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ORIGINAL PAPER

CHANGES IN THE MICROBIOLOGICAL AND BIOCHEMICAL PROPERTIES OF SOIL CONTAMINATED WITH ZINC*

Jadwiga Wyszkowska[1], Edyta Boros-Lajszner[1], Agata Borowik[1], Jan Kucharski[1], Małgorzata Baćmaga[1], Monika Tomkiel[1]

[1]Department of Microbiology
University of Warmia and Mazury in Olsztyn

Abstract

Zinc is an essential element for all living organisms, but overexposure to this element can have strongly toxic effects. A pot experiment was carried out to evaluate the influence of different zinc concentrations (0, 100, 300, 600, 1200, 2400, 4800 mg Zn^{2+} kg^{-1} of soil) on the soil biological activity by analyzing changes in soil stability over time and by determining the resistance (RS) of microorganisms and soil enzyme activity. The influence of Zn^{2+} on the growth and development of oat and white mustard was evaluated. Overexposure to zinc inhibited the growth of soil microorganisms, the activity of soil enzymes, and the growth and development of plants. Excessive zinc doses cause lower microbial biodiversity and enzyme activity. Bacteria of the genus *Azotobacter* were most sensitive and spore-forming oligotrophic bacteria were least sensitive to excessive zinc doses. *β*-glucosidase was most resistant and arylsulfatase was least resistant to the analyzed element. The resistance of the tested microorganisms and enzymes decreased with an increase in zinc accumulation in the soil environment. White mustard was more sensitive to zinc contamination than oat and zinc doses of 2400 and 4800 mg Zn^{2+} kg^{-1} led to the death of white mustard plants. The results of this study indicate that soil contamination with zinc poses a threat for living organisms. In areas with a higher risk of zinc deposition, the content of this element in soils should be monitored more frequently than prescribed by environmental protection regulations.

Keywords: zinc, soil enzymes, microbes resistance, plants resistance.

prof. dr hab. Jadwiga Wyszkowska, Department of Microbiology, University of Warmia and Mazury in Olsztyn, Plac Łódzki 3, 10-727 Olsztyn, Poland, e-mail: jadwiga.wyszkowska@uwm.edu.pl

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INTRODUCTION

Zinc is an essential biogenic element for all living organisms, and it is found in 300 identified enzymes belonging to six classes. The ubiquitous nature of zinc can be attributed to its specific characteristics, including its tetrahedral coordination geometry, acidity and the fact that zinc does not have redox potential and is not hydrolyzed at low pH (Gapys et al. 2014). Overexposure to zinc in the immediate vicinity of human and animal habitats can lead to poisoning, metabolic disorders and carcinogenic changes (McCall et al. 2000). Zinc modifies the soil microbiome because soil microorganisms are the first to respond to the inhibitory effects of zinc and environmental changes. The analyzed element decreases microbial abundance and biodiversity, which lowers the quality of the environment (Sekler et al. 2007, Wyszkowska et al. 2015). The influence of zinc on soil-dwelling microorganisms is determined by its dose and the microbial species or strains exposed to this element. Some microorganisms are highly sensitive to zinc, whereas others become resistant, which leads to succession. Microbial resistance to zinc can be conditioned by cellular systems which promote the removal of this element, bioaccumulation or enzymatic transformation of zinc to less toxic forms (Ashraf, Adamali 2007, Shen et al. 2015).

Soil contamination with zinc can also disrupt the activity of enzymes responsible for biomass decomposition and nutrient cycling (e.g. P, N, S, C) (Rahmansyah et al. 2009, Saha et al. 2012, Igbinosa 2015). Biochemical activity is a robust indicator of soil fertility and quality. Enzyme activity is a measure of microbial metabolism (Igbinosa 2015, Sethi, Gupta 2014). Enzyme activity levels are monitored to determine changes induced by heavy metals in the pedosphere. This is an important consideration because heavy metals are not biodegraded regardless of their toxicity. They have a long half-life, and they tend to accumulate inside living organisms (Behbahaninia et al. 2009). Plants are also sensitive indicators of changes induced by zinc in the soil environment (Chibuike, Obiora 2014, Wyszkowski, Modrzewska 2015). Overexposure to zinc can negatively affect plant growth and decrease yield.

The negative effects of zinc accumulation in the soil environment require effective remedy measures, in particular in an era of rapid industrialization and urbanization. In this study, the influence of zinc on the biological activity of soil was examined by analyzing changes in soil stability over time and by determining the resistance (RS) of microorganisms and soil enzymes. The influence of Zn^{2+} on the growth and development of oat and white mustard was evaluated. The resulting parameters can be used in biological monitoring programs in degraded areas.

MATERIALS AND METHODS

Experimental design

Soil samples were collected from the humus horizon (depth of 0-20 cm). They had the textural composition of sandy loam (sand fraction – 72%, silt fraction – 21%, clay fraction – 7%) and were classified as Eutric Cambisol based on the World Reference Base of Soil Resources (2014).

After the determination of the physicochemical properties of soil, a pot experiment was conducted under controlled conditions, in a greenhouse of the University of Warmia and Mazury in Olsztyn (north-eastern Poland). 3.5 dm^3 polyethylene pots were filled with 3 kg samples of air-dry soil (sandy loam) in five replications. Before the experiment, soil was passed through a 5 mm mesh sieve and contaminated with the following doses of zinc: 0, 100, 300, 600, 1200, 2400, 4800 mg Zn^{2+} kg^{-1} of soil, applied in the form of an aqueous solution of zinc chloride ($ZnCl_2$). The experimental plants were oat (*Avena sativa* L.) cv. Chwat and white mustard (*Sinapis alba*) cv. Rota. Detailed soil characteristics and conditions of the experiment are given in the work Wyszkowska et al. (2016).

Laboratory analyses

The numbers of bacteria *Azotobacter* were determined on the medium described by Fenglerowa (1965), oligotrophic bacteria and spore forming oligotrophic bacteria were determined on the Onta and Hattori (1983) medium. The size of microbial populations was determined with the use of a colony counter. Oligotrophic bacteria and spore-forming oligotrophic bacteria were incubated at a temp. of 28°C for 21 days, and *Azotobacter* bacteria – for 3 days. Enzyme activity was determined by the following methods: acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) – by the method of Alef et al. (1998), arylsulfatase (EC 3.1.6.1) and *β*-glucosidase (EC 3.2.1.21) – according to the procedure described by Alef and Nannipieri (1998). The detailed procedure for analyzing of enzyme activity was described by Wyszkowska et al. (2013*a*). Enzymatic activity was expressed in mM *p*-nitrophenol (PNP) kg^{-1} soil DM h^{-1}. Both microbiological and biochemical analyses were performed on two dates: 25 and 50 day study period.

Based on the determined microbial counts (bacteria of the genus *Azotobacter*, oligotrophic bacteria and spore-forming oligotrophic bacteria), enzyme activity (acid phosphatase, alkaline phosphatase, *β*-glucosidase and arylsulfatase) and crop yield (oat and white mustard), soil resistance to zinc (Orwin, Wardle 2004).

Statistics

The results were analyzed statistically in the Statistica 12.5 program (StatSoft, Inc., 2015). Homogeneous groups were identified by the Tukey's

range test at a significance level of $p = 0.05$. Factors specified percentage of observed variance (η^2), principal component analysis (PCA) and coefficients of linear correlation between the zinc dose vs. microbial counts, enzyme activity, crop yield and the physicochemical properties of soil were calculated.

RESULTS

Soil-dwelling microorganisms

Microbial counts were most correlated with zinc dose: bacteria of the genus *Azotobacter* – in 75%, oligotrophic bacteria – in 63%, and spore-forming oligotrophic bacteria – in 57% (Table 1). In soil containing 100 mg Zn^{2+} kg^{-1},

Table 1

Percentage of observed variability factors η^2

Variable factors	Microorganisms*			Enzymes**			
	Az	Olig	Olig/p	Pac	Pal	Glu	Aryl
Dose Zn^{2+}	74.907	62.537	57.230	54.005	93.565	68.944	83.368
Plant species	3.362	2.574	5.429	6.031	1.233	10.973	1.472
Soil incubation time	0.127	20.849	3.102	22.593	0.026	2.141	5.584
Dose Zn^{2+} · Plant species	10.996	2.213	1.548	4.776	2.488	7.932	2.337
Dose Zn^{2+} · Soil incubation time	4.207	7.442	7.053	4.379	1.251	3.586	5.175
Plant species · Soil incubation time	0.882	2.228	18.415	2.727	0.229	1.908	0.203
Dose Zn^{2+} · Plant species · Soil incubation time	4.060	2.030	3.757	5.460	1.191	4.408	1.013

* Az – *Azotobacter* spp., Olig – oligotrophic bacteria, Olig/p – spore-forming oligotrophic bacteria,
** Pac – acid phosphatase, Pal – alkaline phosphatase, Glu – β-glucosidase, Aryl – arylsulfatase

the RS values of *Azotobacter* and spore-forming oligotrophic bacteria were higher in pots sown with white mustard, and oligotrophic bacteria – in soil sown with oat (Table 2). The negative effects of zinc on microorganisms were exacerbated in treatments exposed to 300 do 4800 mg Zn^{2+} kg^{-1} DM soil. The resistance of *Azotobacter*, oligotrophic bacteria and spore-forming oligotrophic bacteria was significantly negatively correlated with zinc dose. Bacteria of the genus *Azotobacter* were most sensitive to zinc contamination, and their resistance decreased already in response to a zinc dose of 300 mg Zn^{2+} kg^{-1} DM soil. In pots sown with white mustard, *Azotobacter* bacteria were not determined in soil on day 50. Oligotrophic bacteria and spore-forming oligotrophic bacteria were also sensitive to high zinc doses. Regardless of the time of zinc accumulation in soil, doses of 300 mg Zn^{2+} kg^{-1} DM soil decreased RS values by 35% in spore-forming oligotrophic bacteria and by 15% in oligotrophic bacteria on average relative to the treatment with the lowest zinc dose

Table 2

Microbial resistance index (RS) to soil contamination with zinc

Dose Zn^{2+} (mg kg^{-1} soil)	Plant species			
	oat		white mustard	
	soil incubation time, days			
	25	50	25	50
Azotobacter				
100	0.690^{a}	0.902^{a}	0.992^{a}	0.800^{a}
300	0.465^{b}	0.269^{b}	0.341^{b}	0.000^{b}
600	0.000^{c}	0.000^{c}	0.000^{c}	0.000^{b}
1200	0.000^{c}	0.000^{c}	0.000^{c}	0.000^{b}
2400	0.000^{c}	0.000^{c}	0.000^{c}	0.000^{b}
4800	0.000^{c}	0.000^{c}	0.000^{c}	0.000^{b}
$\bar{x}$	0.193	0.195	0.222	0.133
r^{*}	-0.802*	-0.781*	-0.795*	-0.655*
Oligotrophic bacteria				
100	0.887^{a}	0.734^{a}	0.926^{a}	0.371^{a}
300	0.881^{a}	0.502^{b}	0.460^{b}	0.330^{b}
600	0.483^{b}	0.327^{c}	0.314^{c}	0.296^{c}
1200	0.418^{b}	0.166^{d}	0.193^{d}	0.233^{d}
2400	0.281^{c}	0.127^{e}	0.130^{d}	0.192^{e}
4800	0.137^{d}	0.123^{e}	0.038^{e}	0.162^{f}
$\bar{x}$	0.515	0.330	0.344	0.264
r^{*}	-0.968*	-0.941*	-0.923*	-0.992*
Spore-forming oligotrophic bacteria				
100	0.823^{a}	0.806^{a}	0.714^{a}	0.972^{a}
300	0.733^{a}	0.322^{b}	0.626^{b}	0.528^{b}
600	0.477^{b}	0.253^{bc}	0.612^{b}	0.514^{b}
1200	0.334^{bc}	0.241^{bc}	0.542^{c}	0.408^{bc}
2400	0.234^{bc}	0.148^{bc}	0.531^{c}	0.303^{c}
4800	0.218^{c}	0.079^{c}	0.288^{d}	0.089^{d}
$\bar{x}$	0.470	0.308	0.552	0.469
r^{*}	-0.942*	-0.846*	-0.717*	-0.934*

Identical letters in columns denote homogeneous groups, separately for different microbial groups.

* r – coefficient of correlation significant at $p = 0.05$, $n = 17$.

(100 mg Zn^{2+} kg^{-1}). In pots sown with white mustard, the RS values of oligotrophic bacteria and spore-forming oligotrophic bacteria decreased by 39% and 32%, respectively. Higher zinc doses had a stronger inhibitory effect on

microbial proliferation. In oat treatments and in pots sown with white mustard, a zinc dose 4800 mg Zn^{2+} kg^{-1} DM soil decreased RS values by 80% in spore-forming oligotrophic bacteria and by 84% in oligotrophic bacteria. The influence of zinc on microorganisms and enzymes was determined by PCA. The distribution of variance between the first two principle components is presented in Figure 1. Microbial abundance and the activity of all enzymes

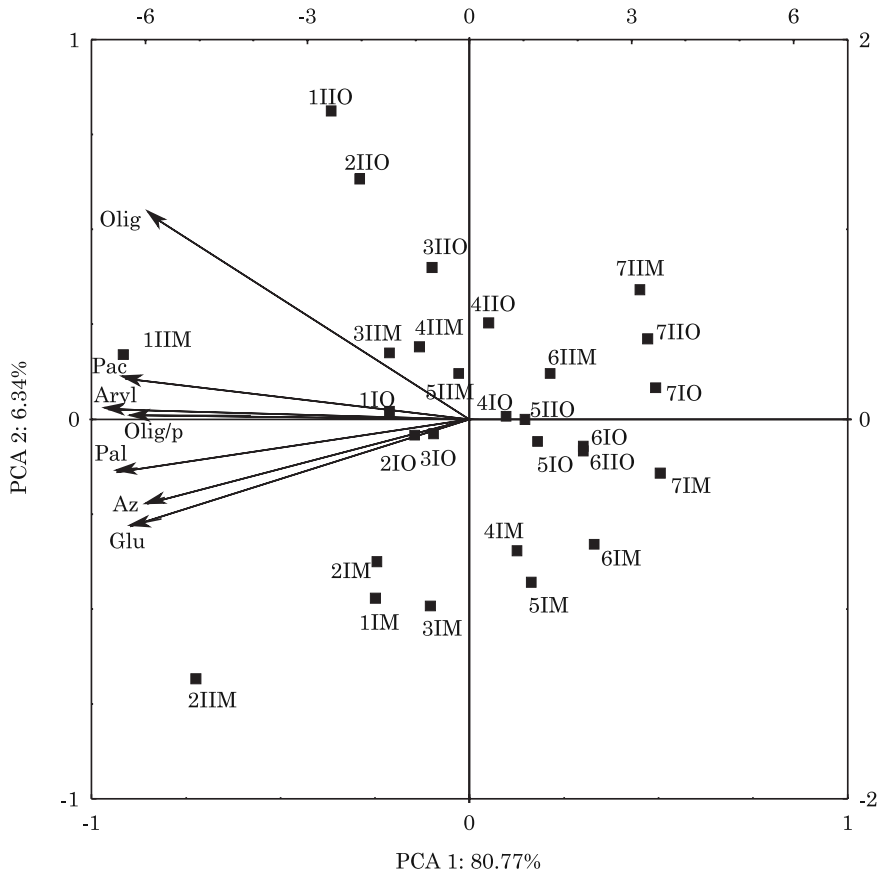

Fig. 1. Principal component analysis of microbial abundance and enzyme activity in soil contaminated with zinc. Dose Zn^{2+} (mg kg^{-1} of soil): 1 – 0, 2 –100, 3 – 300, 4 – 600, 5 – 1200, 6 – 2400, 7 – 4800. Soil incubation time: I – 25 days, II – 50 days. Plant species: O – oat; M – white mustard. Soil microorganisms: Az – *Azotobacter* spp., Olig – oligotrophic bacteria, Olig/p – spore-forming oligotrophic bacteria. Soil enzymes: Pac – acid phosphatase, Pal – alkaline phosphatase, Glu – β-glucosidase, Aryl – arylsulfatase

were negatively correlated with the first principal component, which explained 81% of variance. Zinc doses of 300 to 4800 Zn^{2+} kg^{-1} inhibited the biological activity of soil measured by microbial counts and enzyme activity, which is demonstrated by the distribution of variables in the plane. The position of vectors describing microbial abundance and enzyme activity relative to the

cases representing the highest contamination levels also points to the negative influence of zinc overexposure on the soil microbiome. The inhibitory effect of zinc on the microbiological activity of soil was observed throughout the experiment in both oat and white mustard treatments. These results indicate that microbial abundance and enzyme activity are determined by environmental and anthropological factors as well as by the type of the analyzed microorganisms and enzymes.

Soil enzymes

Similarly to microbial abundance, the activity of soil enzymes was most highly influenced by the applied zinc dose: acid phosphatase – in 54%, alkaline phosphatase – in 94%, β-glucosidase – in 69%, and arylsulfatase – in 83% (Table 1). The RS values of all enzymes were significantly negatively correlated with zinc dose (Table 3). Regardless of the date of analysis, the average resistance of β-glucosidase, acid phosphatase and alkaline phosphatase was higher in pots sown with oat than in white mustard treatments. The RS values of the tested enzymes decreased already in response to a dose of 300 mg Zn^{2+} kg^{-1} DM soil, and the noted decrease was exacerbated with a further increase in zinc dose. In oat treatments exposed to the highest zinc dose (4800 mg Zn^{2+} kg^{-1} DM soil), the average resistance of arylsulfatase was lowered by 93%, alkaline phosphatase – by 82%, β-glucosidase – by 73% and acid phosphatase – by 71% relative to samples exposed to the lowest zinc dose (100 mg Zn^{2+} kg^{-1} DM soil), regardless of date (Table 3). In pots sown with white mustard, exposure to 4800 mg Zn^{2+} kg^{-1} DM soil lowered the RS values of arylsulfatase by 97%, alkaline phosphatase – by 88%, β-glucosidase – by 81%, and acid phosphatase – by 59%. The negative effect of zinc on all enzymes persisted throughout the experiment. Its inhibitory influence was exacerbated rather than reduced with the time of deposition in soil. The adverse effect of zinc on the activity of acid phosphatase was minimized only in soil sown with oat on day 50 relative to day 25. Irrespective of the period of zinc accumulation and soil and crop species, the tested enzymes were arranged in the following order (from most to least sensitive): arylsulfatase > alkaline phosphatase > acid phosphatase > β-glucosidase. The coefficients of correlation between zinc dose vs. microbial counts, enzyme activity, crop yield and the physicochemical properties of soil confirm the negative impact of zinc on the biological activity of soil (Tables 4, 5). In oat treatments, zinc dose was significantly negatively correlated with microbial abundance and enzyme activity (Table 4).

Evaluation of plant growth and development

Oat yield was significantly positively correlated with microbial abundance, enzyme activity and physicochemical properties of soil, excluding hydrolytic acidity, total nitrogen content, available zinc and total zinc content (Table 5). In treatments sown with white mustard, a significant negative

Table 3

Enzyme resistance index (RS) to soil contamination with zinc

Dose Zn^{2+} (mg kg^{-1} soil)	Plant species			
	oat		white mustard	
	soil incubation time, days			
	25	50	25	50
Acid phosphatase				
100	0.763^{a}	0.956^{a}	0.353^{d}	0.596^{a}
300	0.660^{b}	0.875^{b}	0.681^{a}	0.492^{b}
600	0.556^{c}	0.852^{b}	0.624^{ab}	0.466^{c}
1200	0.503^{d}	0.756^{c}	0.599^{c}	0.426^{d}
2400	0.300^{e}	0.428^{d}	0.341^{d}	0.185^{e}
4800	0.189^{f}	0.315^{e}	0.253^{e}	0.137^{f}
$\bar{x}$	0.495	0.697	0.475	0.384
r^{*}	-0.987*	-0.945*	-0.455	-0.956*
Alkaline phosphatase				
100	0.638^{b}	0.974^{a}	0.882^{a}	0.858^{a}
300	0.981^{a}	0.798^{b}	0.756^{b}	0.418^{b}
600	0.621^{b}	0.525^{c}	0.430^{c}	0.412^{b}
1200	0.604^{b}	0.461^{d}	0.377^{d}	0.385^{c}
2400	0.289^{c}	0.243^{e}	0.235^{e}	0.170^{d}
4800	0.160^{d}	0.134^{f}	0.130^{f}	0.077^{e}
$\bar{x}$	0.549	0.523	0.468	0.387
r^{*}	-0.825*	-0.990*	-0.977*	-0.923*
β-glucosidase				
100	0.879^{a}	0.820^{b}	0.960^{a}	0.962^{a}
300	0.868^{a}	0.972^{a}	0.968^{a}	0.587^{b}
600	0.869^{a}	0.972^{a}	0.848^{b}	0.486^{c}
1200	0.842^{a}	0.768^{bc}	0.831^{b}	0.410^{d}
2400	0.679^{b}	0.743^{c}	0.543^{c}	0.321^{e}
4800	0.203^{c}	0.263^{d}	0.224^{d}	0.139^{f}
$\bar{x}$	0.723	0.756	0.729	0.484
r^{*}	-0.795*	-0.751*	-0.911*	-0.954*
Arylsulfatase				
100	0.602^{a}	0.897^{a}	0.774^{a}	0.947^{a}
300	0.569^{a}	0.584^{b}	0.663^{a}	0.474^{b}
600	0.526^{a}	0.345^{c}	0.384^{b}	0.314^{c}
1200	0.221^{b}	0.295^{cd}	0.352^{b}	0.211^{d}
2400	0.279^{b}	0.139^{de}	0.099^{c}	0.121^{e}
4800	0.064^{b}	0.042^{e}	0.018^{c}	0.034^{f}
$\bar{x}$	0.377	0.384	0.382	0.350
r^{*}	-0.916*	-0.965*	-0.975*	-0.924*

Identical letters in columns denote homogeneous groups, separately for different enzymes.
* r – coefficient of correlation significant at $p = 0.05$, $n = 17$.

Table 4

Coefficients of correlation between variables in soil sown with oat

Variable factors	Az	Olig	Olig/p	Pac	Pal	Glu	Aryl
Dose	-0.904*	-0.991*	-0.986*	-0.955*	-0.955*	-0.758*	-0.997*
Yield	0.667	0.876*	0.837*	0.960*	0.934*	0.882*	0.916*
Az	-	0.940*	0.950*	0.755*	0.831*	0.478	0.896*
Olig	0.940*	-	0.987*	0.917*	0.940*	0.695	0.986*
Olig/p	0.950*	0.987*	-	0.907*	0.928*	0.654	0.979*
Pac	0.755*	0.917*	0.907*	-	0.962*	0.859*	0.953*
Pal	0.831*	0.940*	0.928*	0.962*	-	0.797*	0.957*
Glu	0.478	0.695	0.654	0.859*	0.797*	-	0.789*
Aryl	0.896*	0.986*	0.979*	0.953*	0.957*	0.789*	-
HAC	-0.721	-0.857*	-0.853*	-0.917*	-0.894*	-0.928*	-0.928*
EBC	0.689	0.859*	0.837*	0.960*	0.926*	0.959*	0.924*
pH	0.817*	0.890*	0.926*	0.912*	0.893*	0.793*	0.943*
C_{org}	0.788*	0.785*	0.843*	0.744	0.661	0.500	0.793*
CEC	0.680	0.855*	0.830*	0.963*	0.927*	0.960*	0.919*
BS	0.569	0.749	0.734	0.885*	0.845*	0.979*	0.843*
N_{total}	-0.500	-0.617	-0.572	-0.746	-0.699	-0.671	-0.625
Zn_{av}	-0.752	-0.893*	-0.885*	-0.977*	-0.967*	-0.906*	-0.945*
Zn_{total}	-0.751	-0.894*	-0.885*	-0.980*	-0.969*	-0.905*	-0.945*

Az – *Azotobacter* spp., Olig – oligotrophic bacteria, Olig/p – spore-forming oligotrophic bacteria, Pac – acid phosphatase, Pal – alkaline phosphatase, Glu – *β*-glucosidase, Aryl – arylsulfatase, HAC – hydrolytic acidity, EBC – sum of exchangeable cations, CEC – cations exchange capacity, BS – base saturation, C_{org} – organic carbon content, N_{total} – total nitrogen content, Zn_{av} – available zinc content, Zn_{total} – total zinc content,* r – coefficient of correlation significant at: $p = 0.05$, $n = 6$.

correlation was noted between yield vs. hydrolytic acidity, total nitrogen content, available zinc and total zinc content. White mustard yield was significantly positively correlated with microbial counts, enzyme activity and the remaining physicochemical parameters of soil.

A decrease in the RS values of oat and white mustard also points to the adverse effects exerted by zinc on the tested crops (Figure 2). The coefficients of correlation between RS values and zinc dose were determined at $r = -0.909$ for oat and $r = -0.881$ for white mustard. Oat plants were more resistant to zinc overdose because doses of 100 mg to 600 mg Zn^{2+} kg^{-1} did not lead to a significant reduction in RS values which remained fairly constant throughout the experiment. The RS values of oat decreased by 81% and 99% only under exposure to zinc doses of 1200 and 4800 mg Zn^{2+} kg^{-1}, respectively, relative to the maximum permissible levels of zinc in soil (100 mg Zn^{2+} kg^{-1} DM soil).

Table 5

Coefficients of correlation between variables in soil sown with white mustard

Variable factors	Az	Olig	Olig/p	Pac	Pal	Glu	Aryl
Dose	-0.841*	-0.949*	-0.967*	-0.983*	-0.990*	-0.959*	-0.993*
Yield	0.996*	0.900*	0.855*	0.848*	0.887*	0.789*	0.899*
Az	-	0.871*	0.817*	0.805*	0.849*	0.745	0.862*
Olig	0.871*	-	0.910*	0.897*	0.927*	0.858*	0.925*
Olig/p	0.817*	0.910*	-	0.954*	0.977*	0.984*	0.965*
Pac	0.805*	0.897*	0.954*	-	0.993*	0.968*	0.991*
Pal	0.849*	0.927*	0.977*	0.993*	-	0.973*	0.996*
Glu	0.745	0.858*	0.984*	0.968*	0.973*	-	0.963*
Aryl	0.862*	0.925*	0.965*	0.991*	0.996*	0.963*	-
HAC	-0.676	-0.816*	-0.966*	-0.886*	-0.909*	-0.971*	-0.893*
EBC	0.602	0.790*	0.942*	0.916*	0.914*	0.978*	0.899*
pH	0.831*	0.901*	0.989*	0.918*	0.953*	0.959*	0.934*
C_{org}	0.804*	0.933*	0.810*	0.772*	0.816*	0.734	0.790*
CEC	0.586	0.782*	0.933*	0.916*	0.911*	0.975*	0.895*
BS	0.510	0.689	0.899*	0.818*	0.829*	0.933*	0.804*
N_{total}	-0.527	-0.572	-0.412	-0.606	-0.572	-0.458	-0.592
Zn_{av}	-0.644	-0.795*	-0.953*	-0.942*	-0.938*	-0.989*	-0.922*
Zn_{total}	-0.593	-0.745	-0.934*	-0.910*	-0.906*	-0.977*	-0.888*

Explanations see Table 4

White mustard was more sensitive to higher contamination with zinc. This was already visible in treatments exposed to 300 mg Zn^{2+} kg^{-1} DM soil, and negative effects of zinc were exacerbated with an increase in its dose. Contamination with the highest zinc doses (2400 and 4800 mg Zn^{2+} kg^{-1}) led to the death of white mustard plants.

DISCUSSION

Soil-dwelling microorganisms

The activity of soil-dwelling microorganisms was determined by zinc dose, crop species and the period of zinc accumulation in soil. Microbial and enzymatic responses to heavy metals are dictated mainly by the level of contamination (Hercer et al. 2016), cell growth and the specific characteristics of microorganisms (Igbinosa 2015). In most cases, the toxic effects of heavy metals, including zinc, generally include growth inhibition and a decrease in

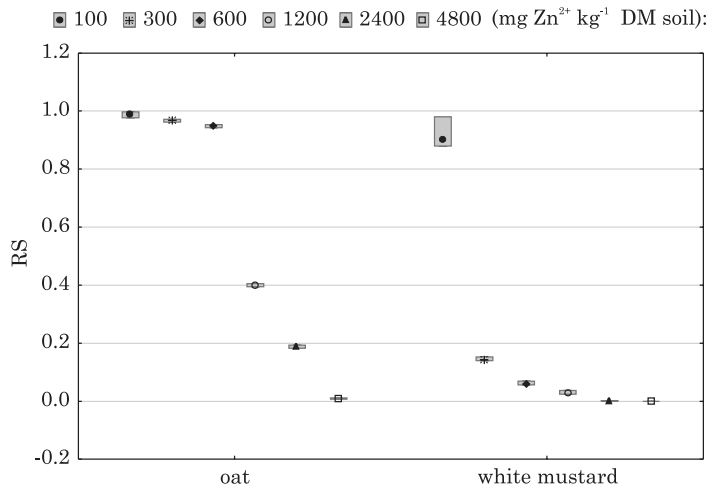

Fig. 2. Plant resistance (RS) to soil contamination with zinc

microbial counts, microbial biomass and microbial activity. The above changes disrupt the balance of the soil ecosystem (Wyszkowska et al. 2013*a*).

In this study, soil contamination with zinc doses of 300 do 4800 mg of Zn^{2+} kg^{-1} of soil inhibited the proliferation and development of *Azotobacter*, oligotrophic and spore-forming oligotrophic bacteria. It should be noted, however, that microorganisms possess high and specific adaptability to adverse environmental conditions. Bacteria of the genus *Azotobacter* were most sensitive to zinc, and they are generally regarded as robust indicators of soil contamination with heavy metals (Borowik et al. 2014). Our findings are consistent with the results of Wang et al. (2010), who observed that Gram-negative bacteria, including *Azotobacter*, were more sensitive to heavy metal pollution than Gram-positive bacteria. In the present study, spore-forming oligotrophic bacteria, most of which are gram-positive, were most resistant to zinc, which is in agreement with the cited authors' findings.

A stimulating effect of moderate zinc doses on microbial populations has been described in the literature (Kucharski et al. 2011, Wyszkowska et al. 2013*b*). In a study by Kucharski et al. (2011), a zinc dose of 300 mg Zn^{2+} kg^{-1} had a positive influence on the growth of organotrophic bacteria, oligotrophic bacteria, actinomyces and fungi. Those observations indicate that microorganisms have highly varied responses to soil contamination with heavy metals, including zinc. Some soil microbes are highly sensitive to toxic substances, whereas others are more resistant. Species-specific variations and the morphological properties of microorganisms have been documented (Zaborowska et al. 2006). Microbial responses were also probably influenced by the accumulation of root secretions in the rhizosphere of oat and white mustard treatments. Root secretions contain glucose, glutamic acid, citric acid and oxalic acid (Renella et al. 2006), which affect microbial activity and

diversity. In this study, the rhizosphere of oat created a more supportive environment for the growth of *Azotobacter* and spore-forming oligotrophic bacteria, whereas the rhizosphere of white mustard was more conducive to the development of oligotrophic bacteria. This can probably be attributed to variations in the chemical composition of the root secretions of the tested plants. Roots secrete carbohydrates, organic acids, amino acids, enzymes and flavones, but also glucosides, saponins and hydrocyanic acid which are toxic for microorganisms. For this reason, crop plants can have stimulating but also toxic effects on soil-dwelling microbes (Zhang et al. 2014).

Soil enzymes

Contamination of the soil ecosystem with zinc also influenced the activity of soil enzymes which responded differently to zinc pollution. According to many authors (Cang et al. 2009, Wang et al. 2010, Sivakumar et al. 2012, Wyszkowska et al. 2013*b*), soil enzymes are reliable indicators of changes in soil under exposure to heavy metals. Castaldi et al. (2009) observed that zinc stimulated alkaline phosphatase and invertase whose activity was positively correlated with zinc dose (300 mg Zn^{2+} kg^{-1}). Their results could be explained by the fact that zinc has a less toxic impact on biochemical parameters in soils with higher organic matter content (Moreno et al. 2009, Boros et al. 2011, Wyszkowska et al. 2013*b*). Phosphatase activity in the soil ecosystem is a measure of the decomposition potential of organic phosphorus compounds and the biological activity of the pedosphere. The activity of those biocatalysts is closely correlated with the organic matter content, total nitrogen content and organic phosphorus content of soil (Tripathy et al. 2014). Studies evaluating the activity of acid phosphatase and alkaline phosphatase demonstrated that zinc can inhibit those enzymes (Hinojosa et al. 2004, Moreno et al. 2009). In some reports, zinc overexposure also lowered the activity of other enzymes, including arylsulfatase (Hinojosa et al. 2004, Mikanova 2006) and β-glucosidase (Castaldi et al. 2009, Moreno et al. 2009). In the present experiment, the activity of the tested enzymes was inhibited by zinc. McCall et al. (2000), Kucharski et al. (2011) and Wyszkowska et al. (2013*b*) also reported close correlations between soil contamination with zinc and the activity of most soil enzymes. In this study, enzyme activity was inhibited in response to abiotic stress induced by zinc. The RS values of acid phosphatase, alkaline phosphatase, β-glucosidase and arylsulfatase decreased with an increase in zinc dose.

In this study, the species of crop plants also influenced the activity of soil enzymes. The tested enzymes were characterized by higher activity levels in treatments sown with oat than white mustard. These observations can be attributed to the phytosanitary properties of white mustard plants and the anti-pathogenic properties of its root secretions which contain organic compounds such as avenacin (Girotto et al. 2013). The mineralization and decomposition of organic matter in soil leads to the accumulation of or-

ganic compounds that can be harmful for microorganisms and can induce changes in the activity of soil enzymes (Zhang et al. 2014).

Evaluation of plant growth and development

Our findings illustrate the devastating consequnces of excessive amounts of zinc, mnifesting themselves as a decrease in the yield of crops. The characteristic symptoms of disruptions in biological mechanisms in the tested crops also included deformation of the root system and leaf chlorosis. Those symptoms were particularly severe in white mustard plants, which could be attributed to differences in zinc uptake and transport from roots to aerial parts. Dicotyledons, including white mustard, are more sensitive to heavy metal pollution because large amounts of toxic elements are transported from roots to aerial parts in initial stages of plant development. Resistance to zinc overexposure is also conditioned by the size of the root system and the ratio between the weight of roots and aerial parts (Wyszkowski, Modrzewska 2015). Resistance can also be determined by plants' sensitivity to zinc, which is associated with processes that limit zinc uptake and transport, and detoxification processes in cell membranes and inside cells (Kabata-Pendias 2004). Higher zinc concentrations inside cells promote the synthesis of low-molecular-weight proteins known as phytochelatins which are characterized by high content of cysteine and glutathione. Phytochelatins are responsible for stabilizing zinc levels in cells.

Plant species resistant to soil contamination with zinc are characterized by specific morphological, anatomical and biochemical structure, which enables them to accumulate and neutralize this element. Plants can also limit their zinc uptake by changing the selective permeability of the cell membrane and excreting zinc from cells (Chibuike, Obiora 2014).

CONCLUSIONS

Zinc overexposure disrupts the soil balance and leads to changes in the biological activity of soil. Excessive zinc doses lower microbial biodiversity and enzyme activity. In the present study, the resistance of the tested microorganisms and enzymes decreased with an increase in zinc accumulation in the soil environment. The inhibitory effects of zinc were observed throughout the experiment in treatments sown with white mustard and oat. White mustard was more sensitive to contamination than oat, and zinc doses of 2400 and 4800 mg Zn^{2+} kg^{-1} led to the death of white mustard plants. The results of this study indicate that the mechanisms responsible for harmful effects of zinc on soil-dwelling microorganisms are complex and require further research, including microbiological, physiological and biochemical analyses.

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