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## **MICROBIOLOGICAL ACTIVITY OF ZINC-CONTAMINATED SOILS**

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### **INTRODUCTION**

The ecological effects of increased accumulation of heavy metals on natural environment have received much attention recently. This problem is encountered not only in industrialized regions, but also in farmlands as a result of the application of mineral fertilizers, crop protection chemicals, composts and sewage used for soil amendment (TERELAK et al. 2000). Due to the increasing significance of zinc in electrotechnology and many other branches of industry (ANDRZEJAK 2001), there is a justified fear that the emission of this metal to the environment may increase.

The increase in heavy metal concentrations in the natural environment (soil, water and air) poses a serious threat to normal growth and development of all living organisms. Soil holds a special position among the components of the biosphere. Numerous polluting substances, including heavy metals, accumulate in soil, which performs the role of a protective filter preventing their further penetration to waters as well as the migration of volatile compounds. Zinc present in excessive quantities becomes a destructive factor, having a toxic effect on humans, animals, plants and microorganisms, as well as an inhibitory effect on soil enzymes (WELP 1999, NOWAK et al. 2000a, NOWAK et al. 2000b, LANDI et al. 2000, WYSZKOWSKA 2002, WYSZKOWSKA, KUCHARSKI 2004).

## ZINC IN THE SOIL ENVIRONMENT

The concentrations of elements found in soil are dependent, to a high degree, on the granulometric composition, type and kind of soil (KOWALKOWSKI, BUSZEWSKI 2002). Podzolic soils developed from sand, light loam and heavy loam contain on average to 30 mg Zn·kg<sup>-1</sup>, 60 mg Zn·kg<sup>-1</sup> and 80 mg Zn·kg<sup>-1</sup>, respectively. Globally, mean zinc concentrations in soil range from 30 to 235 mg·kg<sup>-1</sup> (KABATA-PENDIAS, PENDIAS 1999). Compact loamy, alluvial, silty and loess soils, heavy soils on flysh as well as soils formed on pre-Quaternary carbonate and sulfide rocks are rich in zinc (KABATA-PENDIAS, PENDIAS 1999, TERELAK et al. 2000). The parent rock has a significant effect on the zinc content of soil. Zinc concentrations are usually higher in soils formed on alkaline rocks, compared with soils developed from granite. In brown acid soils the zinc content decreases with depth (SZERSZEŃ et al. 1991).

Soil holds a special position among the components of the biosphere. Numerous polluting substances, including heavy metals, accumulate in soil. The elements that penetrate into soil may undergo sorption by the mineral-organic sorption complex, or biological accumulation. In alkaline soils heavy metal ions may precipitate to form insoluble compounds (FOTYMA, MERCIK 1995). Another group of processes that increase the mobility of elements include desorption, solubility and mineralization of organic compounds (DUBE et al. 2001).

Zinc ions form mobile mineral and organic-minerals bonds in soil (TERELAK et al. 2000), so zinc concentrations in ferruginous and manganese concretions may reach even 2500 mg·kg<sup>-1</sup> and 5500 mg·kg<sup>-1</sup> respectively (KABATA-PENDIAS, PENDIAS 1999). Zinc is characterized by high migratory activity in soil, but is less able than other elements to form stable complexes with organic compounds. This is confirmed by its position in the bioactivity series of soil elements (BARABASZ et al. 1998): according to mobility: B > V > Zn > Ni > Cd > Mn > Cu > Cr > F > Pb, according to the stability of organic complexes: U > Hg > Pb > Cu > Ni > Co > Cd > Zn > Mn > Cr.

The ability of soil to bind heavy metal cations is affected by a variety of physicochemical and biological factors, primarily by soil reaction (BÄATH 1989, KOWALKOWSKI, BUSZEWSKI 2002), oxidation-reduction potential, cation exchange capacity and humus content (ZHANG et al. 1997, KABATA-PENDIAS, PENDIAS 1999).

Zinc solubility is inversely proportional to the presence of organic compounds in soil (ANDRZEJEWSKI, DOREGOWSKA 1986). Fulvic acids exhibit a high sorption capacity for metals. The stability of bonds increases along with an increase in the soil pH, and can be ordered as follows (MERCIK, KUBIK 1995): Fe<sup>3+</sup> > Al<sup>3+</sup> > Cu<sup>2+</sup> > Ni<sup>2+</sup> > Co<sup>2+</sup> > Pb<sup>2+</sup> = Ca<sup>2+</sup> > Zn<sup>2+</sup> > Mn<sup>2+</sup> > Mg<sup>2+</sup>.

In the process of cation binding in soil an important role is also played by clay minerals. BADURA et al. (1984) demonstrated that clay particles which usually have a negative electric charge attract great numbers of cations. Illite (Hydromi-

ca) and kaolinite (Kandite) bind cations according to the following order:  $Pb^{2+} > Cu^{2+} > Zn^{2+} > Ca^{2+} > Cd^{2+} > Mg^{2+}$  and  $Pb^{2+} > Ca^{2+} > Cu^{2+} > Mg^{2+} > Zn^{2+} > Cd^{2+}$ , respectively.

Like other elements, zinc forms three types of speciations in the soil solution: single  $Zn^{2+}$  ions, complexes with inorganic ligands, e.g.  $ZnCl^+$ ,  $ZnOH^+$ , as well as complexes with soluble organic matter (KABATA-PENDIAS, PENDIAS 1999). KABATA-PENDIAS and PENDIAS (1999) reported that the zinc content of arable soils increases gradually at a rate of  $1.5 \text{ mg} \cdot \text{kg}^{-1}$  per  $10^{-1}$  years.

## IMPACT OF ZINC ON SOIL MICROORGANISMS

Zinc is indispensable for the metabolism and growth of microorganisms. Just like iron, copper or cobalt ions, zinc is required in order to maintain ion concentration gradients on both sides of the cytoplasmatic membrane as well as to preserve the stiffness of its structure (STOHS, BAGCHI 1995). In addition, zinc participates in the synthesis of nucleic acids and in the aggregation of ribosomes (CHMIELOWSKI, KŁAPCIŃSKA 1984).

The mechanisms of heavy metal uptake in soil may be divided into three groups:

- 1) related to the specific transport of metal ions with the participation of binding proteins and carriers located in the membrane (CHMIELOWSKI, KŁAPCIŃSKA 1984, BINET et al. 2003),
- 2) related to the synthesis and release of chelating compounds that bind and transport dissolved ions in the environment (CHMIELOWSKI, KŁAPCIŃSKA 1984),
- 3) related to non-specific metal accumulation, i.e. to ion sorption within surface mucus and binding to biopolymers of the cell membrane (CHMIELOWSKI, KŁAPCIŃSKA 1984, LEDIN 2000).

CorA metal ion transporters (MIT) contribute to the non-specific transport of ions, including zinc. The MgtE transport system, found in some Gram-positive and Gram-negative bacteria, also belongs to this family. Substrate-specific transport of  $Zn^{2+}$  ions from the outside to the inside of a cell by RND proteins is also responsible for the outflow of  $Zn^{2+}$ ,  $Cd^{2+}$ ,  $Co^{2+}$  and  $Fe^{2+}$  with the participation of CDF proteins. In such a situation metals undergo mitochondrial detoxication (NIES 1999). If microorganisms do not produce special metabolites able to bind and inactivate a given metal, these processes lead to their death (BADURA 1984, BÄÄTH 1989).

Microorganisms have different defence mechanisms against heavy metals, such as methylation of mercury, tin, arsenic, selenium or tellurium ions which permits the formation of volatile methyl derivatives of metals (CHMIELOWSKI, KŁAPCIŃSKA 1984). Heavy metals tolerance is also dependent on the properties of the cytoplasmatic membrane containing porins – specific proteins that participate in acti-

ve transport (SKŁODOWSKA 2000), as well as on the presence of R plasmid and bacterial mucus – extracellular polymers increasing the viscosity in the environment (BADURA 1984). According to UNZ et al. (1996) and LEDIN (2000), one of the factors activating metal immobilization is an increased concentration of polyphosphates (zinc-binding polymers) in microbial cells.

The introduction of various forms of heavy metals to soil causes abiotic stress that impacts the growth, morphology and metabolism of soil microorganisms due to functional disorders, protein denaturation or cell membrane damage. In consequence, metals may reduce the quantity and activity of microbial biomass (BROOKES et al. 1984). PACHA and GALIMSKA-STYPA (1986) conducted tests on DNA repair ability in *Bacillus subtilis* using salts of cadmium, zinc, copper and lead. These authors found that only cadmium salts had mutagenic properties, while zinc and other metals did not cause DNA damage.

According to SKŁODOWSKA (2000), heavy metal toxicity may manifest in blocking the functional groups in enzymes, dislodging of metals indispensable for proper cell metabolism (cadmium replaces zinc, zinc replaces magnesium) as well as in inducing conformational changes in polymers.

Sensitivity of microorganisms to heavy metal contamination of the soil solution may vary widely (CHMIEŁOWSKI, KŁAPCZYŃSKA 1984, ROST et al. 2001). BARABASZ et al. (1997) reported that the different responses of microorganisms to particular metals resulted from differences in the composition of their metabolites, which formed stable deposits or chelates with metal ions. The influence of metal ions on microorganisms depends also on the kind and concentration of metal, and on the quantity of accompanying ions (CABRERO et al. 1997). NIES (1999) and BINET et al. (2003) demonstrated that zinc uptake by the cell membrane in *Escherichia coli* was possible thanks to the magnesium transport system. Zinc ions behaved along the same lines in *Klebsiella aerogenes* (BADURA 1984).

Many microorganisms, mostly fungi, show high resistance to heavy metals. In the experiment performed by WYSZKOWSKA and KUCHARSKI (2003a) microbial counts increased 2.5-fold in soil contaminated with these xenobiotics (Table 1). JASIEWICZ et al. (1997) did not observe any negative effect of elevated concentrations of zinc, cadmium and lead on the counts of soil fungi, either. In addition,  $Zn^{2+}$  ions had a positive impact on the increment in the biomass of *Fusarium culmorum* and *Fusarium avenaceum* (PRZE•DZIECKI et al. 1991). According to GALUS (1997), some metals (Al, Fe, Mo, Pb) stimulate while other (Cd, Co, Ni, Se) inhibit mycelium growth. SKŁODOWSKA (2000) stressed the role of specific mechanisms responsible for enhanced heavy metal resistance of fungi, as compared with bacteria. These mechanisms include enzymatic transformation of metal ions, formation of vacuoles in which these ions are immobilized in the form of polyphosphates, and increased melamine production.

MISHRA and CHAUDHURY (1996) proved that adsorption of  $Zn^{2+}$  ions by *Penicillium* sp. biomass is affected by various factors, such as soil pH, initial concen-

Table 1  
Tabela 1

Influence of heavy metals on number of microbes in soil (cfu·kg<sup>-1</sup>d.m.)  
Wpływ metali ciężkich na liczebność drobnoustrojów w glebie (jtk·kg<sup>-1</sup>s.m.)  
(WYSZKOWSKA, KUCHARSKI 2003a)

Groups of microorganisms Grupy drobnoustrojów	Kind of metal – Rodzaj metalu																			
	Cr(III)					Zn					Pb					Hg				
	0*	40	80	LSD NIR	0*	500	1000	LSD NIR	0*	500	1000	LSD NIR	0*	500	1000	LSD NIR	0*	10	20	LSD NIR
Oligotrophic bacteria 10 <sup>8</sup> Bakterie oligotroficzne 10 <sup>8</sup>	92.6	51.9	51.3	21.5	92.6	115.6	63.2	24.1	92.6	84.0	48.2	19.9	92.6	89.9	89.9	r.n.				
Copiotrophic bacteria 10 <sup>8</sup> Bakterie koptroficzne 10 <sup>8</sup>	95.7	51.9	49.4	18.4	95.7	85.0	49.3	23.9	95.7	62.2	57.7	16.7	95.7	94.4	89.9	r.n.				
Cellulolytic bacteria 10 <sup>6</sup> Bakterie celulolityczne 10 <sup>6</sup>	36.8	38.7	31.4	5.6	36.8	94.9	92.9	7.4	36.8	57.4	43.4	7.5	36.8	26.5	21.8	4.7				
Ammonifying bacteria 10 <sup>9</sup> Bakterie amonifikacyjne 10 <sup>9</sup>	18.4	17.0	11.5	1.6	18.4	18.4	13.0	1.6	18.4	12.6	7.4	1.5	18.4	16.1	15.9	0.9				
Immobilizing bacteria 10 <sup>8</sup> Bakterie immobilizujące 10 <sup>8</sup>	53.6	44.5	37.1	5.0	53.6	72.0	37.1	5.8	53.6	100.7	86.9	10.0	53.6	54.4	40.8	5.7				
<i>Azotobacter</i> spp. 10 <sup>3</sup>	3.1	0.0	0.0	0.6	3.1	0.0	0.0	0.6	3.1	0.0	0.0	0.6	3.1	0.0	0.0	0.6				
Actinomycetes 10 <sup>9</sup> Promieniotwórcy 10 <sup>9</sup>	12.4	11.3	10.8	0.8	12.4	14.7	8.0	1.7	12.4	12.4	9.0	1.3	12.4	21.0	22.8	1.5				
Fungi 10 <sup>6</sup> Grzyby 10 <sup>6</sup>	7.6	8.7	15.8	1.1	7.6	8.6	19.2	0.8	7.6	5.5	5.5	1.2	7.6	9.3	6.7	1.3				

\* 0 – soil not contaminated with heavy metals – gleba niezanieczyszczona metalami ciężkimi  
r.n. – differences not significant – różnice nieistotne statystycznie

trations of metal ions, biomass quantity and temperature. Higher soil acidity and larger amounts of biomass markedly reduce metal adsorption. According to AHUJA et al. (1999), blue-green algae of the species *Oscillatoria angustissima* can be used as biosorbents for zinc.

Just like fungi, members of the order Actinomycetales are among microorganisms most resistant to heavy metals due to their adaptability, ability to immobilize metal ions in the cell wall or to adsorb them on the surface of hyphae. SMYŁA (1995) determined the sensitivity of *Streptomyces* strains to some heavy metals based on growth inhibition zones, and suggested the following toxicity series: Hg > Cd > Cu > Zn > Ni > Pb.

SKŁODOWSKA (2000) found that mercury and silver are most toxic to bacteria, while *Thiobacillus ferrooxidans* is highly resistant to high concentrations of zinc (17 000 mg·kg<sup>-1</sup> soil) and copper (12000 mg·kg<sup>-1</sup> soil) due to the presence of metallothioneins binding these two elements.

WYSZKOWSKA and KUCHARSKI (2003a) demonstrated that soil contamination with heavy metals contributed to the inhibition of the growth of oligotrophic (Ni > Pb > Cr<sub>(III)</sub> > Cu > Zn > Cd), copiotrophic (Cd > Ni > Cr<sub>(III)</sub> > Zn > Cu), ammonifying (Ni > Pb > Cr<sub>(III)</sub> > Cd > Zn > Hg) and nitrogen-immobilizing (Zn > Cr<sub>(III)</sub> > Hg > Cu) bacteria, as well as Actinomycetales (Cu > Cr<sub>(III)</sub> > Ni > Zn > Pb). A significant negative correlation between microbial counts and the degree of soil contamination with Zn, Pb and Cd was observed by STRZELEC (1997), who found that bacteria of the genus *Azotobacter* were most sensitive to metals (Table 1).

Soil contamination with zinc may modify the qualitative composition of bacteria through selection of less resistant strains or via population adaptation to the existing conditions. In a study by MOFFETT et al. (2003) 400 mg Zn·kg<sup>-1</sup> was introduced to soil. Stress caused by the toxicity of this metal reduced the pool of microbial species, which were dominated by Gram-positive bacteria. According to BADURA (1984), these bacteria are least resistant to heavy metals.

The group of microorganisms most sensitive to soil contamination with heavy metals are nitrifying bacteria (BÄÄTH 1989). The nitrification process (SIMEK 2000) is one of the main links in the nitrogen cycle. It is referred to as biological oxidation of ammonia to nitrite and nitrate. Under anaerobic conditions NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> anions may undergo denitrification or become easily leached from the soil (PAKALE, ALAGAWADI 1995). Both processes are inhibited in the presence of heavy metals. WILSON (as cited in BARABASZ 1986) reported that the nitrification process was limited following zinc introduction to soil (1000 mg·kg<sup>-1</sup>). BENBI et al. (1996) demonstrated that doses of 200 and 400 mg Zn·kg<sup>-1</sup> soil were sufficient to reduce mineral nitrogen concentration, inhibit nitrification and increase NH<sub>4</sub> accumulation.

SINGHA et al. (1998) made an attempt to determine nitrogen metabolism in sewage sludge. These authors found that cadmium is six-fold and eight-fold stronger as an inhibitor of nitrification and ammonification, respectively, compared to

zinc. During sixteen weeks of soil incubation metals reduced the activity of nitrification and ammonification to 86.1% and 44.2% respectively. GUPTA and CHAUDHRY (1994) demonstrated that the transformation of  $\text{N-NH}_4$  to  $\text{N-NO}_3$  may be modified both by ion metals and temperature. The series of metal toxicity was as follows  $\text{Hg} > \text{Zn} > \text{Ni} > \text{Pb}$ .

According to BARABASZ (1986), zinc is not the strongest inhibitor of denitrification since it has lower toxicity against *Pseudomonas* sp. than copper.

## IMPACT OF ZINC ON THE ACTIVITY OF SOIL ENZYMES

The sources of soil enzymes are microorganisms as well as higher organisms: plants and animals (KUCHARSKI 1997). Their adsorption on mineral clays and humus substances is associated with a decrease in activity as well as an increase in stability and resistance to denaturation and proteolysis (GOŁĘBIEWSKA, GRZYB-MIKLASZEWSKA 1991). The activity of soil enzymes is affected by numerous environmental factors, such as temperature, humidity, pH (BADURA et al. 1984), plant cover (GAJDA et al. 2000), and heavy metal emissions (FRANKENBERGER et al. 1983).

Zinc is indispensable for numerous organisms, including microbes, because many enzymes found inside cells require adequate quantities of zinc for proper functioning. Zinc is present in over 300 enzymes representing six different classes (McCALL et al. 2000). Zinc-containing enzymes are, among others, carbonate anhydrase, alkaline phosphatase, carboxypeptidase, dehydrogenases (aldehyde dehydrogenase, 3-phosphoglyceric dehydrogenase, alcohol dehydrogenase, glutamate dehydrogenase), fructosediphosphate aldolase, superoxide dismutase (ŚWIĄTKIEWICZ, KORELESKI 2001), DNA and RNA polymerases, and tRNA transferase (FOTYMA, MERCIK 1995). In the active center of alkaline phosphatase in *Escherichia coli* there are two gram atoms of zinc (BADURA 1984).

According to CORDOVA and ALVAREZ-MONA (1995), the role of zinc as a component of metalloenzymes may be considered in several categories, i.e. catalytic – zinc is needed to stimulate the enzymatic activity of, among others, carbonate anhydrase, carboxypeptidase, thermolysine and aldolase; structural – zinc stabilizes protein structure; regulatory – zinc may be an activator or inhibitor of enzymatic activity.

Among all enzymes found in the soil environment, the most significant role is played by oxidoreductases: dehydrogenases, nitrate reductase, polyphenoloxidases, catalase, peroxidases, hydrolases: esterases, alkaline phosphatase and acid phosphatase, phosphodiesterase, arylphosphatase, cellulase,  $\beta$ -glucosidase, invertase, protease, as well as amidases: urease and pyrophosphatase (KUCHARSKI 1997, MORENO et al. 2001).

The effect of heavy metals on soil enzymes may be direct or indirect. Heavy metals directly influence the activity of free extracellular enzymes, and indirectly –

the biosynthesis of enzymes by microorganisms, the composition of soil microbes (BURNS 1982), mycorrhiza (KRUPA 1996), production of root exudates and the release of enzymes from dead roots (KIELISZEWSKA-ROKICKA 2001).

The determination of enzymatic activity may provide the basis for soil quality evaluation, since enzymes are particularly susceptible to environmental changes (TRASAR-CEPEDA et al. 1998). Heavy metals present in soil in small amounts can stimulate enzymatic activity. However, having exceeded certain threshold values, they contribute to the inhibition of activity of microbes and extracellular enzymes (FRANKENBERGER et al. 1983).

According to JANUSZEK (1999), zinc is more toxic to soil microbes and enzymes than lead. This metal has a lower affinity to organic substance and thus is complexed to organic matter to a lower degree. KUCHARSKI (1992) determined the effect of increasing doses of zinc on the activity of soil microbes and reported that the activity of dehydrogenases and acid phosphatase was markedly reduced at 400 mg Zn·kg<sup>-1</sup>. It should be emphasized that in this study a simultaneous decrease in the counts of oligotrophic and copiotrophic bacteria, Actinomycetales or fungi was not observed.

Dehydrogenases are most sensitive to changes in the soil environment. Their activity measured with TTC as an oxidant reflects the activity of the entire microbial population (KUCHARSKI 1997). WELP (1999) tested the susceptibility of dehydrogenases to heavy metals and determined the following series of elements causing a 50% reduction in the activity of these enzymes: Hg (2mg) > Cu (35 mg) > Cr<sub>(VI)</sub> (71 mg) > Cr<sub>(III)</sub> (75 mg) > Cd (90 mg) > Ni (100 mg) > Zn (115 mg) > As (168 mg) > Co (582 mg) > Pb (652 mg·kg<sup>-1</sup> soil). DOELMAN and HAANSTRA (1989) ordered heavy metals according to their inhibitory activity against phosphatases: Zn > Cu > Cd > Ni > Pb > Cr. This series can be modified by clay minerals which absorb phosphatases and in this way weaken their activity (BOYD, MORTLAND 1985).

NOWAK et al. (2003) ordered the inhibitory effect of heavy metals on acid phosphatase and alkaline phosphatase as follows: Cu<sup>2+</sup> > Al<sup>3+</sup> > Cd<sup>2+</sup> > Zn<sup>2+</sup> > Fe<sup>3+</sup> > Ni<sup>2+</sup> > Pb<sup>2+</sup> > Sn<sup>2+</sup> > Fe<sup>2+</sup> > Co<sup>2+</sup>, and Cd<sup>2+</sup> > Al<sup>3+</sup> > Zn<sup>2+</sup> > Fe<sup>3+</sup> > Cu<sup>2+</sup> > Pb<sup>2+</sup> > Ni<sup>2+</sup> > Fe<sup>2+</sup> > Se<sup>2+</sup> > Co<sup>2+</sup> respectively.

In a study conducted by WYSZKOWSKA and KUCHARSKI (2003b), soil contaminated with different metals (Table 2), including zinc, had lower enzymatic activity than uncontaminated soil, whereas the inhibitory impact of metals on particular enzymes was as follows:

- on dehydrogenases: Cu > Zn > Cr(VI) > Hg > Ni > Cd > Cr(III),
- on urease: Cu > Zn > Ni > Cd > Cr(III) > Cr(VI) > Hg,
- on acid phosphatase: Cu > Ni > Zn > Cd > Cr(III) > Cr(VI) > Hg,
- on alkaline phosphatase: Zn > Cu > Ni > Hg > Cr(VI).

Enzymatic activity is also affected by the kind of a chemical compound containing zinc. BADURA et al. (1984) found that zinc sulphate at a dose of 7500 mg·kg<sup>-1</sup> had a stimulating effect on cellulases, but reduced the activity



Table 2  
Tabela 2

Influence of heavy metals on enzymes activity per 1 kg of soil d.m  
 Wpływ metali ciężkich na aktywność enzymów w 1 kg s.m. gleby  
 (WYSZKOWSKA, KUCHARSKI 2003b)

Kind of enzyme Rodzaj enzymu	Kind of metal – Rodzaj metalu															
	Cr(III)				Zn				Pb				Hg			
	doses of heavy metals (mg·kg <sup>-1</sup> soil) – dawki metali ciężkich (mg·kg <sup>-1</sup> gleby)															
	0*	40	80	LSD NIR	0*	500	1000	LSD NIR	0*	500	1000	LSD NIR	0*	10	20	LSD NIR
Dehydrogenases Dehydrogenazy (cm <sup>3</sup> H <sub>2</sub> ·d <sup>-1</sup> )	5.89	6.69	5.19	0.21	5.89	2.05	0.66	0.07	5.89	5.96	10.60	0.25	5.89	2.60	1.88	0.11
Urease Ureaza (mg N-NH <sub>4</sub> ·h <sup>-1</sup> )	11.88	11.04	10.0	0.29	11.88	6.92	7.10	0.29	11.88	13.28	15.87	0.32	11.88	4.69	1.87	0.44
Acid phosphatase Fosfataza kwasna (mmol PNP·h <sup>-1</sup> )	1.29	0.83	0.75	0.02	1.29	0.89	0.59	0.04	1.29	1.29	1.36	0.02	1.29	0.98	0.97	0.02
Alkaline phosphatase Fosfataza alkaliczna (mmol PNP·h <sup>-1</sup> )	2.62	3.07	2.63	0.07	2.62	1.86	1.09	0.04	2.62	2.95	3.01	0.04	2.62	2.36	2.19	0.04

\* 0 – soil not contaminated with heavy metals – gleba niezanieczyszczona metalami ciężkimi

of dehydrogenases, similarly to the carbonate form. In soil with zinc sulphide dehydrogenase activity increased, while cellulase activity remained unchanged.

The problem of the impact of heavy metals on soil enzymatic activity was also discussed by OLSZOWSKA (1997), who focused on the effects of Zn, Pb and Cd emitted by zinc and lead works. This author showed that dehydrogenases, urease and asparaginase may serve as indicators of stress caused by chemical pollution of the ecosystem. BAÄTH (1989) attributes this role to acid phosphatase.

KUCHARSKI (1994) proved that a high zinc content of soil decreases not only the activity of soil dehydrogenases and phosphatases, but also the activity of glucose dehydrogenase in cells of *Rhizobium leguminosarum*.

## CONCLUSIONS

Beyond a doubt, soil is the most diverse though not the safest reservoir of microorganisms. Soil microbes are exposed to the destructive influence of heavy metals, including zinc. The sources of zinc may be rapid industrialization, mineral fertilization, crop protection chemicals as well as the application of composts or sewage sludge as soil amendments, regardless of possible consequences. The effects of zinc and other heavy metals on soil metabolism is relatively varied. This is related primarily to the granulometric composition of soil, pH levels and oxidation-reduction potential, cation exchange capacity and humus content. It should be also remembered that the natural amount of zinc in soil varies. Compact loamy, alluvial, silty and loess soils are richer in zinc, compared with soils developed from granite as a parental rock.

Excessive quantities of zinc in the soil environment cause abiotic stress, reducing the biodiversity of soil microbes. However, the presence of this element in soil has also some positive aspects. Zinc is required for the metabolism and growth of microorganisms because it stimulates, among others, the synthesis of nucleic acids. Moreover, soil microbes are able to defend themselves against heavy metals making use of more and more advanced mechanisms that protect them against death. Nevertheless, some groups of microorganisms, e.g. members of the genus *Azotobacter* and nitrifying bacteria, are very sensitive and can be used as reliable indicators of soil contamination.

The biological activity of soil can be also estimated using enzymes which perform the function of catalysts in the cycle of organic compounds and strongly respond to heavy metals present in excess in the soil environment. The disturbance of the soil biological equilibrium resulting from zinc contamination is reflected by the activity of dehydrogenases synthesized by living bacterial cells. These enzymes are believed to be the most reliable indicators of soil degradation. However, it should be noted that zinc, being a component of metalloenzymes, can inhibit but also stimulate enzymatic activity. This element is needed for the pro-

per functioning of, among others, carbonate anhydrase, carboxypeptidase, thermolysine and aldolase.

It seems promising that microorganisms are becoming more and more resistant to excessive amounts of heavy metals in the soil environment, and that some of them are successfully used in the process of bioremediation. The analysis of the results obtained by numerous authors suggests that the search for new indicators of soil contamination with the above xenobiotics should be continued.

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#### **MICROBIOLOGICAL ACTIVITY OF ZINC-CONTAMINATED SOILS**

Key words: contamination, zinc, microorganisms, soil enzymes.

#### Abstract

Heavy metals, e.g. zinc, present in soil in excessive quantities become quite persistent contaminants. Thus, they differ from organic pollutants, which can be degraded, to a various degree, by microorganisms. Although the accumulation of heavy metals by microorganisms is widespread, this phenomenon has not been thoroughly investigated. Tolerance limits are not uniform and depend on the biodiversity and duration of ambient concentration, as well as on the physicochemical properties of soil, soil structure, organic matter content, pH and sorptive complex capacity.

Soil contamination is more and more frequently assessed with biological methods, by testing for example the activity of soil enzymes. Such assays, however, can be used to evaluate the quality of soils contaminated with heavy metals, but are less useful for testing soils polluted with organic compounds.

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## AKTYWNOŚĆ MIKROBIOLOGICZNA GLEB ZANIECZYSZCZONYCH CYNKIEM

Słowa kluczowe: zanieczyszczenie gleby, cynk, drobnoustroje, enzymy glebowe.

### Abstrakt

Metale ciężkie, w tym także cynk, gdy dostaną się do gleby w nadmiernych ilościach, stanowią dość trwałe zanieczyszczenie, w przeciwieństwie do różnego rodzaju zanieczyszczeń organicznych, podatnych w większym lub mniejszym stopniu na degradację mikrobiologiczną. Mimo iż akumulacja metali ciężkich przez drobnoustroje jest procesem powszechnym, to nie do końca poznany. Granice tolerancji nie są jednakowe i zależą od ich bioróżnorodności i czasu trwania emisji, a także właściwości fizykochemicznych gleby, jej struktury, zawartości materii organicznej, pH i pojemności kompleksu sorpcyjnego.

Coraz częściej do oceny stanu zanieczyszczenia gleby wykorzystuje się metody biologiczne, w tym aktywność enzymów glebowych, i w nich należy upatrywać szybkiej diagnozy jakości gleb. Wskaźniki te mogą być jednak wykorzystywane tylko do oceny gleb zanieczyszczonych metalami ciężkimi, a są mało przydatne w ocenie jakości gleb zanieczyszczonych związkami organicznymi.