=0.01}$ a = 0.03; b = 0.04; c = 0.03; a x b = 0.06; a x c = 0.05; b x c = 0.06; a x b x c = 0.11								

* – explanation disclose under the table 2

Zinc also had a negative effect on alkaline phosphatase (Table 5, Fig. 1). In the soil which was not supplemented with cellulose, the activity of this enzyme under the influence of zinc contamination (2000 mg Zn kg^{-1}) was 2.2-fold lower than in the control object (uncontaminated); in the cellulose-fertilised soil, the activity of alkaline phosphatase

was depressed by 1.6-fold only. However, cellulose could only slightly increase the activity of alkaline phosphatase. In the cellulose-fertilised soil, the activity of this soil enzyme increased in time and was the highest on day 120. On the other hand, in the soil not fertilised with cellulose, the highest alkaline phosphatase activity was determined on day 90 of the experiment.

Table 5. Activity of alkaline phosphatase in relation to soil contamination with zinc and addition of cellulose (mmol PNP h^{-1} kg^{-1} d.m. soil h^{-1})

Cellulose dose (g kg^{-1})	Zn dose (mg kg^{-1} d.m.)	Soil incubation time (in days)						
		15	30	60	90	120	Average	r
0	0	3.49	4.36	4.29	4.23	3.90	4.05	0.20
	5	3.36	4.49	4.62	5.14	3.16	4.15	-0.03
	500	2.94	3.20	2.97	3.15	2.95	3.04	-0.11
	1000	1.82	2.97	2.63	2.71	2.15	2.46	0.03
	1500	1.76	2.05	2.54	2.2	1.95	2.10	0.21
	2000	1.69	1.96	1.94	1.79	1.87	1.85	0.17
	Average	2.51	3.17	3.17	3.20	2.66	2.94	0.06
	r	-0.94**	-0.97**	-0.93**	-0.94**	-0.92**	-0.97**	
15	0	3.84	3.21	4.49	5.46	4.81	4.36	0.79**
	5	3.97	4.68	4.55	5.85	5.53	4.92	0.87**
	500	3.41	3.54	3.97	4.94	4.94	4.16	0.96**
	1000	2.76	3.30	3.38	3.28	3.64	3.27	0.81**
	1500	1.94	3.17	3.17	2.98	3.32	2.92	0.65**
	2000	1.74	2.46	2.90	2.74	3.38	2.64	0.90**
	Average	2.94	3.39	3.74	4.21	4.27	3.71	0.96**
	r	-0.99**	-0.75**	-0.98**	-0.96**	-0.91**	-0.97**	
LSD*$_{p=0.01}$ a = 0.18; b = 0.16; c = 0.10; a x b = 0.40; a x c = 0.25; b x c = 0.23; a x b x c = 0.57								

* – explanation disclose under the table 1

Alkaline phosphatase was more tolerant to soil acidity than urease. When the soil pH went down from 7.1 to 6.4, the activity of this enzyme was only 1.8-fold weaker; when the soil pH declined from 7.1 to 5.5, the alkaline phosphatase activity was depressed by 2.9-fold only (Table 6). Also acid phosphatase responded negatively to soil acidity (Table 7), although the decline in soil pH from 7.1 to 6.4 and 5.5 did not inhibit its activity as strongly as that of the other enzymes (from 1.4 to 1.6-fold). In conclusion, the tested enzymes can be ordered according to their susceptibility of soil acidity as follows: dehydrogenases > urease > alkaline phosphatase > acid phosphatase.

Table 6. Activity of alkaline phosphatase in relation to soil contamination with zinc and soil pH (mmol PNP kg^{-1} d.m. soil h^{-1})

Soil pH	Zn dose (mg kg^{-1} d.m.)	Soil incubation time (in days)						
		15	30	60	90	120	Average	r
7.1	0	2.16	2.30	2.56	3.00	3.18	2.64	0.99**
	1000	1.36	1.66	1.79	2.07	1.94	1.76	0.87**
	2000	1.02	1.41	1.48	1.58	1.41	1.38	0.63**
	Average	1.51	1.79	1.94	2.22	2.18	1.93	0.93**
	r	-0.97**	-0.97**	-0.97**	-0.98**	-0.97**	-0.97**	
6.4	0	1.60	1.39	1.62	1.88	1.82	1.66	0.81**
	1000	1.09	0.83	0.94	0.94	0.98	0.96	-0.08
	2000	0.80	0.71	0.72	0.78	0.72	0.75	-0.27
	Average	1.16	0.98	1.09	1.20	1.17	1.12	0.51*
	r	-0.99**	-0.94**	-0.96**	-0.93**	-0.96**	-0.95**	
5.5	0	0.76	0.82	0.71	0.96	0.96	0.84	0.76**
	1000	0.64	0.70	0.59	0.60	0.65	0.64	-0.31
	2000	0.62	0.67	0.51	0.50	0.53	0.57	-0.75**
	Average	0.67	0.73	0.60	0.69	0.71	0.68	0.12
	r	-0.92**	-0.94**	-0.99**	-0.95**	-0.97**	-0.96**	
LSD*$_{p=0.01}$ a = 0.03; b = 0.04; c = 0.03; a x b = 0.06; a x c = 0.05; b x c = 0.06; a x b x c = 0.11								

* – explanation disclose under the table 2

Of all the enzymes analysed, acid phosphatase was the most tolerant to zinc contamination of soil (Table 7, 8). The inhibitory influence of zinc on this soil enzyme was the weakest. The activity of acid phosphatase under the influence of 2000 Zn kg^{-1} was depressed by 1.6-fold in the soil not fertilised with cellulose, and 1.7-fold in the cellulose-fertilised soil (Table 8). Cellulose stimulated acid phosphatase, especially in the object not contaminated with zinc (Fig. 1). This stimulation, however, in the soil containing excessive amounts of zinc was much weaker than in the case of the other soil enzymes, although it persisted throughout the whole experiment. On day 120 of the experiment it was much stronger than on day 15.

Table 7. Activity of acid phosphatase in relation to soil contamination with zinc and addition of cellulose (mmol PNP kg^{-1} d.m. soil h^{-1})

Cellulose dose (g kg^{-1})	Zn dose (mg kg^{-1} d.m.)	Soil incubation time (in days)						
		15	30	60	90	120	Average	r
0	0	1.69	2.71	2.02	1.98	2.35	2.15	0.16
	5	1.76	2.94	2.17	2.02	2.22	2.22	-0.09
	500	1.49	2.19	2.15	1.81	2.08	1.94	0.34
	1000	1.39	1.89	1.37	1.46	1.77	1.58	0.17
	1500	1.20	1.74	1.33	1.43	1.74	1.49	0.45*
	2000	1.14	1.42	1.25	1.26	1.70	1.35	0.68**
	Average	1.45	2.15	1.72	1.66	1.98	1.79	0.25
	r	-0.98**	-0.97**	-0.91**	-0.98**	-0.94**	-0.98**	
15	0	1.94	2.90	2.42	2.54	2.82	2.52	0.51**
	5	2.30	3.21	2.67	2.29	2.97	2.69	0.10
	500	1.58	2.21	2.42	1.68	2.39	2.06	0.37
	1000	1.47	1.87	1.81	1.44	2.07	1.73	0.41
	1500	1.22	1.83	1.74	1.35	1.92	1.61	0.41
	2000	1.18	1.63	1.44	1.33	1.79	1.47	0.56**
	Average	1.62	2.28	2.08	1.77	2.33	2.01	0.40
	r	-0.92**	-0.92**	-0.96**	-0.89**	-0.96**	-0.95**	
LSD*$_{p=0.01}$ a = 0.18; b = 0.16; c = 0.10; a x b = 0.40; a x c = 0.25; b x c = 0.23; a x b x c = 0.57								

* – explanation disclose under the table 1

Table 8. Activity of acid phosphatase in relation to soil contamination with zinc and soil pH (mmol PNP kg^{-1} d.m. soil h^{-1})

Soil pH	Zn dose (mg kg^{-1} d.m.)	Soil incubation time (in days)						
		15	30	60	90	120	Average	r
7.1	0	1.48	1.50	1.48	1.55	1.54	1.51	0.82**
	1000	1.23	1.25	1.22	1.19	1.30	1.24	0.32
	2000	1.14	1.15	1.15	1.13	1.10	1.13	-0.82**
	Average	1.28	1.30	1.28	1.29	1.31	1.29	0.61**
	r	-0.97**	-0.97**	-0.95**	-0.92**	-0.99**	-0.97**	
6.4	0	1.39	1.46	1.57	1.66	1.53	1.52	0.70**
	1000	0.91	0.93	0.78	0.79	0.85	0.85	-0.59**
	2000	0.73	0.68	0.68	0.74	0.74	0.71	0.49*
	Average	1.01	1.02	1.01	1.06	1.04	1.03	0.70**
	r	-0.97**	-0.98**	-0.91**	-0.89**	-0.92**	-0.93**	
5.5	0	1.07	0.65	1.31	1.37	1.33	1.15	0.70**
	1000	0.55	0.61	0.70	0.70	0.69	0.65	0.83**
	2000	0.51	0.48	0.65	0.64	0.59	0.57	0.66**
	Average	0.71	0.58	0.89	0.90	0.87	0.79	0.75**
	r	-0.90**	-0.96**	-0.90**	-0.90**	-0.92**	-0.92**	
LSD*$_{p=0.01}$ a = 0.03; b = 0.04; c = 0.03; a x b = 0.06; a x c = 0.05; b x c = 0.06; a x b x c = 0.11								

* – explanation disclose under the table 2

To recapitulate, it can be stated unambiguously that soil contamination with zinc had an inhibitory effect on all the soil enzymes analysed. Their activity was also adversely influenced by soil acidity, but responded positively to cellulose fertilisation. The negative effect of zinc was a product of the direct influence of this metal on the enzymes and the indirect influence through a change in the soil acidity due to the contamination with zinc sulphide (Table 9). Those findings are confirmed by some earlier research [6, 16, 18].

Table 9. Effect of soil contamination with zinc on soil pH_{KCl}

Soil pH	Zn dose (mg kg^{-1} d.m.)	Soil incubation time (in days)				
		15	30	60	90	120
7.1	0	7.1	7.1	7.1	7.0	6.8
	1000	6.9	6.8	6.8	6.7	6.6
	2000	6.6	6.6	6.6	6.5	6.6
6.4	0	6.5	6.5	6.5	6.2	6.1
	1000	5.9	6.0	6.1	5.8	5.8
	2000	5.6	5.7	5.8	5.6	5.5
5.5	0	5.5	5.6	5.6	5.6	5.5
	1000	5.0	5.4	5.4	5.3	5.2
	2000	4.8	5.0	5.2	5.1	4.8
$LSD^*_{p=0.01}$ a = 0.02; b = 0.04; c = 0.02; a x b = 0.06; a x c = 0.04; b x c = 0.06; a x b x c = 0.10						

* – explanation disclose under the table 2

The most intolerant to the effect of zinc were dehydrogenases, which according to Trasar-Cepeda *et al.* [15] and Kucharski [6] are the most objective reflection of the biological state of soil. The inhibitory effect of zinc on dehydrogenases was also determined by Welp [16], who determined that 115 Zn kg^{-1} of soil was responsible for 50% depression of the activity of these enzymes. The negative effect of zinc contamination on the other soil enzymes has been reported in the relevant literature. Reports can be found on zinc effect on urease [17] and phosphatases [10].

CONCLUSIONS

1. Zinc contamination of soil contributed to the depressed activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase. Doses of zinc exceeding 5 mg kg^{-1} had negative influence on the enzymatic activity of soil, and the effect was stronger as the soil contamination degree was higher. Dehydrogenases and urease proved to be less tolerant to zinc contamination than phosphatases.
2. The soil enzymes were negatively affected not only by zinc contamination but also by increasing soil acidity. According to the intolerance to soil acidity, the soil enzymes can be put in the following order: dehydrogenases > urease > alkaline phosphatase > acid phosphatase.
3. Cellulose added to soil (15g kg^{-1}) was found to be a good factor in the improvement of soil biochemical properties, although it did not limit the negative effects produced by zinc.
4. The inhibitory influence of zinc on the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase persisted throughout the whole period of the experiments (120 days).

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