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ORIGINAL PAPER

MICROBIOME OF SOIL CONTAMINATED WITH ZINC*

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Abstract

The accumulation of heavy metals in the environment can contribute to soil homeostasis disturbance. However, their adverse effect can be reduced by introducing neutralizing substances to soil. Therefore, an experiment was carried out to determine the effect of varied doses of zinc on the microbiological and enzymatic properties of soil. Additionally, the neutralizing effect of liming applied to zinc-contaminated soil was evaluated with regard to the microbiological and biochemical properties of the soil. Loamy sand of pH_{KCl} 5.6 was used as the soil material. Calcium carbonate was applied in doses neutralizing the hydrolytic acidity of soil, which amounted to 0, 1 and 2 HAC, respectively. Next, zinc ($ZnCl_2$) was introduced to soil, in the amounts of 0, 250, 500, 750, 1000 and 1250 mg kg^{-1} DM of soil. In weeks 2 and 20 of the experiment, the counts of microorganisms (organotrophic bacteria, copiotrophic bacteria, oligotrophic bacteria, actinomycetes and fungi) and activity of enzymes (dehydrogenases, catalase, urease, acid phosphatase, alkaline phosphatase and β-glucosidase) were determined in soil samples. Based on observations of the proliferation of microorganisms, changes in the diversity of the soil microbiome exposed to zinc were evaluated with the use of the colony development (CD) index and ecophysiological biodiversity (EP) index. Excessive amounts of zinc demonstrated a negative effect on the biological parameters of soil. It brought about a reduction in the count of microorganisms, although the highest resistance to zinc was demonstrated by copiotrophic bacteria and fungi. Additionally, this element revealed an inhibitory effect on the activity of soil enzymes and colony development index, as well as on the ecophysiological biodiversity (EP) index of microorganisms. The addition of calcium carbonate to the soil neutralized the negative effects of zinc on its microbiological and biochemical properties.

Keywords: zinc, liming, soil enzymes, soil microbiome, microbial diversity.

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INTRODUCTION

Upsetting the equilibrium between individual elements of environment can lead to disturbances in the functioning of the entire ecosystem. Soil is a very important element of the environment for its use by humans. Therefore, the highest emphasis is placed on soil fertility, which determines the yields obtained. In turn, soil productivity is conditioned by the biological processes occurring in it (Vrščaj et al. 2008). Soil microorganisms form a living, integral part of each soil environment. Processes occurring in soil with the participation of its microbiome can be disturbed by the toxic effect of xenobiotics, including heavy metals, on living organisms (Knopf, König 2010). Kucharski et al. (2011) and Wyszkowska et al. (2016) demonstrated the adverse effect of zinc on biological soil parameters, such as the count of microorganisms and their biodiversity and biochemical processes occurring in soil.

Another important factor determining the effect of heavy metals on soil functions is their availability in soil, which is closely related to the physicochemical properties of the soil (Modrzewska, Wyszkowski 2014). Transformation of the biologically inassimilable form to the assimilable one can be related to some disturbance of the physicochemical properties of soil, i.e. pH, cation exchange capacity, share of organic matter and silty minerals, content of Fe, Al, Mn oxides and redox potential (Aydinalp, Marinova 2003). Therefore, the alkaline reaction of soils and a high content of carbonates result in decreasing mobility of heavy metals, as a result of which they do not pose a direct threat to ecosystems affected by their presence (Navarro et al. 2008). A practical application of this fact is soil liming, which significantly decreases the bioassimilability of heavy metals (including zinc) in soil (Garau et al. 2007).

The aim of the research was to determine the relations occurring between the content of zinc in soil and microbiological and biochemical properties of soil. Additionally, the effectiveness of soil supplementation with calcium carbonate in mitigating the negative effects of zinc on the soil microbiome and enzyme activity was evaluated.

MATERIAL AND METHODS

The laboratory experiment was conducted in three replications. The soil material used in the experiment was loamy sand (Eutric Cambisols) collected from the uppermost humus horizon (0-20 cm) at the Educational and Research Station in Tomaszkowo (north-eastern Poland, 53.71610 N, 20.41670 E). The characteristics of the soil used in the experiment are presented in Table 1. Glass beakers were filled with 100 g air-dried soil material of <2 mm in the graining diameter. Before setting up the experiment, a liming dose was established based on the soil hydrolytic acidity. Calcium

Table 1

Physicochemical properties of soil

Parameter	Unit	Value
Soil texture <0.002 0.002-0.05 0.05-2.00	(%)	 1.490 17.98 80.56
pH	-	5.600
Total organic carbon	(g kg^{-1} DM)	10.00
Total nitrogen		0.580
Available cations P K Mg	(mg kg^{-1} DM)	 96.32 179.1 50.17
Total exchangeable cations K^+ Ca^{2+} Na^+ Mg^{2+}	(mg kg^{-1} DM)	 217.7 568.6 100.3 64.52
Hydrolytic acidity (HAC)	(mM(+) kg^{-1} DM)	18.66
Total exchangeable bases (TEB)		40.00
Cation exchange capacity (CEC)		58.66
Base saturation (BS)	(%)	68.19
Total zinc	(mg kg^{-1} DM)	22.68
Available zinc		9.130

carbonate was added to the soil in an amount neutralizing the hydrolytic acidity: 0 Hh, 1 Hh, 2 Hh. Next, zinc was applied to the samples in the form of $ZnCl_2$ in the following doses: 0, 250, 500, 750, 1000 and 1250 mg Zn^{2+} kg^{-1} soil. Samples were thoroughly homogenized. Incubation was conducted at 25°C for 2 and 20 weeks, keeping the humidity of soil at 50% capillary water capacity. After the set incubation time, the number of the following groups of microbes was determined: organotrophic bacteria (Bunt, Rovira 1955), oligotrophic and copiotrophic bacteria (Ohta, Hattori 1983), actinomycetes (Parkinson et al. 1971) and fungi (Martin 1950). The activity of selected enzymes: dehydrogenase, catalase, urease, acid phosphatase, alkaline phosphatase, β-glucosidase was also tested. The detailed procedure for determining the activity of the examined enzymes was discussed in Borowik et al. (2014). Additionally, physicochemical analyses of soil were performed, such as measuring the granulometric composition of soil with a laser particle size analyser Mastersizer 2000, pH, hydrolytic acidity, sum of exchangeable base cations, organic carbon content and total nitrogen content according to the method described in Carter (1993), the content of exchangeable cations (K^+, Na^+, Ca^{2+}, Mg^{2+}) according to the methodology reported by Harris (2006) and the total zinc content and the content of assimilable zinc using atomic absorption spectroscopy (FAAS).

The results were statistically analysed using Statistica 12 software (StatSoft, Inc. 2014). The percentage of factors contributing to the observed η^2 variability was determined for dependent variables and independent variables. Data concerning the activity of enzymes and counts of soil microorganisms were analysed using the method of multivariate principal component analysis (PCA). Based on the count of microorganisms (organotrophic bacteria, actinomycetes and fungi), the colony development (CD) index was determined (Sarathchandra et al. 1997), as well as the ecophysiological biodiversity (EP) index (De Leij et al. 1993).

RESULTS AND DISCUSSION

The results indicate that zinc significantly contributed to upsetting the microbiological equilibrium of soil (Table 2). A particularly highly significant effect of a zinc dose was recorded with regard to the count of fungi (in 81.92%). The count of other groups of microbes depended to a higher degree on the soil incubation time – from 26.34% (actinomycetes) to 60.15% (organotrophic bacteria). Soil liming demonstrated the highest effect on the number of organotrophic bacteria (in 11.59%) and actinomycetes (in 15.55%).

The counts of the groups of soil microorganisms examined in the study are visualized in Figure 1 according to the principal component analysis

Table 2

Percentage share of factors for observable variability η^2

Factors	Parameters										
	Org	Cop	Olig	Act	Fun	Deh	Cat	Ure	Pac	Pal	Glu
Dose of Zn	7.829	22.13	23.92	15.08	81.92	76.08	70.00	41.05	47.35	92.15	55.23
Dose of $CaCO_3$	11.59	0.146	5.689	15.55	0.799	2.284	1.319	4.721	32.13	2.158	0.434
Incubation time	60.15	47.81	28.52	26.34	1.092	3.730	10.88	6.954	1.567	1.264	0.858
Dose of Zn · dose of $CaCO_3$	6.163	10.48	12.60	7.497	5.379	2.352	1.693	13.38	8.526	2.892	4.097
Dose of Zn · incubation time	6.687	5.662	11.90	14.35	5.538	5.418	10.34	3.853	0.340	0.463	3.048
Dose of $CaCO_3$ · incubation time	1.169	1.182	1.835	11.32	2.008	2.295	2.533	6.890	2.827	0.185	30.32
Dose of Zn · dose of $CaCO_3$ · incubation time	5.338	8.736	12.80	9.416	2.949	7.142	1.158	21.37	5.723	0.819	5.136
Error	1.074	3.854	2.736	0.447	0.315	0.699	2.077	1.782	1.537	0.069	0.877

Zn – zinc, Org – organotrophic bacteria, Cop – copiotrophic bacteria, Olig – oligotrophic bacteria, Act – actinomycetes, Fun – fungi, Deh – dehydrogenases, Cat – catalase, Ure – urease, Pac – acid phosphatase, Pal – alkaline phosphatase, Glu – β-glucosidase

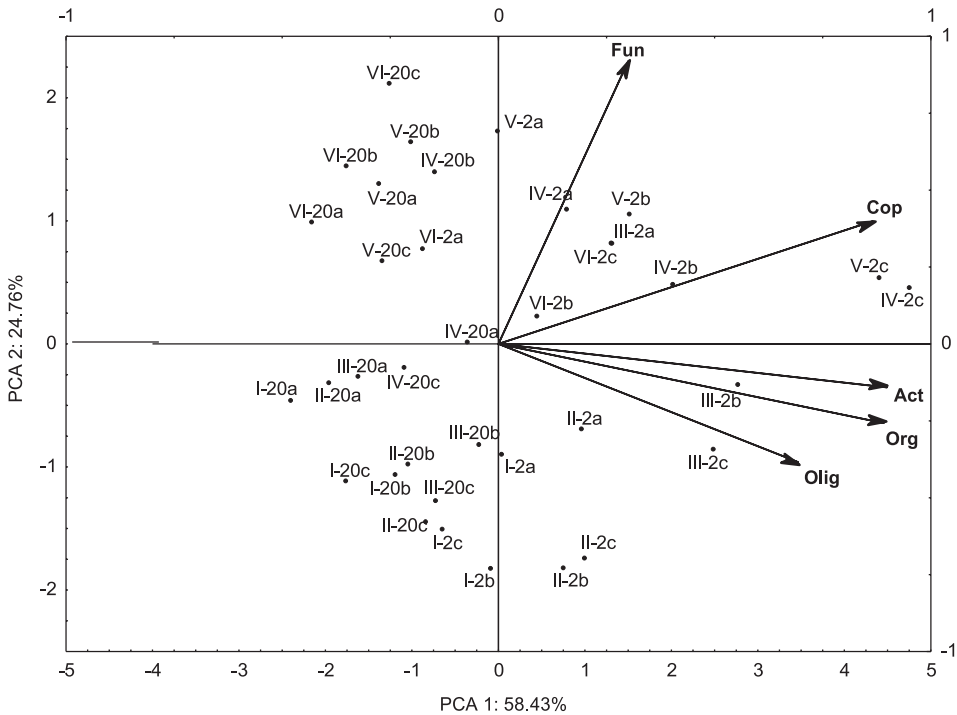

Fig. 1. Counts of selected groups of microorganisms in zinc-contaminated soil supplemented with calcium carbonate (PCA). Abbreviations of microbiological parameters described under Table 2. Dose of zinc (mg Zn^{2+} kg^{-1} DM soil): I – 0, II – 250, III – 500, IV – 750, V – 1000, VI – 1250; dose of $CaCO_3$ (corresponding to HAC): a – 0, b – 1, c – 2; incubation time: 2 – two weeks, 20 – twenty weeks

(PCA). Three groups of microorganisms (organotrophic bacteria, oligotrophic bacteria and actinomycetes) were stimulated by zinc, but the highest contamination of soil with this element (1250 mg Zn^{2+} kg DM of soil) had an inhibitory effect on their development. Zinc in moderate amounts can contribute to the stimulation of soil microorganisms (Wyszkowska et al. 2013*a*). On the other hand, high doses of zinc, i.e. between 1200 and 2400 mg Zn^{2+} kg^{-1} DM of soil, lead to a reduced growth of soil microorganisms (Wyszkowska et al. 2016). In our own research, soil contamination with zinc (doses between 250 and 1250 mg Zn^{2+} mg kg^{-1} DM of soil) did not demonstrate a negative effect on the development of copiotrophic bacteria or fungi.

The application of liming to soil, both uncontaminated and contaminated with zinc, resulted in the stimulation of the growth of organotrophic bacteria, oligotrophic bacteria and actinomycetes. The count of microorganisms decreased over the soil incubation time. As a result of changes occurring in the soil environment under the influence of zinc, soil microorganisms more resistant to this metal could emerge, eliminating more susceptible species (Wyszkowska et al. 2016).

The Pearson's correlation coefficients also confirm that soil contamination with zinc had a highly significant adverse effect on the count of the examined group of microorganisms (Table 3). The exception was organotrophic bacteria, in which this trend was unobserved. The number of organotrophic bacteria and actinomycetes was highly significantly positively correlated with the sum of exchangeable base cations, cation exchange capacity and base saturation, but was negatively correlated with hydrolytic acidity. The count of organotrophic bacteria was also positively correlated with pH of the soil.

Contamination of soil with zinc contributed to changes in the soil microbiome's biodiversity. The value of the colony development (CD) index of organotrophic bacteria decreased along with the zinc content in soil (Figure 2). Liming at the level of 1 HAC of soil contaminated only with 1000 and 1250 mg Zn^{2+} kg^{-1} DM of soil resulted in an increase in the value of this index, while a dose of $CaCO_3$ corresponding to 2 HAC mitigated the stress related to zinc in all variants of pollution. In addition, for actinomycetes, only a slight decrease in the CD index value was found, while the response of fungi was not evident. Liming only slightly changed the CD index for actinomycetes, while for fungi, liming of uncontaminated soil and soil contaminated with zinc in the amount of 250 and 500 mg Zn^{2+} kg^{-1} DM of soil contributed to reducing the rate of proliferation of this group of microorganisms. Zinc applied in excessive doses lowered the values of the ecophysiological biodiversity (EP) index of microbes (Figure 3). All groups of microorganisms under examination responded negatively to increased zinc doses, which was proven by declining values of the EP index. The most significant changes caused by the heavy metal content were found among fungi. The reaction of organotrophic bacteria and actinomycetes was less evident. Liming at the level of 2 HAC contributed to increasing the value of the ecophysiological biodiversity index of fungal diversity in the soil contaminated with zinc. The diversity of actinomycetes was also larger in samples with 1000 and 1250 mg Zn^{2+} kg^{-1} DM of soil subject to $CaCO_3$ at the levels of 1 and 2 HAC. Zinc can effect changes in the structure of microorganisms, which can also develop resistance to zinc. Zinc ions, eliminating susceptible microorganisms, can also support the development of microorganisms resistant to its presence in soil (Lock, Janssen 2005). Additionally, soil liming can lead to modifications in the soil microbiome structure (Garau et al. 2007) and the change of pH in this environment brings about a change in the living conditions of microorganisms (Geisseler, Scow 2014).

The activity of soil enzymes was influenced by all examined factors (Table 2). The dose of zinc changed the activity of enzymes ranging from 41.05% (urease) to 92.15% (alkaline phosphatase). The soil incubation time had the greatest effect on the activity of catalase (in 10.88%) and urease (in 6.95%), while liming affected most strongly the activity of acid phosphatase (in 32.13%).

The PCA analysis (Figure 4) shows that zinc demonstrated a strong inhibitory effect on soil enzyme activity. The highest activity of the tested en-

Table 3

Correlation coefficients between examined variables and enzyme activity, counts of microorganisms and soil physicochemical properties

Parameters	Zn	Org	Cop	Olig	Act	Fun	Deh	Cat	Ure	Pac	Pal	Glu	pH	HAC	TEB	CEC
Org	-0.611**															
Cop	0.631**	-0.419**														
Olig	0.001	0.041	0.005													
Act	-0.561**	0.449**	-0.412**	0.411**												
Fun	0.881**	-0.680**	0.739**	0.103	-0.586**											
Deh	-0.866**	0.656**	-0.605**	-0.188	0.388**	-0.774**										
Cat	-0.839**	0.727**	-0.580**	0.091	0.549**	-0.845**	0.800**									
Ure	-0.581**	0.032	-0.172	0.016	0.309*	-0.337*	0.245	0.277*								
Pac	-0.819**	0.410**	-0.457**	-0.102	0.359**	-0.712**	0.536**	0.541**	0.678**							
Pal	-0.506**	0.719**	-0.285*	0.392**	0.820**	-0.575**	0.393**	0.708**	0.141	0.273*						
Glu	-0.403**	0.513**	-0.199	0.476**	0.756**	-0.387**	0.218	0.400**	0.394**	0.230	0.787**					
pH	-0.133	0.653**	-0.032	0.098	0.268	-0.201	0.280*	0.507**	-0.205	-0.120	0.691**	0.393**				
HAC	0.203	-0.685**	0.031	-0.124	-0.351**	0.224	-0.334*	-0.521**	0.130	0.080	-0.739**	-0.479**	-0.978**			
TEB	-0.292*	0.741**	-0.151	0.108	0.342*	-0.345*	0.429**	0.636**	-0.132	0.003	0.746**	0.436**	0.982**	-0.971**		
CEC	-0.323*	0.752**	-0.196	0.100	0.333*	-0.388**	0.461**	0.671**	-0.130	0.037	0.737**	0.412**	0.968**	-0.944**	0.995**	
BS	-0.245	0.691**	-0.037	0.116	0.368**	-0.241	0.347*	0.523**	-0.052	-0.014	0.740**	0.508**	0.963**	-0.993**	0.959**	0.930**

Abbreviations of microbiological and biochemical parameters described under Table 2.
HAC – hydrolytic acidity, TEB – total exchangeable bases, CEC – cation exchange capacity, BS – base saturation (*p = 0.05, **p = 0.01; n = 53)

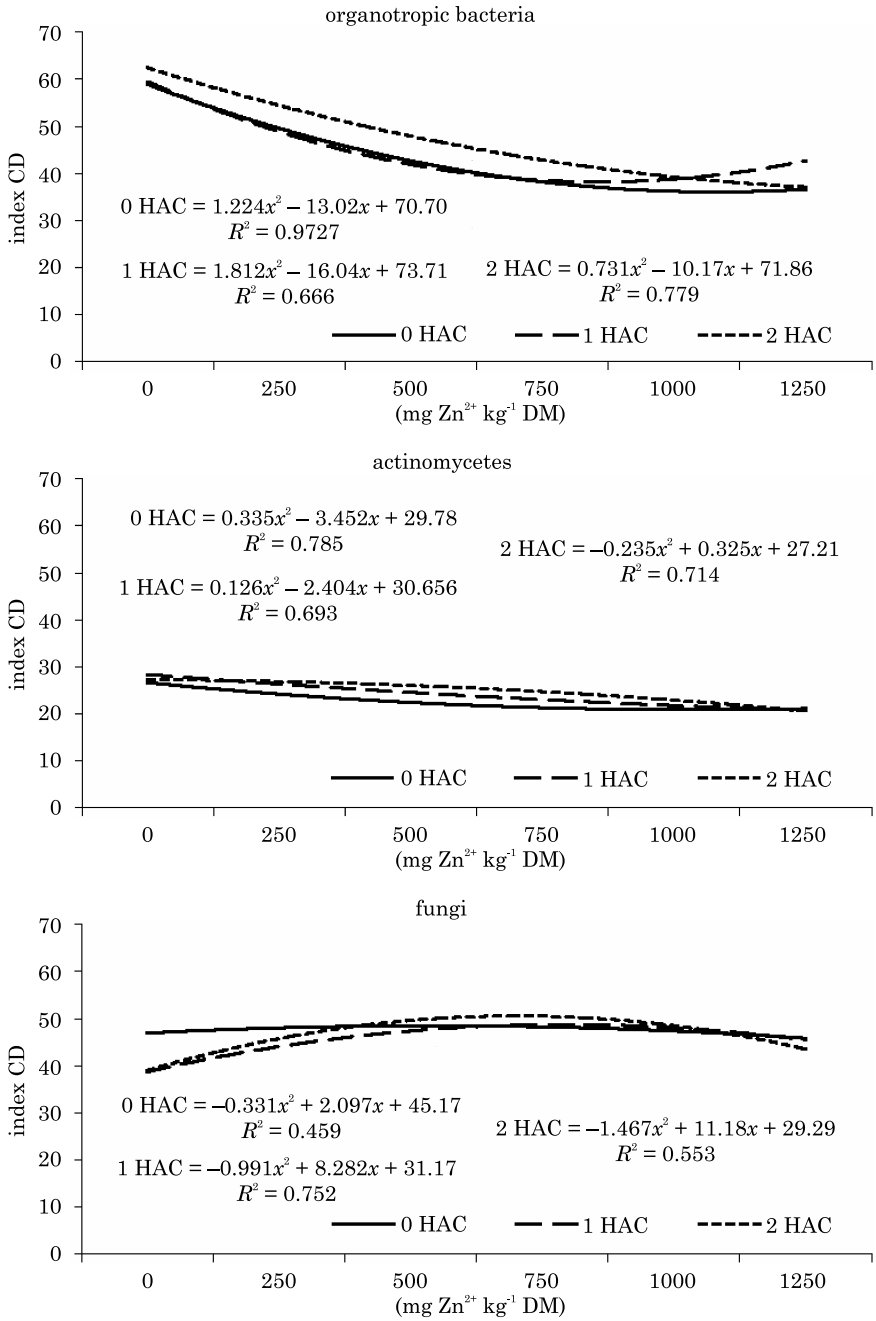

Fig 2. Colony development (CD) index for microorganisms in zinc-contaminated soil supplemented with calcium carbonate

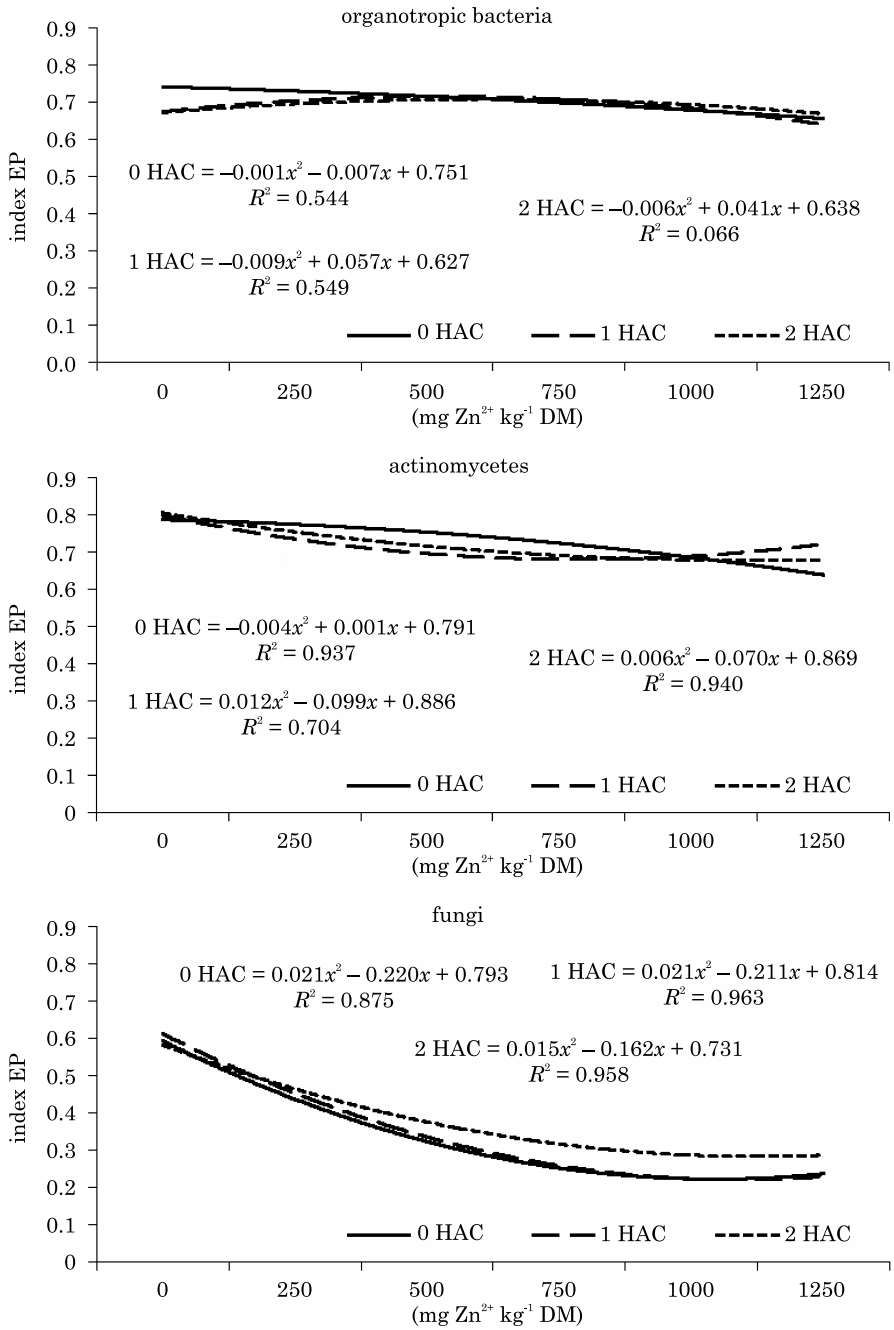

Fig. 3. Ecophysiological biodiversity (EP) index for microorganisms in zinc-contaminated soil supplemented with calcium carbonate

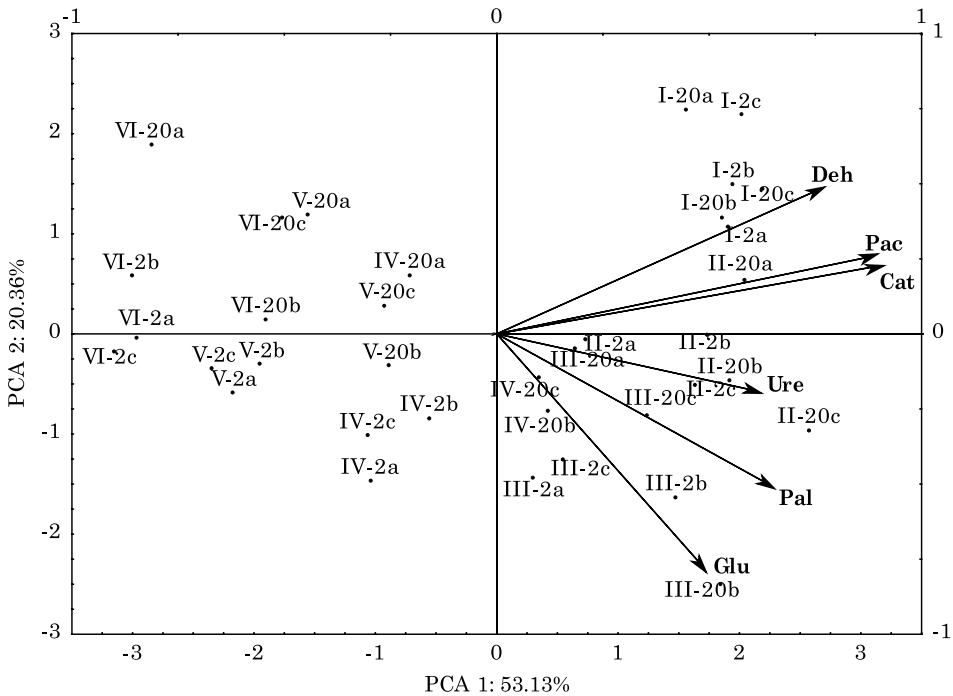

Fig. 4. Activity of selected enzymes in zinc-contaminated soil supplemented with calcium carbonate (PCA). Abbreviations of biochemical parameters described under Table 2. Dose of zinc (mg Zn^{2+} kg^{-1} DM soil): I – 0, II – 250, III – 500, IV – 750, V – 1000, VI – 1250; dose of $CaCO_3$ (corresponding to HAC): a – 0, b – 1, c – 2; incubation time: 2 – two weeks, 20 – twenty weeks

zymes was found in soil uncontaminated with zinc and subject to 250 and 500 mg Zn^{2+} kg^{-1} DM of soil. The projection of cases in relation to the position of vectors describing enzymes indicates a linear relationship with their activity, decreasing along with the growth of the zinc content in soil.

Numerous references (Chaperon, Sauvé 2007, Wyszkowska et al. 2013*b*, Borowik et al. 2014, Wyszkowska et al. 2016) have also confirmed the negative effect of zinc ions on soil enzyme activity. In a study carried out by Lee et al. (2009), soil respiration and the activity of urease and dehydrogenases were negatively correlated with the assimilable zinc content. In our research, dehydrogenases proved more susceptible to zinc, while *β*-glucosidase demonstrated the highest resistance. Additionally, the distribution of cases in relation to vectors justifies the conclusion that liming contributed to mitigating the stress due to to the presence of zinc in soil. Soil supplementation with calcium carbonate to the highest degree mitigated the toxic effect of zinc on the activity of dehydrogenases, catalase, urease and acid phosphatase. Garau et al. (2007) found that liming effects the stimulation of dehydrogenase and urease activity in comparison to control soil. Lee et al. (2009) also observed an increase in soil respiration and activity of dehydrogenases, urease and

phosphatase in soil subjected to liming. The activity of dehydrogenases, urease, acid phosphatase and *β*-glucosidase was the highest at week 20. This could have resulted from the rapid response and high susceptibility of these enzymes to the effects of excessive zinc doses at week 2 of the experiment. Another consequence could have been the emergence of microorganisms characterized by higher tolerance to excessive doses of the examined heavy metal.

The Pearson's correlation coefficients presented in Table 3 also indicate that soil contamination with zinc was negatively correlated with the activity of all tested enzymes. Additionally, the examined physicochemical parameters of soil were positively correlated with the activity of dehydrogenases, alkaline phosphatase and *β*-glucosidase. The only exception was hydrolytic activity, which was significantly negatively correlated with the activity of those enzymes.

The addition of calcium carbonate caused an increase of pH in soil (Figure 5). The highest growth of pH value was observed in samples to which

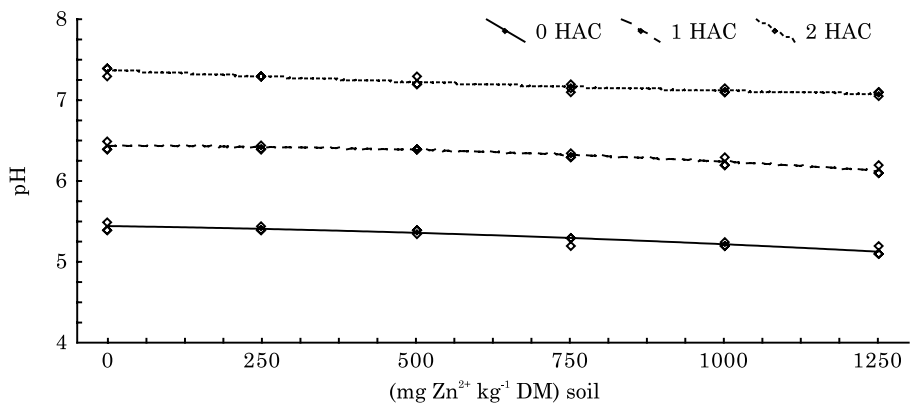

Fig. 5. Changes in pH of soil contaminated with zinc and supplemented with calcium carbonate

calcium carbonate was applied in the 2 HAC dose. Soil pH was positively correlated with the sum of exchangeable base cations, cation exchange capacity and base saturation, but was negatively correlated with hydrolytic acidity (Table 3). Basta, McGowen (2004) confirmed that calcium carbonate effects an increase in soil pH and a decrease of the amount of bioassimilable zinc. This could be caused by conditions conducive to the formation of carbonates with heavy metals. Since these are the conditions in which $ZnCO_3$ can be formed, by changing physicochemical properties of soil, liming influences the conditions for microbial growth and enzyme activity.

CONCLUSIONS

Contamination with zinc had an effect on the biological condition of soil. The current study demonstrated that an excessive amount of zinc in soil resulted in decreasing the count of microorganisms and caused changes in the microbiome's diversity. Additionally, zinc revealed an inhibitory effect on soil enzyme activity. Nevertheless, zinc applied to soil in the amounts of 250 to 500 mg Zn^{2+} kg^{-1} DM of soil stimulated the proliferation of microorganisms. Fungi demonstrated higher tolerance to the presence of zinc in soil, but zinc was responsible, to the highest degree, for a decrease in the ecophysiological diversity of this group of microorganisms. Soil liming contributed to the limitation and reduction in fungi diversity. Based on the results, it can be concluded that liming reduces stress related to the presence of zinc in soil and the activity of soil enzymes, while stimulating the growth of microorganisms.

REFERENCES

Aydinalp C., Marinova S. 2003. *Distribution and forms of heavy metals in some agricultural soils*. Pol. J. Environ. Stud., 12: 629-633.

Basta N.T., McGowen S.L. 2004. *Evaluation of chemical immobilization treatments for reducing heavy metal transport in a smelter-contaminated soil*. Environ. Pollut., 127(1): 73-82. DOI: 10.1016/S0269-7491(03)00250-1

Borowik A., Wyszkowska J., Kucharski J., Baćmaga M., Boros-Lajszner E., Tomkiel M. 2014. *Sensitivity of soil enzymes to excessive zinc concentrations*. J. Elem., 19(3): 637-648. DOI: 10.5601/jelem.2014.19.2.456

Bunt J.S., Rovira A.D. 1955. *Microbiological studies of some subantarctic soils*. J. Soil Sci., 6: 119-128. DOI: 10.1111/j.1365-2389.1955.tb00836.x

Carter M.R. 1993. *Soil sampling and methods of analysis*. Can. Soc. Soil Sci., Lewis Publishers, London.

Chaperon S., Sauvé S. 2007. *Toxicity interaction of metals (Ag, Cu, Hg, Zn) to urease and dehydrogenase activities in soils*. Soil Biol. Biochem., 39(9): 2329-2338. DOI: 10.1016/j.soilbio.2007.04.004

De Leij F.A.A.M., Whipps J.M., Lynch J.M. 1993. *The use of colony development for the characterization of bacterial communities in soil and on roots*. Microb. Ecol., 27: 81-97. DOI: 10.1007/BF00170116

Garau G., Castaldi P., Santona L., Deiana P., Melis P. 2007. *Influence of red mud, zeolite and lime on heavy metal immobilization, culturable heterotrophic microbial populations and enzyme activities in a contaminated soil*. Geoderma, 142(1-2): 47-57. DOI: 10.1016/j.geoderma.2007.07.011

Geisseler D., Scow K.M. 2014. *Long-term effects of mineral fertilizers on soil microorganisms – A review*. Soil Biol. Biochem., 75: 54-63. DOI: 10.1016/j.soilbio. 2014.03.023

Harris D.C. 2006. *Quantitative chemical analysis*. Michelson Laboratory Chine Lake USA. WH Freeman and Company 7th ed., 1008.

Knopf B., König H. 2010. *Biomethylation of heavy metals in soil and terrestrial invertebrates*. In: *Soil heavy metals*. Sherameti I., Varma A. (eds.) Springer, Berlin/Heidelberg.

Kucharski J., Wieczorek K., Wyszkowska J. 2011. *Changes in the enzymatic activity in sandy loam soil exposed to zinc pressure*. J. Elem., 16(4): 577-589. DOI: 10.5601/jelem. 2011.16.4.07

Lee S.H., Lee J.S, Choi Y.J., Kim J.G. 2009. *In situ stabilization of cadmium, lead, and zinc*

contaminated soil using various amendments. Chemosphere, 77(8): 1069-1075. DOI: 10.1016/j.chemosphere.2009.08.056

Lock K., Janssen C.R. 2005. *Influence of soil zinc concentrations on zinc sensitivity and functional diversity of microbial communities*. Environ. Pollut., 136(2): 275-281. DOI: 10.1016/j.envpol.2004.12.038

Martin J. 1950. *Use of acid rose bengal and streptomycin in the plate method for estimating soil fungi*. Soil Sci., 69: 215-233. DOI: 10.1097/00010694-195003000-00006

Modrzewska B., Wyszkowski M. 2014. *Trace metals content in soils along the State Road 51 (northeastern Poland)*. Environ. Monit. Assess, 186(4): 2589-2597. DOI: 10.1007/s10661--013-3562-z

Navarro M.C., Pérez-Sirvent C., Martínez-Sánchez M.J., Vidal J., Tovar P.J., Bech J. 2008. *Abandoned mine sites as a source of contamination by heavy metals: A case study in a semi--arid zone*. J. Geochem. Explor., 96(2-3): 183-193. DOI: 10.1016/j.gexplo. 2007.04.011

Ohta H., Hattori T. 1983. *Oligotrophic bacteria on organic debris and plant roots in a paddy field*. Soil Biol. Biochem., 1: 1-8. DOI: 10.1016/0038-0717(83)90111-6

Parkinson D., Gray F.R.G., Williams S.T. 1971. *Methods for studying the ecology of soil microorganisms*. Blackweel Scientific Publications Oxford and Einburg, IBP Handbook, 19: 116 pp.

Sarathchandra S.U., Burch G., Cox N.R. 1997. *Growth patterns of bacterial communities in the rhizoplane and rhizosphere in white clover* (*Trifolium repens* L.) *and perennial ryegrass (Lolium perenne* L.*) in long-term pasture*. Appl. Soil Ecol., 6: 293-299. DOI: 10.1016/S0929--1393(97)00015-2

StatSoft Inc. 2014. *Statistica (data analysis software system), version 12.0*. Available at www.statsoft.com.

Vrščaj B., Poggio L., Ajmone-Marsan F. 2008. *A method for soil environmental quality evaluation for management and planning in urban areas*. Landscape Urban Plan., 88(2-4): 81-94. DOI: 10.1016/j.landurbplan.2008.08.005

Wyszkowska J., Borowik A., Kucharski M., Kucharski J. 2013*a*. *Effect of cadmium, copper and zinc on plants, soil microorganisms and soil enzymes*. J. Elem., 18(4): 769-796. DOI: 10.5601/jelem.2013.18.4.455

Wyszkowska J., Borowik A., Kucharski J., Baćmaga M., Tomkiek M., Boros-Lajszner E. 2013*b*. *The effect of organic fertilizers on the biochemical properties of soil contaminated with zinc*. Plant Soil Environ., 59(11): 500-504.

Wyszkowska J., Boros-Lajszner E., Borowik A., Baćmaga M., Kucharski J., Tomkiel M. 2016. *Implication of zinc excess on soil health*. J. Environ. Sci. Healt B, 51(5): 261-270. DOI: 10.1080/10934529.2015.1128726