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ORIGINAL PAPERS

RESISTANCE OF DEHYDROGENASES, CATALASE, UREASE AND PLANTS TO SOIL CONTAMINATION WITH ZINC*

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Abstract

Pot trials on growing plants were conducted in order to determine the resistance of dehydrogenases, catalase and urease as well as the plants themselves to soil contamination with zinc. The experimental variables were: the type of soil (loamy sand and sandy loam), degree of soil pollution with zinc from 0 to 600 mg Zn^{2+} kg^{-1} d.m., and plant species (oat, spring rape and yellow lupine). Samples of soil were tested to determine the activity of dehydrogenases, catalase and urease as well as its physicochemical properties. Based on the enzymatic activity of soil and the dry matter of harvested plants, the resistance of enzymes and each of the crops was determined to excessive amounts of zinc in soil with different grain-size distribution. It was concluded that zinc contamination significantly inhibited the activity of dehydrogenases, catalase and urease. With respect to their sensitivity to zinc, the enzymes were arranged in the following order: dehydrogenases > urease > catalase. The plant species and grain-size distribution of soil determined the resistance of the enzymes to zinc pollution. Dehydrogenases were most resistant to zinc in soil cropped with oat, urease – in soil under spring rape and catalase – in soil sown with yellow lupine. Dehydrogenases and urease were more resistant to the adverse influence of zinc in sandy loam than in loamy sand, contrary to catalase, which was less vulnerable in loamy sand than in sandy loam. Tolerance of plants to zinc pollution proved to be a species-specific characteristic. Yellow lupine was most sensitive to excess zinc in soil, while oat was most resistant to the said contamination out of the three examined plant species.

Key words: zinc, activity of enzymes, resistance index, oat, yellow lupine, spring rape, soil contamination with zinc.

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OPORNOŚĆ DEHYDROGENAZ, KATALAZY, UREAZY I ROŚLIN NA ZANIECZYSZCZENIE GLEBY CYNKIEM

Abstrakt

W badaniach wegetacyjnych wazonowych, których celem było określenie oporności dehydrogenaz, katalazy i ureazy oraz roślin na zanieczyszczenie gleby cynkiem, czynnikami zmiennymi w doświadczeniu były: rodzaj utworu glebowego (piasek gliniasty i glina piaszczysta), stopień zanieczyszczenia gleby cynkiem (od 0 do 600 mg Zn^{2+} kg^{-1} s.m. gleby) oraz gatunek uprawianej rośliny (owies, rzepak jary i łubin żółty). W próbkach gleby określono aktywność dehydrogenaz, katalazy i ureazy oraz właściwości fizykochemiczne. Na podstawie aktywności enzymów oraz plonu suchej masy roślin określono oporność enzymów oraz poszczególnych gatunków roślin na nadmierne ilości cynku w glebach o zróżnicowanym składzie granulometrycznym. Stwierdzono, że zanieczyszczenie cynkiem hamuje istotnie aktywność dehydrogenaz, ureazy i katalazy. Enzymy pod względem wrażliwości na cynk uszeregowano następująco: dehydrogenazy > ureaza > katalaza. Gatunek roślin oraz skład granulometryczny gleby determinował oporność enzymów na zanieczyszczenie cynkiem. Dehydrogenazy najbardziej oporne na negatywne działanie cynku były w glebie pod uprawą owsa, ureaza – rzepaku jarego, a katalaza – łubinu żółtego. Dehydrogenazy i ureaza są bardziej oporne na działanie cynku w glinie piaszczystej niż w piasku gliniastym, a katalaza odwrotnie – bardziej oporna w piasku gliniastym niż w glinie piaszczystej. Wrażliwość roślin na zanieczyszczenie cynkiem okazała się być cechą gatunkową. Spośród badanych roślin najbardziej wrażliwy na nadmiar cynku w glebie był łubin żółty, a najmniej – owies.

Słowa kluczowe: cynk, aktywność enzymów, indeks oporności, owies, łubin żółty, rzepak jary, zanieczyszczenie gleby cynkiem.

INTRODUCTION

Zinc is an essential element for all organisms, in which it performs a number of metabolic functions. It occurs in world soils within the range of concentrations from 35 mg to 12,400 mg kg^{-1} of soil (Kabata-Pendias, Pendias 2001). This element can occur in excessive amounts in soils in industrial regions and as a point pollutant in soils lying in agricultural regions (Boussen et al. 2013, Bringmark et al. 2013, Famera et al. 2013). However, when present in excess of the threshold limit, zinc becomes a destructive agent, producing toxic effects on humans and animals (Cordova, Alvarez-Mona 1995, Takeda 2000) and plants (Shumaker, Begonia 2005, Boros et al. 2011, Guala et al. 2013) as well as on soil microorganisms (Landi et al. 2000, Mikanova et al. 2001, Renella et al. 2005, Borowik et al. 2011) and enzymes (Landi et al. 2000, Boros et al. 2011, Kucharski et al. 2011, Trevisan et al. 2012, Wyszkowska et al. 2013). Many enzymes present inside cells could not function properly without this element. In fact, zinc occurs in over 300 enzymes, which belong to six different classes (McCall et al. 2000, Sekler et al. 2007). Its role as a component of metal enzymes should be analyzed in the context of catalytic, structural and regulatory functions. This means that zinc can be essential for the activity of some enzymes, e.g. carbon anhydrase, thermolysine, alkaline phosphatase, dehydrogenases: 3-phosphoglyceric aldehyde, alcohol and glutamine dehydrogenases, and fructobisphosphate aldolase, su-

peroxide disumtase, DNA and RNA polymerase, tRNA transferase. Zinc can stabilize their protein structure and act either as their activator or inhibitor (Cordova, Alvarez-Mona 1995, Sekler et al. 2007). These natural functions of zinc could be disrupted when the element occurs in excessive quantities in nature.

Too much zinc in the environment interferes with the metabolism of soil and poses a threat to the proper development of all organisms (De Brouwere et al. 2007, Mertens et al. 2007, Wyszkowska et al. 2008, Moreno 2009). Excessive amounts of zinc depress soil fertility and reduce the activity of enzymes (Renella et al. 2005, Mikanova 2006, Djukic, Mandic 2006, Gulser, Erdrogan 2008, Vogeler et al. 2008, Lee et al. 2009, Jiang et al. 2010, Coppolecchia et al. 2011), which are an important biomarker of the quality of soil (Hinojosa et al. 2008, Borowik et al. 2013, Wyszkowska et al. 2013). Thus, being able to recognize all underlying conditions of the effects produced by zinc on the natural environment is important from both the cognitive and utilitarian point of view. For this reason, the present experiment has been conducted in order to determine the impact of zinc present in excessive amounts in soil on the activity of dehydrogenases, catalase and urease. Another objective has been to determine the resistance of these enzymes to soil contamination with zinc.

MATERIAL AND METHODS

Design of the experiment

The trials were set up with five replications and conducted in a greenhouse, in polyethylene pots each with the capacity of 3.5 dm^3. The following variables were tested:

1) soil type: loamy sand and sandy loam;
2) degree of soil contamination with zinc, in mg Zn^{2+} kg^{-1} d.m. of soil: 0, 150, 450, 600;
3) species of the grown plant: oat (*Avena sativa* L.), spring rape (*Brassica napus* L.) and yellow lupine (*Lupinus luteues* L.).

Soil (3 kg per pot) was placed in a polyethylene container, prior to putting into single pots, and polluted with zinc in the form of zinc chloride. NPKMg fertilizers were added. Soil was carefully mixed with the added ingredients and packed into the pots. The same level of fertilization with microelements was used in all the treatments, consisting of N – 100 (yellow lupine was not fertilized with nitrogen), P – 35, K^+ – 100, Mg^{2+} – 20 mg kg^{-1} d.m. of soil. Nitrogen was added in the form of $CO(NH_2)_2$, phosphorus – as KH_2PO_4, potassium – as KH_2PO_4 and KCl, and magnesium – as $MgSO_4 \cdot 7H_2O$. Once in the pots, soil was added water up to the moisture content equal 60% of capillary water capacity. Finally, the following plants were sown: oat cv. *Kasztan*, spring rape cv. *Huzar* and yellow lupine cv. *Mister*.

After emergence, the plants were thinned, leaving the following per pot: 12 oat, 8 spring rape and 5 yellow lupine plants. The plants were left to grow for 50 days. After harvest, the dry matter yield produced by the plants was determined.

Soil

Two types of soil, both belonging to Eutric Cambisols and collected from the arable humic horizon, were used in the experiment. With respect to their grain-size distribution, the soils were loamy sand and sandy loam. The physicochemical and chemical properties of the soils are specified in Table 1.

Table 1

The physico-chemical and chemical properties of the soil

Property	Loamy sand	Sandy loam
pH_{KCl}	6.7	6.8
Soil texture (μm)	(g kg^{-1})	
2000 - 50	755.6	479.2
50 - 2	229.2	487.1
2 < 0	15.2	33.7
	(mmol(+) kg^{-1})	
HAC	7.8	5.2
TEB	98.7	131.4
CEC	106.5	136.6
	%	
BS	92.7	96.2
	(g kg^{-1})	
$C_{organic}$	11.0	9.0
N_{total}	0.97	1.14
	(mg kg^{-1})	
$K_{exchangeable}$	180	168
$Na_{exchangeable}$	28	57
$Ca_{exchangeable}$	1429	2214
$Mg_{exchangeable}$	80	50
Zn_{total}	26.8	37.2

Explanations: HAC – hydrolytic acidity, TEB – sum of exchangeable bases Ca^{++}, Mg^{++}, K^{+}, and Na^{+}, CEC – cation exchange capacity, BS – base saturation

Determination of the physicochemical and chemical properties of soils

Before the proper experiment, the following properties of the soils were determined:

1) grain-size composition of the soil with laser method using a Mastersizer 2000 m;
2) pH – by potentiometry in aqueous KCl solution of the concentration of 1 mol KCl dm^{-3} (ISO 10390, 2005);
3) hydrolytic acidity (HAC) and total exchangeable base cations (BS) according to Kappen, (Klute 1996);
4) content of organic carbon (C_{org}) – with Tiurin's method (Kawada 1957);
5) content of total nitrogen according to the method by Kjeldahl (ISO 11261:1995),
6) content of zinc by flame atomic absorption spectrometry (PN-ISO 11047:2001).
7) content of potassium, sodium and calcium exchangeable cations by flame photometry, and the content of magnesium cations by atomic absorption spectrophotometry (BS EN ISO 11260:2011).

Additionally, after harvesting the crops, the analyses specified in points 1 to 5 were repeated in vegetative trials. The results of these determinations were used to calculate correlation coefficients between the activity of soil enzymes and the physicochemical and chemical properties of soil.

Determination of the activity of soil enzymes

Twice during the whole experiment (on day 25 and 50), soil samples were taken from each replicate for determination of the activity of dehydrogenases (EC 1.1), urease (EC 3.5.1.5) and catalase (EC 1.11.1.6). Dehydrogenases were determined according to the method elaborated by Öhlinger (1996). TTC (2,3,5-Triphenyl tetrazolium chloride) prepared as 3% aqueous solution served as the substrate for dehydrogenases. Soil was incubated for 24 hours at 37°C. Extinction of produced TFF (triphenyl fomazan) was measured on a Perkin-Elmer Lambda (485 nm) spectrophotometer. The results were converted into µmol of produced TFF kg^{-1} d.m. of soil h^{-1}.

The activity of catalase was determined according to the protocol described by Alef and Nannipieri (1998). The substrate was composed of 0.3% aqueus solution of H_2O_2. Soil incubation was carried out for 20 minutes at 20°C. Soil filtrate was titrated with 0.02 M aqueous solution of $KMnO_4$, and the results were converted into mol O_2 kg^{-1} d.m. of soil h^{-1}.

The activity of urease was measured according to the procedure described by Alef and Nannipieri (1998). The substrate for urease was 10% aqueous solution of urea. Soil was incubated for 3 hours at 37°C. The results were presented in mmol of produced N-NH_4 kg^{-1} d.m. of soil h^{-1}.

Activities of all the enzymes were presented as means from the dates of determinations.

Calculation methods

The resistance of dehydrogenases, catalase and urease and the tolerance of plants to zinc were derived from the data describing the activity of soil

enzymes and volumes of harvested yields. The following formula, proposed by Orwin and Wardle (2004) was applied:

$$RS = 1 - \frac{2\left|D_0\right|}{C_0 + \left|D_0\right|},$$

RS – resistance,

C_0 – value of analyzed parameter in control soil,

P_0 – value of analyzed parameter in polluted soil,

$D_0 = C_0 - P_0$.

The RS index ranges from -1 to +1. The RS equal 1 means complete resistance.

Additionally, the extent of the toxic influence of zinc on dehydrogenases, catalase and urease was computed through the determination of a rate of the metal causing a 20% (ED_{20}) decrease in the activity of these enzymes.

Statistical analysis

The results were submitted to statistical processing, which involved the determination of homogenous groups with Tukey's test at the level of significance p=0.01. Pearson's simple correlation coefficients were calculated between the degree of soil contamination and the activity of enzymes, as well as the activity of enzymes versus the harvest of plants or the physicochemical properties of soil. For the assessment of the influence of zinc on the activity of soil enzymes, the principal component analysis (PCA) was employed. All statistical calculations were aided by the software Statistica 10.0 (StatSoft, Inc... 2012).

RESULTS AND DISCUSSION

Activity and resistance of enzymes

The activity of the tested enzymes was determined, to a different degree, by the soil contamination with zinc (Table 2). Zinc was a stronger inhibitor of dehydrogenases and urease than of catalase. Inhibition of the activity of dehydrogenases and urease by excess zinc in soil was also reported by Kanito et al. (2001) or Boros et al. (2011). In turn, Renella et al. (2005) showed that zinc had a less destructive influence on soil enzymes than other metals. Similarly to the findings of the present study, the effect of zinc on different enzymes was not identical. In the authors' own research, zinc had the weakest negative influence on catalase. According to Epelde et al. (2008), the inhibitory effect of zinc can be counteracted by a proper selection of plants. In the experiment discussed herein, oat was a plant that acted

Table 2

Enzyme activity in soil contaminated with zinc

Dose of Zn^{2+} (mg kg^{-1} soil)	Kind of soil					
	loamy sand			sandy loam		
	kind of plant					
	oat	spring rape	yellow lupine	oat	spring rape	yellow lupine
Dehydrogenases (μmol TFF kg d.m. of soil h^{-1})						
0	16.923^{a}	12.954^{bcd}	12.945^{bcd}	16.528^{a}	11.839^{d}	12.368^{cd}
150	13.476^{bc}	8.644^{ef}	7.501^{gh}	13.732^{b}	9.392^{e}	8.163^{fg}
450	7.951^{fg}	4.403^{ij}	3.502^{jk}	9.766^{e}	7.602^{fgh}	7.659^{fg}
600	6.471^{h}	3.331^{jk}	3.072^{k}	7.552^{fgh}	7.215^{gh}	5.260^{i}
Average	11.205	7.333	6.755	11.894	9.012	8.363
Catalase (mol O_2 kg^{-1} d.m. of soil h^{-1})						
0	0.239^{ij}	0.238^{ij}	0.223^{jk}	0.474^{a}	0.475^{a}	0.494^{a}
150	0.249^{i}	0.238^{ij}	0.208^{kl}	0.421^{cd}	0.419^{cd}	0.423^{bc}
450	0.219^{k}	0.212^{k}	0.179^{mn}	0.438^{b}	0.423^{bc}	0.406^{de}
600	0.207^{kl}	0.192^{lm}	0.167^{n}	0.368^{h}	0.399^{ef}	0.387^{fg}
Average	0.228	0.220	0.194	0.425	0.429	0.428
Urease (mmol $N\text{-}NH_4$ kg^{-1} d.m. of soil h^{-1})						
0	1.351^{i}	0.758^{l}	1.251^{j}	3.129^{a}	2.761^{d}	3.063^{b}
150	0.888^{k}	0.686^{m}	0.798^{l}	3.092^{ab}	2.975^{c}	2.926^{c}
450	0.612^{n}	0.480^{o}	0.445^{o}	2.086^{g}	2.306^{f}	2.520^{e}
600	0.473^{o}	0.361^{p}	0.320^{p}	1.416^{h}	1.470^{h}	1.302^{i}
Average	0.831	0.571	0.703	2.431	2.378	2.453

Same letters within a given enzyme, both in columns and in rows, are assigned to homogenous groups.

protectively towards dehydrogenases and urease. However, it was capable of protecting the enzymes only at the lowest degree of soil contamination (150 mg Zn^{2+} kg^{-1}). These relationships can be more easily traced by looking at Figures 1 and 2. The alleviating effect produced by plants with respect to the toxic influence of heavy metals on soil metabolism has been mentioned by other scholars (Renella et al. 2006, Epelde et al. 2008, Jiang et al. 2010, Wyszkowska et al. 2010). Figure 1 indicates that in loamy sand the first two components represent 83.38% of the variance of original variables, with the first component corresponding to 64.44% and the second one – to 64.44% of the said variance. Vectors reflecting the activity of dehydrogenases under all the plants, activity of urease in soil cropped with oat and canola and catalase in soil sown with oat almost reached the edges of the unit circle (a circle of the radius equal 1), which means that they are very well represented by the

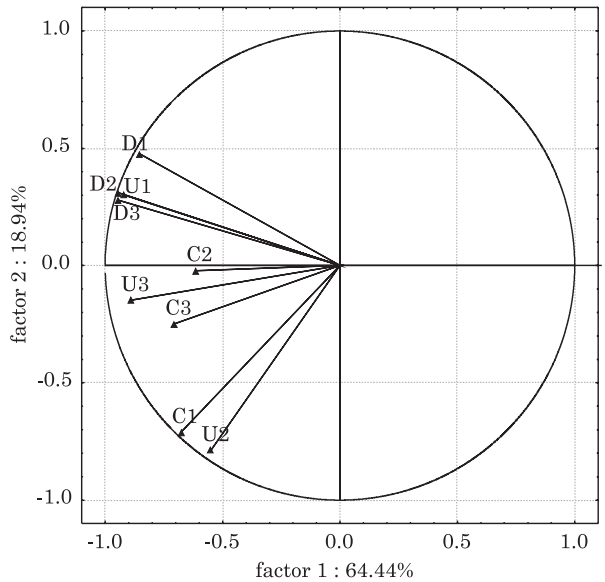

Fig. 1. Enzyme activity in loamy sand contaminated with zinc – PCA method. Vectors represent the analyzed variables: D – dehydrogenases, C – catalase, U – urease, 1 – oat, 2 – spring rape, 3 – yellow lupine

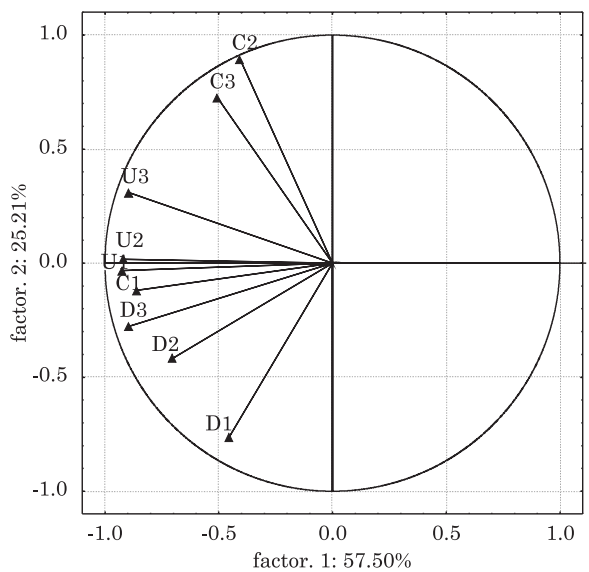

Fig. 2. Enzyme activity in sandy loam contaminated with zinc – PCA method. Vectors represent the analyzed variables: D – dehydrogenases, C – catalase, U – urease, 1 – oat, 2 – spring rape, 3 – yellow lupine

first two components, which create a set of coordinates. Vectors showing the activity of dehydrogenases, catalase and urease in soil under yellow lupine are located the closest to the first principal component, which proves that the response of these enzymes to soil contamination with zinc was similar and negative, with the original variables being most closely reflected by dehydrogenases and most weakly by catalase.

Also, we were able to detect the negative influence of zinc in soil under spring rape, especially directed against dehydrogenases and urease, with the correlation between dehydrogenases and urease being weaker than in soil under yellow lupine. Vectors representing original variables for dehydrogenases and urease in soil under oat are approximately the same, but the one corresponding to catalase is much more distant. The first principal variable most strongly determined the activity of dehydrogenases in soil under spring rape and most weakly affected the activity of catalase in soil under the same crop. All the values of the vectors representing the activity of the enzyme shaped by the first principal component were negative and ranged from -0.551 for U2 to -0.945 for D1. The second principal component most strongly shaped the U2 and C1 vectors, which assumed negative values.

The contribution of the first and second principal component in reflecting the original variable in sandy loam is slightly different (Figure 2). In this type of soil, both principal components represent 82.71% of variance, with the first one responsible for 57.50% of variance and the second one corresponding to 25.21%. Vectors U1, U2, U3, C1, D2 and D3 gathered along the axis representing the first principal component. They are characterized by high negative adjustment. Vectors C2, C3 and D1 lie along the axis corresponding to the second principal component. Vectors C2 and C3 are characterized by high positive adjustment, while vector D1 is highly negatively adjusted.

Values of the RS index for dehydrogenases (Table 3) show that the resistance of these enzymes to zinc decreased in response to an increasing degree of soil contamination with this element. The mean values suggest that dehydrogenases in sandy loam were more resistant to zinc pollution than the same enzymes in loamy sand. Dehydrogenases can be classified as very sensitive to excess zinc in soil because the RS values even in soils with the lowest dose of zinc ranged from 0.434 to 0.641 in loamy sand and from 0.494 to 0.705 in sandy loam. Dehydrogenases in soil under oat were the most resistant, while those in soil under yellow lupine were the most sensitive to zinc. Dehydrogenases determined in soil under spring rape were characterized by intermediate resistance.

The resistance of catalase (Table 3) to the influence of excessive quantities of zinc in soil was much higher than that of dehydrogenases. In loamy sand with 150 mg Zn^{2+} kg^{-1}, the RS for this enzyme varied from 0.880 to 0.955; in sandy loam, it ranged from 0.789 to 0.804. Following the application of the smallest dose of zinc to soil, no significant effect of the plant spe-

Table 3

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

Dose of Zn^{2+} (mg kg^{-1} soil)	Kind of soil					
	loamy sand			sandy loam		
	kind of plant					
	oat	spring rape	yellow lupine	oat	spring rape	yellow lupine
Dehydrogenases (μmol TFF kg d.m. of soil h^{-1})						
150	0.641^{b}	0.497^{c}	0.434^{d}	0.705^{a}	0.693^{a}	0.494^{c}
450	0.330^{e}	0.211^{h}	0.177^{i}	0.437^{d}	0.505^{c}	0.452^{d}
600	0.254^{g}	0.151^{j}	0.156^{ij}	0.292^{f}	0.496^{c}	0.270^{fg}
Average	0.408	0.286	0.256	0.478	0.565	0.405
Catalase (mol O_2 kg^{-1} d.m. of soil h^{-1})						
150	0.885^{b}	0.955^{a}	0.880^{b}	0.804^{c}	0.789^{c}	0.791^{c}
450	0.802^{c}	0.800^{c}	0.667^{fg}	0.776^{c}	0.806^{c}	0.681^{ef}
600	0.787^{c}	0.672^{ef}	0.602^{i}	0.637^{gh}	0.722^{d}	0.701^{de}
Average	0.824	0.809	0.717	0.739	0.772	0.724
Urease (mmol N-NH_4 kg^{-1} d.m. of soil h^{-1})						
150	0.543^{g}	0.772^{c}	0.651^{e}	0.568^{f}	0.854^{b}	0.935^{a}
450	0.406^{i}	0.468^{h}	0.455^{h}	0.540^{g}	0.720^{d}	0.663^{e}
600	0.313^{k}	0.316^{k}	0.304^{k}	0.305^{k}	0.361^{j}	0.261^{l}
Average	0.421	0.518	0.470	0.471	0.645	0.619

Same letters within a given enzyme, both in columns and in rows, are assigned to homogenous groups.

cies on the RS of catalase was appeared. In loamy sand cropped with oat and yellow lupine, the values of this index were approximately the same, but in soil under spring rape, the RS of catalase reached as much as 0.955. Catalse was relatively highly resistant in soils polluted with 600 mg Zn^{2+} kg^{-1}, which was demonstrated by rather high values of the RS, within 0.602 to 0.787. The RS of catalase, in contrast to dehydrogenases, was not usually affected by the type of soil.

Urease (Table 3) was more sensitive to excessive amounts of zinc in soil than catalase but more resistant than dehydrogenases. This conclusion is supported by the intermediate values of the RS of urease, between the values obtained by dehydrogenases and catalase. In the loamy sand polluted by the smallest zinc rate (150 mg kg^{-1}), the RS value ranged from 0.543 to 0.722; in the analogous treatment on sandy loam, the range was from 0.568 to 0.935. In both types of soil, urease in soil under oat was the least resistant to the above contamination rate; it demonstrated the highest tolerance in loamy sand cropped with spring rape, while the highest RS value in sandy

loam was determined under yellow lupine.

The highest dose of zinc (600 mg kg^{-1}) significantly decreased the RS values. Whereas in loamy sand, the RS index did not depend on the species of cultivated crop, it reached the highest value (0.361) in sandy loam cropped with spring rape, being the lowest in soil under yellow lupine. Within the zinc doses of 150 mg - 450 mg Zn^{2+} kg^{-1} urease was more tolerant in sandy loam than in loamy sand, but this relationship disappeared when soil had been polluted with 600 mg Zn^{2+} kg^{-1}. While analyzing the above results, we should remember that the activity of soil enzymes in soils unpolluted by heavy metals is higher when leguminous plants rather than cereals are grown (Velmourougane et al. 2013).

With respect to the resistance to zinc contamination, the analyzed enzymes can be ordered as follows: catalase > urease > dehydrogenases. These results coincide with the ED_{20} for zinc (Table 4). The ED_{20} index had the highest values for catalase in loamy sand and sandy loam, while being the lowest for dehdyrogenases, except in soil under oat. Regarding dehydrogenases and urease, higher values of the zinc ED_{20} index were recorded in sandy loam than in loamy sand. No such relationship was detected in the case of catalase. However Qu et al. (2013) found more inhibition of soil urease activity by zinc than dehydrogenase.

Table 4

The dose of zinc (mg Zn^{2+} kg^{-1} d.m. of soil) decreases by 20% the activity of soil enzymes (ED_{20})*

Enzyme	Kind of soil					
	loamy sand			sandy loam		
	kind of plant					
	oat	spring rape	yellow lupine	oat	spring rape	yellow lupine
Dehydrogenases	169^{c}	116^{d}	96^{e}	209^{b}	243^{a}	171^{c}
Catalase	788^{a}	655^{b}	465^{e}	529^{c}	648^{b}	493^{d}
Urease	131^{e}	247^{d}	111^{f}	277^{c}	362^{a}	301^{b}

* Homogenous groups in rows labelled with identical letters.

It is interesting to notice that enzymes of the same class, i.e. dehydrogenases and catalase, respond to zinc contamination. Catalase is more resistant than dehydrogenases, which may be a result of the specific nature of dehydrogenases. The disproportions between these enzymes could also be caused by the fact that dehydrogenases appear in soil as an integral part of intact cells and are an indicator of the rate of respiratory metabolism carried out by soil microorganisms (Praveen-Kumar, Tarafdar 2003), whereas catalase, having been partially released from cells, shows some stability in soil owing to its sorption on the surface of loamy minerals or association with soil's organic colloid (Calamai et al. 2000).

Despite the higher activity of all the enzymes in sandy soil, that is soil

with a higher sorption capacity, than in loamy sand, characterized by lower sorption, which is in accord with numerous studies (Calamai et al. 2000, Karlen et al. 2003, Wyszkowska et al. 2010), dehydrogenases and urease were determined to be more resistant to zinc in sandy loam than in loamy sand, in contrast to catalase, which was more resistant in loamy sand. The fact that urease was more tolerant to the presence of zinc in sandy loam than in loamy sand could be attributed to the protective role played by organic and mineral colloids towards this enzyme (Abraymana 1993) or to stronger sorption of zinc (Adamoa et al. 2003, Qu et al. 2013). And although the catalytic efficiency of enzymes associated with colloids is typically lower than enzymes alone, in the free state or inside cells, they are more resistant to periodic changes of the conditions in a given ecosystem, and they mostly decide about the directions of biochemical transformations in soil, thus shaping soil fertility (Calamai et al. 2000, Praveen-Kumar, Tarafdar 2003). On the other hand, a higher resistance of dehydrogenases in sandy loam than in loamy sand can be a result of both stronger sorption of zinc in more compact soil and a higher number and diversity of microorganisms.

Resistance of plants

Large differences in the sensitivity response to excessive doses of zinc in soil were observed not only among enzymes but also among plant species (Table 3). Of the three crops grown on loamy sand, oat was most resistant while lupine was most sensitive to zinc. The RS of oat ranged from 0.825 to 0.891 and was not correlated with the degree of soil contamination with zinc; on the other hand, the RS of yellow lupine decreased from 0.575 (150 mg Zn^{2+} kg^{-1}) to 0.035 (600 mg Zn^{2+} kg^{-1}) and that of spring rape declined from 0.939 to 0.315, respectively. The least resistant to the influence of zinc was also yellow lupine grown on sandy loam, while the resistance of oat and spring rape grown in pots with 150 mg and 450 mg Zn^{2+} kg^{-1} was similar. However, spring rape was more resistant to zinc than oat in treatments consisting of loam polluted with 600 mg Zn^{2+} kg^{-1}. Varied resistance of particular plant species to excessive quantities of zinc and other heavy metals in soil, mentioned in relevant references (Karlen et al. 2003, Hua et al. 2008, Zalewska 2012, Nadgórska-Socha et al. 2013, Tran, Popova 2013, Wyszkowski, Radziemska 2013). Is attributed to the anatomical structure and physiological characteristics of plants, a thesis supported by the high resistance to zinc contamination demonstrated by oat, a plant which tolerates well stress conditions in the environment.

When soil was polluted with zinc, the dependence between yields of plants and activity of soil enzymes was not unambiguous (Table 5). The correlation between the activity of dehydrogenases and urease versus the yield of oat on loamy sand was negative, being positive on sandy loam. Positive correlation also occurred between the activity of all the tested enzymes in loamy sand and the yields of spring rape and yellow lupine. The same re-

Table 5

Index of plant resistance (RS) depending on zinc pollution

Dose of Zn^{2+} (mg kg^{-1} soil)	Kind of soil					
	loamy sand			sandy loam		
	kind of plant					
	oat	spring rape	yellow lupine	oat	spring rape	yellow lupine
150	0.856^{bc}	0.939^{a}	0.575^{f}	0.719^{de}	0.685^{e}	0.190^{h}
450	0.825^{c}	0.341^{g}	0.041^{i}	0.702^{de}	0.734^{de}	0.066^{i}
600	0.891^{ab}	0.315^{g}	0.035^{i}	0.566^{f}	0.738^{d}	0.061^{i}
Average	0.858	0.531	0.217	0.662	0.719	0.105

lationship was maintained between the enzymes in sandy loam and yields of yellow lupine. In contrast, the activity of dehydrogenases and catalase in sandy loam was negatively correlated with yields of spring rape.

Interactions of enzymes and plants with physicochemical properties of soil

The activity of enzymes was not univocally correlated with certain physicochemical properties of soil (Table 6). In loamy sand under all the tested crops, the activity of dehydrogensases, catalase and urease was positively correlated with pH and negatively with hydrolytic acidity, but in sandy loam, a significant positive correlation was found only between soil pH and urease in soil cropped with yellow lupine. Literature (Wang et al. 2006, Zaborowska et al. 2006) suggests that in general there is positive correlation between enzymes and pH of soil, as observed in loamy sand.

The activity of catalase in sandy loam under oats and urease in soil under yellow lupine was positively correlated with hydrolytic acidity. An opposite correlation was determined between the activity of dehydrogenases, catalase and urease in loamy sand and in sandy loam, versus the content of organic carbon and total nitrogen or the base cations saturation. The content of C_{org} and N_{total} was negatively correlated with enzymes in sandy loam sown with spring rape and yellow lupine, while being positively correlated with enzymes in loamy sand under any of the tested crops. Furthermore, the degree of soil's saturation with base cations was positively correlated with the activity of enzymes in loamy sand, but in sandy loam, the correlation was univocally negative, not only in treatments with oat, as suggested by the negative values of TEB and CEC.

The physicochemical properties significantly shape the activity of soil enzymes (Hinojosa et al. 2004, Kucharski et al. 2011, Wyszkowska et al. 2008), but differences in the correlations between the activity of dehydrogenases, catalase and urease versus some soil properties of loamy sand and sandy loam could be a result of the degree of buffering of these soils, a claim

Table 6

Pearson's correlation coefficients between enzymatic activity and plants yield and physicochemical properties of soil

Variable	Kind of plant								
	oat			spring rape			yellow lupine		
	Deh	Cat	Ure	Deh	Cat	Ure	Deh	Cat	Ure
Loamy sand									
Plants yield	-0.675*	-0.245	-0.771**	0.931**	0.942**	0.967**	0.963**	0.984**	0.968**
$C_{organic}$	-0.885**	-0.571*	-0.920**	-0.150	-0.556*	-0.408	-0.967**	-0.855**	-0.951**
N_{total}	-0.854**	-0.603*	-0.850**	-0.199	-0.536	-0.417	-0.967**	-0.913**	-0.973**
pH	0.984**	0.771**	0.999**	0.913**	0.962**	0.969**	0.999**	0.936**	0.989**
HAC	-0.972**	-0.961**	-0.900**	-0.990**	-0.904**	-0.965**	-0.987**	-0.898**	-0.975**
TEB	0.311	0.711**	0.098	-0.134	0.233	0.085	-0.426	-0.143	-0.342
CEC	-0.011	0.449	-0.228	-0.285	0.091	-0.064	-0.662**	-0.411	-0.592*
BS	0.920**	0.988**	0.815**	0.795**	0.902**	0.881**	0.986**	0.957**	0.994**
Sandy loam									
Plants yield	0.803**	0.499	0.928**	-0.787**	-0.899**	-0.217	0.944**	0.986**	0.634*
$C_{organic}$	0.801**	0.599*	0.608*	0.878**	0.642*	0.849**	0.966**	0.913**	0.937**
N_{total}	0.899**	0.931**	0.768**	0.750**	0.810**	0.897**	0.959**	0.898**	0.932**
pH	0.306	-0.071	0.530	-0.120	0.204	-0.597*	0.401	0.318	0.828**
HAC	0.336	0.697**	0.451	-0.171	-0.041	0.475	0.069	-0.094	0.564**
TEB	-0.986**	-0.727**	-0.934**	-0.112	-0.495	0.049	-0.531	-0.618*	0.036
CEC	-0.985**	-0.724**	-0.936**	-0.105	-0.486	-0.008	-0.490	-0.592*	0.085
BS	-0.967**	-0.766**	-0.860**	-0.136	-0.303	0.562*	0.257	0.410	-0.276

Explatnations: Deh – dehydrogenases, Cat – catalase, Ure – urease, HAC – hydrolytic acidity, TEB – sum of exchangeable bases Ca^{++}, Mg^{++}, K^{+}, and Na^{+}, CEC – cation exchange capacity, BS – base saturation;

* significant for P=0.05, ** significant for P=0.01, n=12

supported by the fact that the resistance of dehydrogenses and urease to zinc contamination was higher in sandy loam, that is in soil with higher sorption capacity, than in loam sand, characterized by lower sorption. Of certain importance are the interferences caused by zinc with the growth of the examined plant species, which are manifested by amounts of root excretions produced by plants, inducing a certain level of biochemical activity of soil (Dijkstra et al. 2006, Perez-de-Mora et al. 2006, Renella et al. 2005, Tejada et al. 2008).

CONCLUSIONS

1. Soil contamination with zinc within the range of doses 150 mg to 600 mg kg^{-1} of soil significantly inhibits the activity of dehydrogenases, urease and catalase. With respect to their sensitivity to zinc, the analyzed enzymes can be ranked as follows: dehydrogenases > urease > catalase.

2. Dehydrogenases were most resistant to the negative influence of zinc in soil under oat; urease – in soil cropped with spring rape, and catalase – in soil sown with yellow lupine.

3. Dehydrogenases and urease were more resistant to the effect of zinc in sandy loam than in loamy sand, in contrast to catalase, which was more resistant in loamy sand than in sandy loam.

4. Tolerance of plants to zinc contamination of soil is a species-specific trait. Among the analyzed plants, yellow lupine is the most sensitive to the presence of zinc in soil, while oat seems to be most tolerant to this contaminant.

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