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CHANGES IN THE ENZYMATIC ACTIVITY IN SANDY LOAM SOIL EXPOSED TO ZINC PRESSURE

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Abstract

One of the bioindicators most often applied to assess the quality of soil is its enzymatic activity. Undesirable changes in the activity of enzymes can imply excessive presence of substances which are harmful to soil environment, such as heavy metals. Being a heavy metal, zinc is also an element essential for maintaining proper functions of live organisms. The purpose of this study has been to determine the significance of changes occurring in moderately heavy soil under the influence of zinc.

The experiment was carried out in three replicates under laboratory conditions. Sandy loams of pH 5.5 and 7.0 were used for the trials. The soils were contaminated with zinc according to the following design: control (natural content), raised content (70 mg Zn^{2+} kg^{-1}), weakly polluted (200 mg Zn^{2+} kg^{-1}), moderately polluted (500 mg Zn^{2+} kg^{-1}), heavily polluted (1,500 mg Zn^{2+} kg^{-1}) and very heavily polluted soil (5,000 mg and 10,000 mg Zn^{2+} kg^{-1}). The soil samples prepared as above were brought to the moisture content of 50% maximum water capacity and incubated at 25°C for 120 days. On day 30, 60 and 120, the activity of dehydrogenases, β-glucosidase, urease, acid phosphatase and arylsulphatase was determined. Based on these determinations, the following indices were calculated: ED_{50}, the index for resistance (RS) and the index for resilience (RL).

The tests have demonstrated that as the rate of soil contamination with zinc increased, the activity of all the analyzed enzymes was significantly depressed. The negative influence of zinc contamination on the activity of particular enzymes, irrespective of the soil pH, persisted throughout the whole experiment. In respect of their sensitivity to zinc, the enzymes can be ordered as follows: arylsulphatase > dehydrogenases > acid phosphatase > urease > β-glucosidase. Zinc contamination caused lasting changes in the soil environment, but the return to the state of equilibrium was the quickest in the case of dehydrogenases (RL = 0.276), less rapid for arylsulphatase (RL = 0.173) and the slowest for acid phosphatase (RL = 0.064). In contrast, the activity of urease, instead of regenerating, was increasingly disturbed (RL = 0.350). Soil acidification was the factor that most evidently exacerbated the negative influence of zinc on the activity of β-glucosidase and arylsul-

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phatase. Values of ED50 for the activity of particular enzymes were varied. In the soil of pH 7.0, they ranged from 3,324 mg Zn^{2+} kg^{-1} for β-glucosidase to 412 mg Zn^{2+} kg^{-1} for dehydrogenases, and in the soil of pH 5.5, they varied from 1,008 Zn^{2+} kg^{-1} for β-glucosidase to 280 mg Zn^{2+} kg^{-1} for arylsulphatase.

Key words: zinc, ED_{50}, index for resistance (RS), index for resilience of (RL), soil contamination, activity of enzymes.

ZMIANY AKTYWNOŚCI ENZYMATYCZNEJ W GLINIE PIASZCZYSTEJ PODDANEJ PRESJI CYNKU

Abstrakt

Aktywność enzymatyczna gleby stanowi jeden z najczęściej wykorzystywanych biowskaźników do oceny jakości gleby. Niekorzystne zmiany w aktywności enzymów mogą świadczyć o nadmiernej zawartości substancji szkodliwych dla środowiska glebowego, do których należą również metale ciężkie. Cynk jest też niezbędnym składnikiem do prawidłowego funkcjonowania organizmów żywych. Celem badań było określenie istotności zmian zachodzących w glebie o średniej kategorii agrotechnicznej ciężkości pod wpływem cynku.

Doświadczenie wykonano w warunkach laboratoryjnych, w 3 powtórzeniach. Do badań wykorzystano gliny piaszczyste o pH 5,5 oraz o pH 7,0, które zanieczyszczono cynkiem wg następującego schematu: kontrola (zawartość naturalna), zawartość podwyższona (70 mg Zn^{2+} kg^{-1}), słabe zanieczyszczenie (200 mg Zn^{2+} kg^{-1}), średnie zanieczyszczenie (500 mg Zn^{2+} kg^{-1}), silne zanieczyszczenie (1500 mg Zn^{2+} kg^{-1}) i bardzo silne zanieczyszczenie (5000 mg oraz 10 000 mg Zn^{2+} kg^{-1}). Tak przygotowane próbki gleby uwilgotniono do poziomu 50% maksymalnej pojemności wodnej i inkubowano w temp. 25°C przez 120 dni. W 30., 60. oraz 120. dniu określono aktywność dehydrogenaz, β-glukozydazy, ureazy, fosfatazy kwaśnej oraz arylosulfatazy. Na ich podstawie obliczono wskaźniki ED_{50}, oporności (RS) i powrotu do równowagi (RL).

W wyniku badań stwierdzono, że wraz ze zwiększeniem stopnia zanieczyszczenia gleby cynkiem aktywność wszystkich badanych enzymów ulegała istotnemu zmniejszeniu. Negatywny wpływ zanieczyszczenia cynkiem na aktywność enzymatyczną gleby, niezależnie od jej pH, utrzymywał się przez cały okres trwania badań. Enzymy pod wpływem wrażliwości na zanieczyszczenie gleby cynkiem można uszeregować następująco: arylosulfataza > dehydrogenazy > fosfataza kwaśna > ureaza > β-glukozydaza. Zanieczyszczenie cynkiem powodowało długotrwałe zmiany w środowisku glebowym, ale najszybciej do stanu równowagi powracały dehydrogenazy (RL = 0,276), nieco wolniej arylosulfataza (RL = 0,173), i najwolniej fosfataza kwaśna (RL = 0,064). Natomiast aktywność ureazy nie tyko nie regenerowała się, ale stan zaburzenia ulegał pogłębieniu (RL = -0,350). Zakwaszenie gleby w największym stopniu wzmagało negatywne działanie cynku na aktywność β-glukozydazy i arylosulfatazy. ED_{50} dla aktywności poszczególnych enzymów było zróżnicowane. W glebie o pH 7,0 wahało się od 3324 mg Zn^{2+} kg^{-1} dla β-glukozydazy do 412 mg Zn^{2+} kg^{-1} dla dehydrogenaz, a w glebie o pH 5,5 od 1008 mg Zn^{2+} kg^{-1} dla β-glukozydazy do 280 mg Zn^{2+} kg^{-1} dla arylosulfatazy.

Słowa kluczowe: cynk, ED_{50}, wskaźnik oporności (RS), wskaźnik powrotu do równowagi (RL), zanieczyszczenie gleby, aktywność enzymów.

INTRODUCTION

The intensive growth of industries and civilization progress have raised levels of heavy metals in soil, a development which is now a serious problem (SEIFERT, DOMKA 2005) affecting not just plants and animals (BROOKES 1995) but microorganisms as well (KUCHARSKI et al. 2000, WYSZKOWSKA, KUCHARSKI 2003, WYSZKOWSKA et al. 2001). Excessively high concentrations of these elements have an adverse effect on soil fertility. Elevated levels of heavy metals produce negative influence on the physicochemical and biological properties of soils (GILLET, PONGE 2002, KUCHARSKI, WYSZKOWSKA 2004, WYSZKOWSKA et al. 2007), including the activity of enzymes and the nitrification process (KUCHARSKI et al. 2009).

Soil enzymes are produced by plants and animals, but the dominant ones are secreted by microorganisms (NIELSEN, WINDING 2002). Consequently, counts of microorganisms in soil are a very important indicator of soil quality. Excessive content of zinc has a negative impact on counts of oligotrophic bacteria, *Azotobacter* and *Arthrobacter* bacteria as well as fungi (WYSZKOWSKA, KUCHARSKI 2003).The harmful influence of heavy metals on soil ecosystems is compounded by the fact that in practice – unlike organic compounds – heavy metals do not undergo transformations, that is, they are not degraded and consequently their accumulation in soil is burdensome to each biotope and to the whole biocenosis as well (ADRIANO et al. 2004).

Soil enzymes are involved in the circulation of biogenic elements (NIELSEN, WINDING 2002). This fact is of great importance from the point of view of ecology, as the activity of microorganisms and enzymes they secrete enable the circulation of elements in nature (BROOKES 1995, TRASAR-CEPEDA et al. 2000) and the transformation of the substances they contain into forms available to other autochthonous organisms. A low level of soil contamination with heavy metals may just impede the metabolic processes of microorganisms, but amounts surpassing several-fold the permissible levels will become a sole reason why soils are degraded due to depressed microbial activity (KUCHARSKI, WYSZKOWSKA 2004, NIELSEN, WINDIG 2002, WYSZKOWSKA et al., 2008, 2009). Analysis of the enzymatic activity in soil is an important bioindicator of soil quality as it enables us to detect some unfavourable changes occurring in soil environment (VISSER, PARKINSON 1992, HINOJOSA et al. 2008).

Zinc is one of those heavy metals which living organisms need as a trace element (MICHALAK 2006). It is found in the structure of metalloenzymes (MCCALL et al. 2000) and it plays many important physiological functions, but when present in excessive amounts, zinc may cause destabilisation of ecosystems. Thus, it seems essential to gain thorough knowledge of the effects zinc exerts on soil, including its influence on the activity of soil enzymes.

The objective of the present study has been to determine changes in the activity of dehydrogenases, β-glucosidase, urease, arylsulphatase and acid phosphatase in sandy loam soil of different reaction, exposed to zinc pressure.

MATERIAL AND METHODS

The experiment was run in three replicates under laboratory conditions. Sandy loams of pH 5.5 and 7.0 were used for the trials. The soils were sampled from the arable humus horizon, from the depth of 5-25 cm. More detailed specification of the soils is presented in Table 1. A portion of 100 g air dry soil was placed in each of 100 cm^3 glass beakers. Zinc in the form of aqueous solution of $ZnCl_2$ salt was added to the soil samples in the following doses (in mg Zn^{2+} kg^{-1} d.m. of soil): 0 (natural content – 0º), 70 (raised

Table 1

Some physicochemical properties of soil used in the experiment

Kind of soil	Grain-size composition (mm)			C_{org}	pH_{KCl}	Hh	S	T	V
	2-0.05	0.05-0.002	<0.002	(g)		mmol(+) kg^{-1} of soil d.m.			(%)
Sandy loam	72	21	7	7.05	7.0	16.05	75.00	91.05	82.37
Sandy loam	74	16	10	5.75	5.5	33.50	35.33	68.83	51.33

C_{org} – organic carbon content per 1 kg of soil d.m., pH_{KCl} – soil reaction, Hh – hydrolytic acidity, S – exchangeable soil capacity, T – total exchangeable soil capacity, V – soil base saturation

content – Iº), 200 (weak contamination – IIº), 500 (moderate contamination – IIIº), 1,500 (heavy contamination – IVº), 5,000 (very heavy contamination – Vº), 10,000 (a dose two-fold higher than very heavy contamination – VIº). Afterwards, the soil samples were thoroughly mixed and brought to the moisture content equal 50% of the maximum water capacity. The soil samples thus prepared were covered with plastic film and placed in a heater (25ºC). The experiment was set up in three replicates. During the incubation, the soil moisture was monitored regularly and water losses were supplemented. The soil samples were incubated for 30, 60 and 120 days. On each of these dates, the activity of the following enzymes was determined in three replications: acid phosphatase (EC 3.1.3.2), arylsulphatase (EC 3.1.6.1), β-glucosidase (EC 3.2.1.21), urease (EC 3.5.1.5) and dehydrogenases (EC 1.1.1.1).

The substrates used for determination of the activity of particular enzymes were 4-nitrophenylphosphate disodium for acid phosphatase, potassium-*p*-nitrophenylsulphate for arylsulphatase, *p*-nitrophenyl-β-D-glucopyranoside for β-glucosidase, urea for urease and 2,3,5-triphenyltetrazolium chloride

(TTC) for dehydrogenases. The activity of acid phosphatase, arylsulphatase and β-glucosidase was expressed in mmol *p*-nitrophenol (PNP) kg^{-1} d.m. h^{-1}; of urease – in mmol $N\text{-}NH_4$ kg^{-1} h^{-1}, and of dehydrogenases – in µmol triphenyl formazan (TPF) kg^{-1} d.m. h^{-1}. The activity of all the enzymes, except dehydrogenases, was determined according to the protocol described by ALEF and NANNIPIERI (1995). In turn, the activity of dehydrogenases was assessed as explained by ÖHLINGER (1996). The results of the analyses were processed statistically with a multi-factor analysis of variance Anova, using Statistica software. ED_{50} as well as the index for resistance (RS) and the index for resilience (RL) were calculated for each enzyme according to ORWIN and WARDLE (2004). The activity of the enzymes as well as RS were given as means from all the dates of analyses, whereas the RL index was calculated for the samples tested after 120 days of incubation.

RESULTS AND DISCUSSION

The results of the determinations suggest that the presence of zinc in soil significantly modifies the biological properties of soil. The extent to which zinc affects the enzymatic activity in soil largely depends on the soil contamination degree and soil reaction.

The activity of dehydrogenases in the soil of pH 7.0 containing a naturally occurring amount of zinc was 10.06 µmol TFF kg^{-1} d.m. h^{-1}, and in the soil of pH 5.5, it was 7.89 µmol TFF kg^{-1} h^{-1} (Figure 1). In the soil of pH 7.0, the activity of these enzymes fell by 12% when a dose of 200 mg Zn^{2+} kg^{-1} was added and by 65% – when the rate of zinc rose to 500 mg Zn^{2+} kg^{-1}. The same rates of zinc added to the soil of pH 5.5 inhibited the activity of dehydrogenases more strongly, i.e. by 30 and 71%, respectively. Higher doses of the contaminant (1,500 to 10,000 mg Zn^{2+} kg^{-1}) had a nearly identical influence on dehydrogenases in the soils of both reactions, by depressing their activity within 96 to 98%.

WYSZKOWSKA et al. (2006a, 2006b) demonstrated 30% inhibition of the activity of dehydrogenases in response to a rate of zinc as low as 50 mg Zn^{2+} kg^{-1}. The same experiment showed that the activity of dehydrogenases decreased as the zinc contamination of soil increased. The effect produced by this element on dehydrogenases, like that of other heavy metals (WYSZKOWSKA et al. 2001), depends not only on its concentration in soil but also on a whole range of soil properties, such as levels of heavy metals (HINOJOSA et al. 2004). The results obtained in the present study coincide with the report presented by BIELIŃSKA (2007) and confirm the suggestions made by NIELSEN and WINDIG (2002), who claimed that dehydrogenases might be a good indicator of the microbiological activity of soil since they are classified as intercellular enzymes (BROOKES 1995).

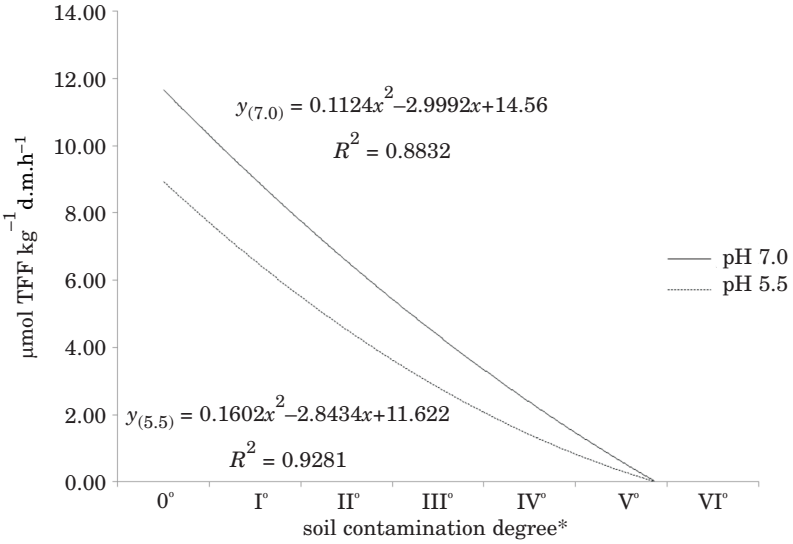

Fig. 1. Activity of dehydrogenases in soil contaminated with zinc, µmol TFF kg^{-1} d.m. h^{-1}
*zinc doses (mg Zn^{2+} kg^{-1} of soil d.m.): 0° – 0, I° – 70, II° – 200, III° – 500, IV° – 1,500, V° – 5,000, VI° – 10,000

Urease, another enzyme tested in our trials, was similar to dehydrogenases in that it was very sensitive to soil contamination with zinc and to soil acidification (Figure 2). In acid sandy loam not contaminated with zinc, the activity of this enzyme was up to 47% lower than in sandy loam of neutral reaction. In both soils, as the zinc concentration rose, the activity of urease decreased. However, the activity of that enzyme seemed more grossly disturbed in the soil of pH 7.0 than 5.5. In the former soil, zinc depressed the activity of urease by an average 58%, regardless of the degree of contamination, while in the latter type of soil – the average decrease was 49%. The negative effect of excess zinc on the activity of urease in soil has also been reported elsewhere (Balyaeva et al. 2005, Gülser and Erdoğan 2008, Stuczyński et al. 2003, Wyszkowska et al. 2006a,b). Both, the authors' own studies and the above references prove that excess zinc in soil can cause disorders in the nitrogen cycle, in which urease plays an important role (Nielsen, Winding 2002).

Acid phosphatase responded to soil acidification differently than urease or dehydrogenases, although its reaction to zinc contamination was similar to that of the other enzymes (Figure 3). The activity of phosphatase in uncontaminated sandy loam of pH 7.0 was 56% lower than in soil of similar grain size composition but of lower pH (5.5). Differences between the soils in the scale of the negative effect of zinc on acid phosphatase were significant up to the contamination dose of 200 mg Zn^{2+} kg^{-1} but became less evident under the influence of increasing rates of the pollutant (500-10,000

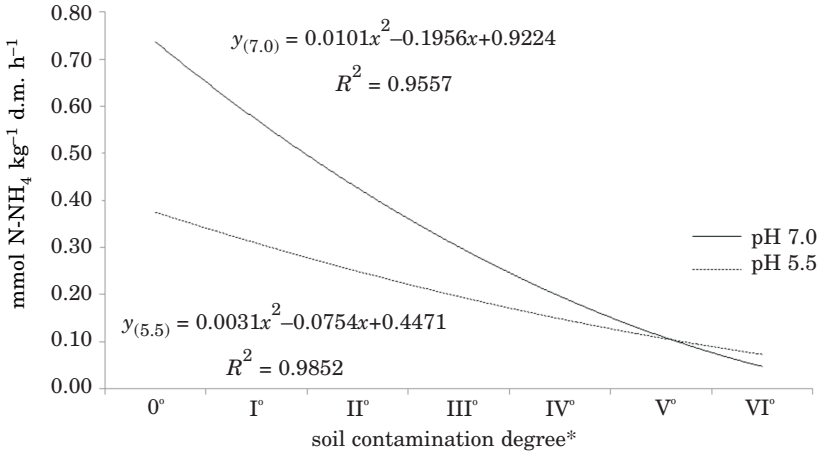

Fig. 2. Activity of urease in soil contaminated with zinc, mmol $N\text{-}NH_4$ kg^{-1} d.m. h^{-1}
*The key is given under Fig. 1

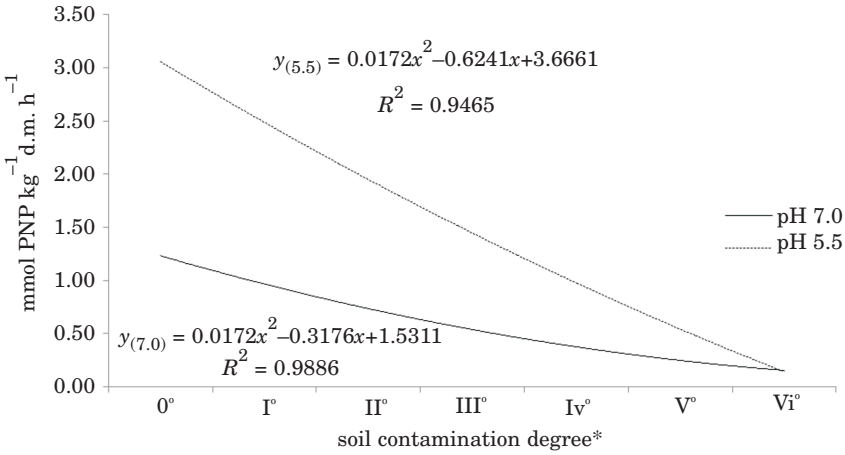

Fig. 3. Activity of acid phosphatase in soil contaminated with zinc, mmol PNP kg^{-1} d.m. h^{-1}
*The key is given under Fig. 1

mg Zn^{2+} kg^{-1}). In the soil of neutral reaction, a dose of zinc as low as 70 mg Zn^{2+} kg^{-1} depressed the activity of this enzyme by 26%, whereas a higher dose such as 200 mg Zn^{2+} kg^{-1} lowered it by 42%. In the soil of acid reaction, the activity of urease was depressed by 7 and 20%, respectively. About 50% inhibition of the activity of urease appeared in both types of soil in response to a dose of 500 mg Zn^{2+} kg^{-1}. Higher rates of the contaminant (1,500-10,000 mg Zn^{2+} kg^{-1}) depressed the activity of urease within the range of 74% to 88%.

Among the analyzed enzymes, acid phosphatase was characterized by the highest variability in respect to the soil reaction, analogously to its behaviour in an experiment reported by STUCZYŃSKI et al. (2003), and its response to zinc contamination was similar to the results obtained by BALYAEVA et al. (2005), GÜLSER and ERDOĐAN (2008) and KUPERMAN and CARREIRO (1997).

The activity of β-glucosidase in sandy loam of pH equal 7.0, without additional zinc, was 0.466 mmol PNP and in sandy loam of pH 5.5 it reached 0.505 mmol PNP kg^{-1} d.m. h^{-1} (Figure 4). Evident inhibition of the activity of this enzyme occurred in moderately polluted soils (500 mg Zn^{2+} kg^{-1}): by 26% in soil of pH 7.0 and by 49% in soil of pH 5.5. Strong contamination (10,000 mg Zn^{2+} kg^{-1}) depressed the activity of β-glucosidase by 85% (pH 7.0) and 79% (pH 5.5). Likewise, HINOJOSA et al. (2004) determined that the activity of β-glucosidase was 84% lower in response to heavy soil contamination with zinc, whereas KUPERMAN and CARREIRO (1997) noted the inhibition of this enzyme reaching as much as 97%. The fact that the activity of β-glucosidase was more strongly inhibited in the experiments completed by the above authors than in the present study could be related to some interaction of zinc contamination with other heavy metals.

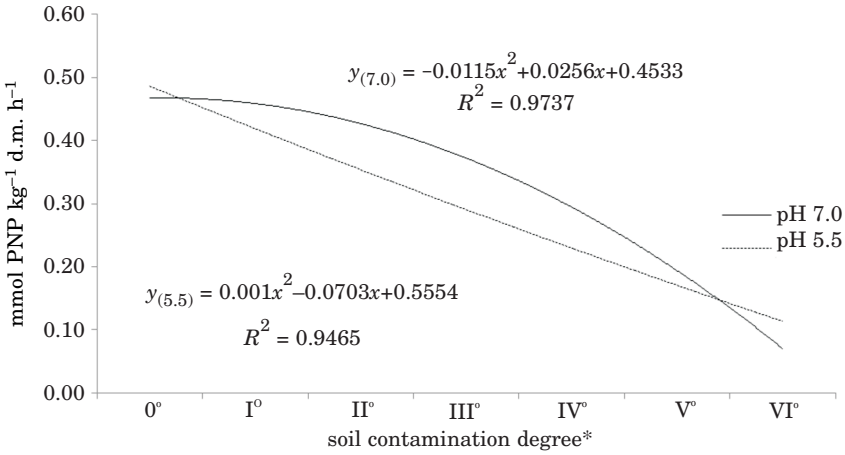

Fig. 4. Activity of β-glucosidase in soil contaminated with zinc, mmol PNP kg^{-1} d.m. h^{-1}
*The key is given under Fig. 1

The activity of arylsulphatase (Figure 5), in contrast to β-glucosidase, was higher in the soil of neutral (0.417 mmol PNP kg^{-1}) rather than acid reaction (0.203 mmol PNP kg^{-1}). In both soils, negative correlation appeared between the degree of zinc contamination and the activity of arylsulphatase. Depending on the concentration of zinc, the activity of this enzyme was inhibited by 36 to 98% in the former soil; in the latter one – it fell by 15 to 85%. These results are only partly congruent with the research reported by STUCZYŃSKI et al. (2003), who demonstrated 41% inhibition in the activity

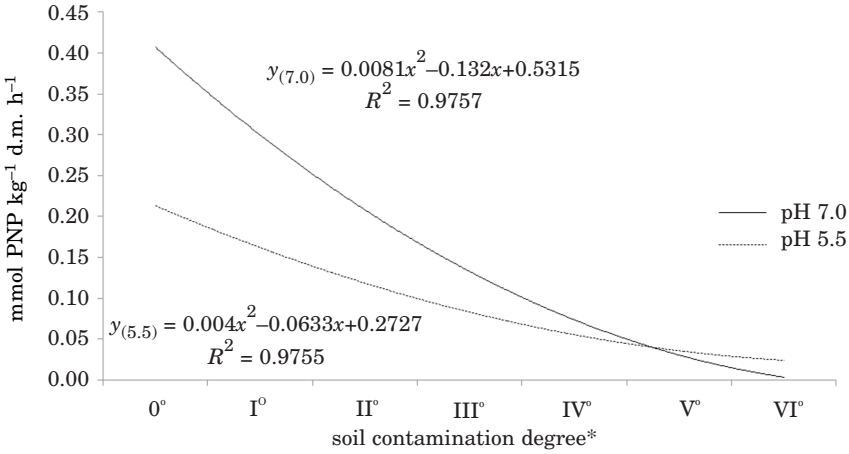

Fig. 5. Activity of arylsulphatase in soil contaminated with zinc, mmol PNP kg^{-1} d.m. h^{-1}
*The key is given under Fig. 1

of arylsulphatase under the influence of 980 mg Zn^{2+} kg^{-1} in soil of pH 7.4 and complete inhibition under the effect of 390 mg Zn^{2+} kg in soil of pH 5.1.

The value of the index for resistance (RS) to zinc proves that the sensitivity of particular enzymes to this metal is varied (Table 2). Irrespective of soil pH, in terms of RS the enzymes can be ordered as follows: β-glucosi-

Table 2

Index of soil enzyme resistance (RS) depending on zinc pollution

mg Zn^{2+} kg^{-1} of soil d.m.	Activity									
	dehydrogenases		urease		acid phosphatase		β-glucosidase		arylsulphatase	
	soil pH									
	7.0	5.5	7.0	5.5	7.0	5.5	7.0	5.5	7.0	5.5
	RS									
70	0.914	0.978	0.889	0.846	0.591	0.864	0.900	0.580	0.621	0.544
200	0.793	0.539	0.522	0.543	0.407	0.673	0.967	0.653	0.500	0.373
500	0.209	0.172	0.287	0.376	0.328	0.341	0.582	0.345	0.291	0.186
1,500	0.018	0.018	0.091	0.220	0.151	0.117	0.436	0.325	0.065	0.090
5,000	0.011	0.013	0.085	0.174	0.102	0.085	0.282	0.207	0.046	0.081
10,000	0.008	0.009	0.063	0.125	0.071	0.062	0.081	0.119	0.014	0.064
Average	0.326	0.288	0.323	0.381	0.275	0.357	0.541	0.372	0.256	0.223
r	-0.623	-0.582	-0.629	-0.696	-0.752	-0.684	-0.866	-0.818	-0.720	-0.641

r – correlation coefficient

dase (0.457) > urease (0.352) > acid phosphatase (0.316) > dehydrogenases (0.307) > arylsulphatase (0.240). However, dehydrogenases, β-glucosidase and arylsulphatase were more resistant to the contamination in neutral soil whereas acid phosphatase and urease – in acid one. Another study by WYSZKOWSKA et al. (2009) proves that resistance of particular soil enzymes to the influence of heavy metals is varied although there is a certain regularity that repeats, namely heavy metals inhibit more strongly the activity of dehydrogenases than that of urease.

ED_{50} for the analyzed enzymes was likewise varied Table 3). In the soil of pH 7.0, it ranged from 3,324 mg Zn^{2+} kg for β-glucosidase to 412 mg Zn^{2+} kg^{-1} for dehydrogenases, and in soil of pH 5.5. – from 1,008 mg Zn^{2+} kg^{-1} for β-glucosidase to 280 mg Zn^{2+} kg^{-1} for arylsulphatase. The above values demonstrate that the response of β-glucosidase and arylsulphatase was the highest to the zinc contamination dependent on the pH of the environment.

Table 3

The dose of zinc (mg Zn^{2+} kg^{-1} of soil) decreases by 50% the activity of soil enzymes (ED_{50})*

Soil pH	Activity				
	dehydrogenases	urease	acid phosphatase	β-glucosidase	arylsulphatase
	ED_{50}				
7.0	412*a*	422*b*	425*b*	3324*a*	432*a*
5.5	357*b*	508*a*	510*a*	1008*b*	280*b*

*homogenous groups in the columns labelled with identical letters

The values of RL suggest that zinc contamination causes lasting changes in soil environment (Table 4), but dehydrogenases are the first to return to equilibrium (RL = 0.276); arylsulphatase is somewhat less resilient (RL = 0.173) and acid phosphatase takes the longest to return to the normal state of balance (RL = 0.064). In contrast, the activity of urease, instead of regenerating, becomes increasingly disturbed (RL = -0.350). The fact that dehydrogenases return to the state of equilibrium sooner may be associated with the succession of microorganisms. Dead cells are replaced by new ones, more resistant to zinc contamination and dead microorganisms serve as energy substrate for new microbial assemblages. Disproportions between the resumed activity of dehydrogenases and the inferior activity of urease might be due to the fact that dehydroogenases express exclusive intercellular activity while urease shows both extra- and intercellular activity.

Table 4

Index of soil enzymes resilience (RS) depending on zinc pollution

mg Zn^{2+} kg^{-1} of soil d.m.	Activity									
	dehydrogenases		urease		acid phosphatase		β-glucosidase		arylsulphatase	
	soil pH									
	7.0	5.5	7.0	5.5	7.0	5.5	7.0	5.5	7.0	5.5
70	-0.291	0.405	-1.000	-0.667	0.663	0.307	-0.710	0.496	-0.101	0.391
200	0.909	0.427	-0.500	-0.643	0.435	-0.090	0.599	0.980	0.164	0.498
500	0.403	0.111	0.080	-0.444	0.462	-0.416	0.319	0.046	0.114	0.141
1,500	0.262	0.177	-0.111	-0.256	0.159	-0.375	0.049	-0.115	0.093	0.230
5,000	0.266	0.184	-0.111	-0.224	0.127	-0.323	0.142	0.002	0.043	0.237
10,000	0.268	0.187	-0.109	-0.216	0.133	-0.321	-0.007	0.045	-0.005	0.274
Average	0.303	0.248	-0.292	-0.408	0.330	-0.203	0.065	0.242	0.051	0.295
r	-0.062	-0.406	0.397	0.727	-0.711	-0.386	-0.022	-0.442	-0.267	-0.250

r – correlation coefficient

CONCLUSIONS

1. Soil contamination with zinc in doses from 70 to 10,000 mg kg^{-1} d.m. of soil causes highly significant inhibition of the activity of dehydrogenases, arylsulphatase, urease, acid phosphatase and β-glucosidae.

2. In respect of their sensitivity to soil contamination with zinc, the enzymes can be ordered as follows: arylsulphatase > dehydrogenases > acid phosphatase > urease > β-glucosidae.

3. Zinc contamination causes persistent changes in soil environment, but dehydrogenases are the first to return to the normal state of equilibrium (RL = 0.276), while arylsulphatase takes longer (RL = 0.173) and acid phosphatase is the least resilient (RL = 0.064). Urease, instead of having its activity improved in time, becomes increasingly disturbed (RL = -0.350).

4. Soil acidification reinforces the negative effect of zinc contamination most evidently in respect of the activity of β-glucosidase and arylsulphatase.

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