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DEVELOPMENT OF MICROORGANISMS AND OXIDATION
OF SOME ORGANIC COMPOUNDS IN SOIL POLLUTED
WITH HEAVY METALS

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Abstract. Number of bacteria, actinomycetes and especially fungi in soil polluted with heavy metals was lower than in the nonpolluted one. The respiratory substrates influenced in a different way the physiological properties of microorganisms in both soils. The respiratory activity of the polluted soil was lower than that of nonpolluted one.

It is realized, that heavy metals contamination of agricultural and forest soils can not be appreciated without elucidating its effects on soil microorganisms. Heavy metals may affect not only the balance among the total number but also between species of bacteria [6,7,16].

Heavy metals are biologically essential and are required for normal plant growth and development. Other heavy metals have not been proven to be required for plant growth and development and are designed biologically not essential. Essential are for example copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn). As non-essential are considered cadmium (Cd), lead (Pb), mercury (Hg) and others. At a sufficient concentration or dose all heavy metals are toxic to plants and microorganisms [20].

It is suggested that heavy metals deposited from the atmosphere to forest systems are accumulated in the forest floor [8,19]. Since this accumulation is in soil with maximum root activity, maximum activity of soil micro- and macrobiota, it is appropriate to consider rhizosphere regula-

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tion of heavy metal availability and/or toxicity to roots and other biotic components of the soil ecosystem [20].

Therefore the aim of this work was to study the development of bacteria and fungi and the enzymatic activity measured by hydrogenase activity (the samples were taken from the upper part of the soil – up to 10 cm).

It is known that the biochemical activity of microorganisms affect both growth and development of the plants.

MATERIALS AND METHODS

Our studies were performed with soils taken from two surfaces. One in the region heavily polluted with industrial emissions (Brynica, chief-forestry Świerklaniec). Trees (*Pinus sylvestris* L.) were 22 years old. The soils were compared with those not polluted with industrial emissions (chief-forestry Herby). Trees (mainly *Pinus sylvestris* L. with mixture of *Betula verrucosa* Ehrh., *Quercus sessilis* Ehrh., *Larix europaea* DC.) were 27 years old. Both experimental surfaces contained podzolized soils derived from Quaternary outwash coarse sands.

Samples of the soils were taken from upper layers (up to 10 cm) and sieved through 1 mm sieve.

The heavy metals in the extract (1 mol/dm³ HCl) were determined as given by Lityński *et al.* [13].

Microbiological studies were performed after adjusting the soil samples to 60 % water holding capacity. The general number of bacteria and fungi was determined before and after 24 h of incubation with different respiratory substrates.

Bacteria: The number of bacteria was determined using the modified medium of Allen [1]. Its composition was: soil extract – 1000 ml, K₂HPO₄ – 0.5 g, actidione – 50 mg, nystatin – 100 mg, agar – 15 g.

Actinomycetes: For the determination of actinomycetes the Bacto Actinomycetes Isolation (Difco) was used.

Fungi: For the determination of fungi the Martin's medium [4] was applied.

Microorganisms were grown on plates for 7 days at 26°C and their number was calculated per gram of fresh and dry soil. Similarly calculated was the number of microorganisms grown for 24 h in the presence of the following respiratory compounds: glucose, sodium acetate, sodium pyruvate, casein hydrolyzate and soluble starch, used at concentration of 5 mg/ml each. Incubation with the above substrates was carried out in Eppendorf's tubes at 37°C.

The effect of the above mentioned substrates on the development of the following physiological groups was studied:

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ammonifiers – were enumerated on the modified Allen's [1] medium: peptone – 2.0 g, glucose – 1.0 g, K_2HPO_4 – 0.2 g, $MgSO_4 \times 7H_2O$ – 0.2 g, $FeSO_4 \times 7H_2O$ – trace, H_2O dist. – 1000 ml. 5 ml aliquots of the medium were inoculated with soil dilutions 1:10 to 1:1. After 7 days of incubation at 26°C the presence of ammonia was tested with the Nessler's reagent;

denitrifiers – this group of microorganisms was studied on the modified Allen's [1] medium: glucose – 10 g, KNO_3 – 1.0 g, K_2HPO_4 – 0.5 g, agar – 15 g, H_2O dist. – 1000 ml. After 7 days of growth at 26°C the plates were flooded with Lugol's solution and the zones of clearing around the colonies were recorded;

amylolytic organisms – these organisms were grown in a medium: peptone – 5.0 g, $FeSO_4 \times 7H_2O$ – 0.1 g, glucose – 0.5 g, soluble starch – 5.0 g, agar – 15 g, H_2O dist. – 1000 ml. After 7 days of growth at 26°C the plates were flooded with Lugol's solution and the zones of clearing around the colonies were recorded;

cellulolytic microorganisms – for this purpose the Dubos medium [12] with Whatman No. 3 filter paper was used. The tubes inoculated with the soil suspension were cultured during 30 days at 26°C. Subsequently the growth of microorganisms on the filter paper strips was observed as well as the loss of biomass of the strips was recorded.

The number of appropriate physiological groups was determined by the Most Probable Number Enumeration System (J. Bennett, P. Woerner & R. Yost – Nif TAL Project).

Dehydrogenase activity: The dehydrogenase activity was determined according to Russel [18].

Statistical evaluation of the results was performed using 2-factor analysis of variance (ANOVA 2).

RESULTS AND DISCUSSION

The results of our studies are shown in Tables 1–4 and Figs. 1, 2. Among all respiratory substrates used in the control soil ("0" zone of pollution with heavy metals), the strongest effect exhibited casein hydrolyzate (Table 2). This substrate stimulated the development of bacteria and actinomycetes but retarded fungi. This could be an indirect effect through antagonistic action of the former groups. In the control samples (without respiratory substrates) most numerous among the physiological groups were amylolytic organisms and least numerous the cellulolytic one. Not numerous were also the denitrifiers. The respiratory substrates used affected least the cellulolytic and amylolytic organisms. Glucose, acetate and pyruvate significantly stimulated the development of ammonifying and denitrifying organisms (Table 2).

As shown in Table 3, the number of microorganisms in the particular

TABLE 1. SOME CHEMICAL PROPERTIES OF HUMUS HORIZONS OF THE INVESTIGATED SOILS

Zone of industrial pollution	Locality	pH		Org. C %	Total N %	C/N	Ca	Mg	P	K	Pb	Zn	Cu	Cd
		H ₂ O	KCl											
nonpolluted (0)	Herby	4.8	3.7	3.8	0.24	15.8	19.5	1.0	2.8	2.9	60.5	16.0	4.8	0.5
heavy industrial pollution (III)	Brynica	6.1	4.9	0.7	0.05	14.0	11.5	0.4	0.6	1.8	149.0	130.0	4.7	2.4

TABLE 2. TOTAL NUMBER OF MICROORGANISMS AND NUMBER OF PHYSIOLOGICAL GROUPS IN SOIL NONPOLLUTED BY INDUSTRIAL EMISSIONS (thousands/g. of fresh soil)

Microorganisms and physiological groups	Control (without substrate)	Glucose	Sodium acetate	Casein hydrolyzate	Sodium pyruvate	Soluble starch
Bacteria	416	1127	1383	20530+	7600	3200
Actinomycetes	540	1170	730	7567	77	63
Fungi	883	277-	57-	76-	117-	96.7-
Ammonifiers	75	2500+	2500+	2500+	0.95	0.95
Denitrifiers	1.5	2500+	2500+	2500+	0.065	0.65
Amylolytic organisms	950	95	950	950	95	950
Cellulolytic organisms	0.025	0.025	0.025	0	0.095	0.095

Explanation: + stimulation ($p \leq 0.05$)
- inhibition ($p \leq 0.05$)

TABLE 3. TOTAL NUMBER OF MICROORGANISMS AND NUMBER OF PHYSIOLOGICAL GROUPS IN SOIL POLLUTED BY INDUSTRIAL EMISSIONS (thousands/g. of fresh soil)

Microorganisms and physiological groups	Control (without substrate)	Glucose	Sodium acetate	Casein hydrolyzate	Sodium pyruvate	Soluble starch
Bacteria	233	7933.3+	633	4670+	450	11870+
Actinomycetes	180	4933.3+	1067	2970	1900	6530+
Fungi	16.7	6.7	6.0	3.3	4.0	3.0
Ammonifiers	4.5	2500+	950	2500+	2500+	2500+
Denitrifiers	25.0	0	200	11.5	0	0
Amylolytic organisms	4.5	950	450	2500	950	9500+
Cellulolytic organisms	0.045	0.0095	0.0045	0	0	0.025

Explanation: + stimulation ($p \leq 0.05$)

TABLE 4. COMPARISON OF RESPIRATORY ACTIVITY OF TWO SOILS (ENDOGENIC RESPIRATORY * PROPER RESPIRATORY * RESPIRATORY SUBSTRATE)

Soil nonpolluted (0 zone)	Soil industrially polluted (III zone)
2.2963 b	0.6272 a

Explanation: values indicated as a and b differ statistically at $p \leq 0.05$

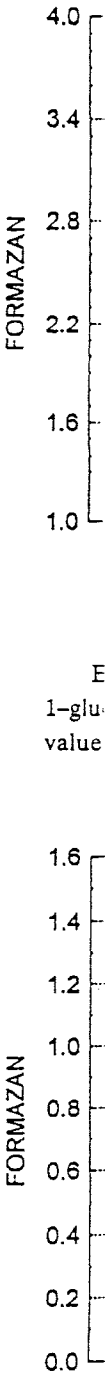


Fig. 2.

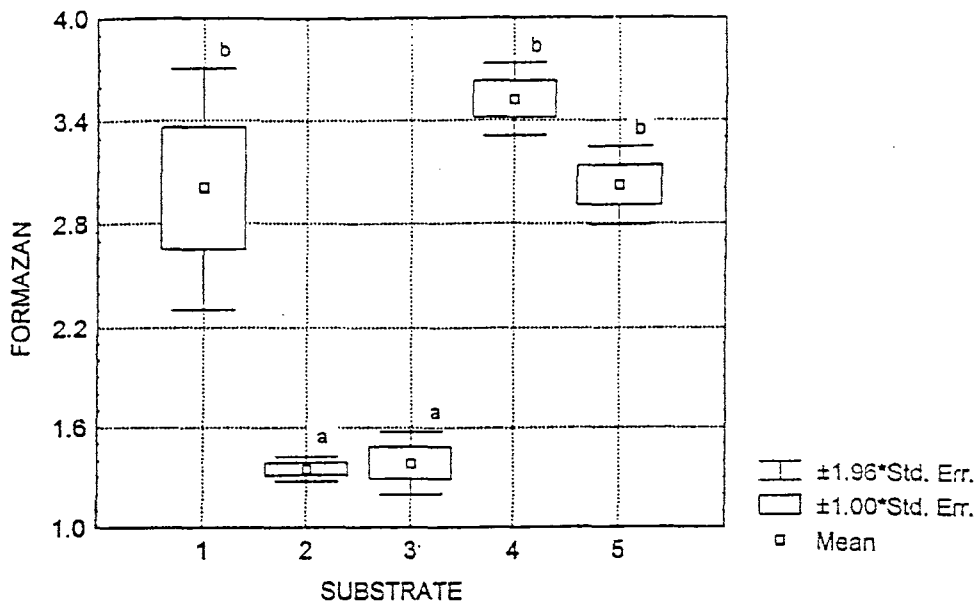


Fig. 1. Activity of dehydrogenases (μg formazan/ml) in soil nonpolluted

Explanations:
 1–glucose, 2–sodium acetate, 3–casein hydrolysate, 4–sodium pyruvate, 5–soluble starch
 values indicated as "a" and "b" differ statistically at $p \leq 0.05$

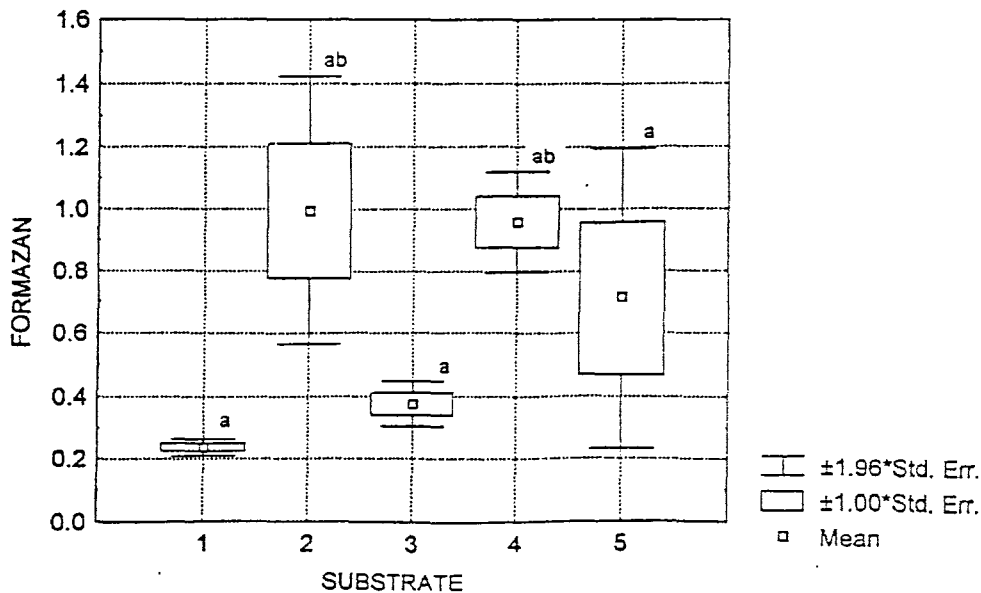


Fig. 2. Activity of dehydrogenase (μg formazan/ml) in soil polluted with heavy metals

SOILS
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 oil
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 .7 2.4

GROUPS IN
 Soluble starch
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 6530+
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 2500+
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 9500+
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physiological groups, except of denitrifiers, in the polluted soil was lower than in the nonpolluted one. Glucose and starch stimulated significantly the development of actinomycetes and bacteria (bacteria were stimulated also by casein hydrolyzate) in soil polluted with heavy metals. All the substrates used did not influence the development of fungi. Ammonifying organisms were stimulated by glucose, casein hydrolyzate, pyruvate and starch, and amylolytic organisms by casein hydrolyzate and starch. Respiratory substrates did not influence the development of denitrifying and cellulolytic organisms.

Both soils differed statistically in respiratory activity. This activity was higher in the nonpolluted than the polluted soil (Table 4).

The respiratory activity of the nonpolluted soil (expressed as μg formazan/ml) is presented on Fig. 1. The highest respiratory activity was found in the soil amended with glucose, pyruvate and starch (*ca* 3 μg of formazan/ml). Least activity was noted in the presence of casein hydrolyzate and acetate (*ca* 1 μg of formazan/ml).

The respiratory activity of the polluted soil is presented on Fig. 2. The respiratory activity of this soil was lower (statistically significant) than of the nonpolluted one. The highest was noted in the presence of acetate and pyruvate and the lowest with glucose, casein hydrolyzate and starch. However, these differences were statistically not significant. We also observed that the activity of dehydrogenase, in the soil polluted, in the presence of respiratory substrates did not differ significantly from the endogenous (without substrates) one. In the control soil (nonpolluted), the activity of dehydrogenase in the presence of respiratory substrates was higher than endogenous one.

Accumulation of heavy metals in the soil may decrease the role of decomposition of organic matter – as a result of diminished soil microbial activity [11,13]. But the effects of heavy metal pollution on the soil microbes are not always unambiguous and depend on many ecological factors like pH, soil organic matter content and cations exchange capacity of soil [3].

In model studies on the action of heavy metals on pure cultures of different microorganisms, a great variability in reactions of these organisms has been stated [5,10,17]. Heavy metals contamination of soils has become a serious problem in environmental pollution. Bacteria are generally recognized as the first organisms to be affected by these compounds [21]. In field studies the heavy metals pollution is undoubtedly never due to one single metal. This makes the conclusions regarding the toxicity of heavy metals to organism hard to draw [2].

In our studies heavy metals were considered because of their importance in the natural environment and their possible involvement in the acid rain syndrome and because of their widespread occurrence as products of man activity.

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It appears from our studies, that the polluted soil contained less microorganisms than the nonpolluted one. Also the respiratory activity was higher in the nonpolluted soil than in the polluted one. Both soils differed in organic carbon content and pH (Table 1). Thus, it is hard to say how these factors affect the influence of the heavy metals on microorganisms. Heavy metals, after being deposited in soil, undergo various transformations, forming salts or complexes more or less soluble in water. They can be bound by mineral and organic colloids, as well as with organic acids and amino acids present in soil, thus forming complexes of different complexing strength [3]. It is also hard to, say which metal is most harmful to the microorganisms, as pollution is never due to one single metal [2]. However, reduction of bacteria and fungi due to heavy metals was reported by Maliszewska *et al.* [14] and Ohyama *et al.* [16]. Fungi appear to be more tolerant to heavy metals than bacteria. However, in our studies the fungi were more numerous in the nonpolluted soil than in polluted one. Several studies on the effect of heavy metals on hydrolytic enzymes showed differences in the degree of inhibition of these enzymes. However, most of the enzymes were affected in a similar way. Low enzymatic activity in soil can be due to low concentration of the enzyme, by masking of active groups, by protein denaturation, by other effects on enzyme configuration or by competition with activating metal ions [15]. Tolerance and adaptation of microorganisms to heavy metals are common phenomena and the presence of tolerant fungi and bacteria in polluted environments has frequently been observed [9]. Tyler [23] found the rate of cellulose and starch decomposition to be lower in metal contaminated soil, while the effects on protein and especially glucose degradation were negligible. Also N-mineralization in the field was negatively correlated to pollution levels [22].

We have found, that amylolytic organisms were more numerous in the nonpolluted soil than in the polluted one. The cellulolytic microorganisms were not numerous in both polluted and nonpolluted soil and difference in their number was not statistically significant.

Many different methods were used to measure the effects of heavy metals on the soil ecosystem. We have chosen the development of general microflora, some of their physiological groups (which undoubtedly are important in nutrient cycling) and respiratory activity.

CONCLUSIONS

1. The polluted soil contained less microorganisms than the nonpolluted one. Also the respiratory activity was lower in the polluted soil than in the nonpolluted one.
2. Casein hydrolyzate exhibited the strongest effect on the development of bacteria and actinomycetes in the control soil.
3. In soil polluted with heavy metals glucose and starch stimulated sig-

nificantly the development of actinomycetes and bacteria (bacteria were stimulated also by casein hydrolyzate).

4. Because both soils differed in organic carbon content and pH, thus it is hard to say how these factors affected the influence of the heavy metals on microorganisms.

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ROZWÓJ MIKROORGANIZMÓW I UTLENIANIE NIEKTÓRYCH ZWIĄZKÓW ORGANICZNYCH W GLEBIE SKAŻONEJ METALAMI CIĘŻKIMI

W pracy przedstawiono wyniki badań nad wpływem niektórych substratów oddechowych na liczebność oraz aktywność metaboliczną drobnoustrojów w glebie skażonej emisjami przemysłowymi (III strefa skażeń) oraz w glebie kontrolnej (0 strefa skażeń).

Liczebność bakterii, promieniowców, a szczególnie grzybów w glebie skażonej metalami

ciężkimi była niższa niż w glebie kontrolnej. Substraty oddechowe w różny sposób oddziaływały na właściwości fizjologiczne drobnoustrojów w obydwu glebach. Glukoza i skrobia stymulowały rozwój promieniowców i bakterii, lecz nie grzybów, w glebie skażonej emisjami przemysłowymi. W glebie kontrolnej najsilniejszy wpływ wykazywał hydrolizat kazeiny. Substrat ten stymulował rozwój bakterii i promieniowców, ale hamował rozwój grzybów. Aktywność oddechowa gleby skażonej była niższa niż gleby kontrolnej.

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