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Effect of industrial dusts on the development and activity of micro-organisms in soils of the Niepołomice Forest (southern Poland)

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Abstract — In soils contaminated with industrial dusts (at doses of 0, 100, 500, 1000, and 2000 t km^{-2} $year^{-1}$) containing 177.2—3544.0 kg Zn ha^{-1} or 26.4—528.0 kg Cd ha^{-1} a considerable reduction in the numbers of micro-organisms was found. It was also observed that the respiratory activity of micro-organisms, C_x cellulase, and soil phosphatases varied, depending on the kind and dose of dusts, their time of action, and mineral fertilization of the soils (NPK and liming).

Key words: soil, bacteria, total activity of micro-organisms, bacterial activity, enzymatic activity of soils.

1. Introduction

In recent decades unconsiderated human activity brought about an enormous devastation of the natural environment and consistently led to its further degradation.

All over the world ecologists have undertaken intensive studies on aquatic and land ecosystems, indicating a threat to the life of plant and animal organisms (Babich, Stotzky 1977a, 1978, Geiger et al. 1978, Grodziński, Yorks 1981, Borgman 1983, Bewley, Parkinson 1984, Badura 1985).

One of the more dangerous pollutions of the environment is the emission of dusts and gases to the atmosphere by various kinds of industrial plants. These emissions, containing considerable amounts of sulphur, nitrogen, carbon, and heavy metal compounds, is a serious threat to ecosystems, among them forests (Grodziński, Yorks, 1981, Grodziński et al. 1984), where unfavourable changes have been manifested in the number and structure of trees and herbaceous plants (Myczkowski, Lesiński 1976, Grodzińska et al. 1978).

Out of the total area of 8.6 million hectares of Polish forests about 8% is estimated as seriously threatened by industry. As an example, the forests of Upper Silesia lying around the zinc smelter at Miasteczko Śląskie may be mentioned. Its emission delivers over 300 kg zinc and 200 kg lead per hectare annually. A decrease in the productivity of these forests has been

observed, brought about among other factors, by the excessive accumulation of heavy metals in the timber and also in herbaceous plants (Przybylski 1985). On the other hand, microbiological investigation of the soil in forests lying at various distances from the zinc smelter (the damage zones) did not show any differences in the number or activity of micro-organisms, in spite of the large content of total zinc in the soil. This phenomenon is associated with the buffer properties of the soil environment and in this connection, with the small content of soluble zinc in the soil (Badura et al. 1984b, Badura, Pacha 1984).

Another example of an ecosystem threatened by industrial emissions is the Niepołomice Forest. In this forest area 1 km^2 is exposed to the fallout of about 100 t of dusts annually, in this amount about 3 t Fe, 125 kg Zn, 31 kg Pb, 18 kg Cu, 6 kg Ni, and 1.5 kg Cd, of which about 45% occur in soluble form (Manecki 1984). In the soil, litter, undergrowth, and mesofauna of the Niepołomice Forest a pronounced accumulation of heavy metals was found. In the case of increased acidification of the environment and, hence, of increased toxicity of heavy metals, this accumulation may result in paralysis of the organisms taking part in the decomposition of litter (Grodzińska et al. 1987).

In multiannual experiments carried out in the mixed forests at Niepołomice by Greszta (1988) and Greszta et al. (1987), industrial dusts collected from electrofilters of zinc, cadmium, and aluminium smelters, from an electric power station, and a cement plants, and introduced to the forest floor in doses of 100, 500, 1000, 2000, and 5000 t km^2 year^{-1}, were found to produce considerable changes in the different links of the ecosystem, especially with large doses of zinc, cadmium, and aluminium dusts. In the first year of the experiment, 100% of mosses and plants with a shallow root system disappeared. A delay of 1—3 years was noted in the changes of tree stands, manifested by a reduced increment (by 10—30%) and finally the dieback of trees. With increasing doses of dusts a decrease was also observed in the numbers of bacteria (20—70%), Actinomycetes (20—50%), and fungi (10—60%) and, to a similar degree, inhibition of the enzymatic activity of soils.

Pollution on forest soils also leads to disturbances in the development of micro-organisms, to their qualitative selection (Babich, Stotzky 1980, Titus, Pfister 1982, Badura et al. 1983a, Bewley, Parkinson 1984, Badura, Galimska-Stypa 1986), and to their decreased metabolic activity, this inhibiting the cycling of nutrients (Stickel 1975, Barnhart, Vestal 1983) and the development of plants.

In forest ecosystems the layer of litter and soil constitutes a sub-system where organic matter and pollutants are accumulated. As a consequence of microbiological decomposition over 60% of nutrients of the whole ecosystem are accumulated in the litter (Weaver 1975), this giving evidence of its great role in their cycling. The processes which account for the cycling

of matter and the flow of energy in the ecosystem are particularly sensitive to atmospheric pollutants, which accumulate excessively in the litter (Smith 1974, Jackson, Watson 1977). Hence, the estimated amounts of nutrients and pollutants and, especially, the energy flow in the litter are reliable indictators of the regularity or disturbance of forest ecosystems (Grodziński, Yorks 1981, Zieliński 1984); they may be used in determining the appropriate trophy of the ecosystem, which can ensure its balance (Babich, Stotzky 1983).

Micro-organisms play a very important role in maintaining the balance of the ecosystem. Their heterotrophic activity makes possible the release of nitrogen, carbon, phosphorus, sulphur, and other biogenes, limiting primary production, from organic remnants. They also have a share in maintaining an appropriate structure of the soil (the production of humus) which assures regular aeration of the soil and the cycling of water, these being important factors in root development. Moreover, the active microflora has the ability of utilizing and accumulating the excessive amounts of organic matter in the environment and of accumulating the toxic substances produced by its decomposition, and also allochtonous substances (Alexander 1980).

These basic functions of micro-organisms may be disturbed by industrial deposits, especially heavy metals contained in dusts (Babich, Stotzky 1974, Jordan, Lechevalier 1975, Coughtrey et al. 1979), leading to disturbances in the cycling of matter in ecosystems (Jackson, Watson 1977, Tamm 1977, Grodziński, Yorks 1981). This effect does not depend only on the sensitivity of particular organisms (Babich, Stotzky 1977a) but also on many abiotic factors of the environment (Babich, Stotzky 1977b, 1978) which affect the bioaccessibility of metals and their final toxicity for macro- and micro-organisms (Babich, Stotzky 1983).

In spite of reports concerning the phenomenon of inhibited litter decomposition due to the decreased activity of micro-organisms in forests exposed to industrial pressure (Babich, Stotzky 1974, Tyler 1980, Strojan 1978, Dodd, Lauenroth 1981), it seems justified to undertake studies on the effect of industrial emissions in different forest ecosystems. On the one hand this is dictated by the different specificity of particular ecosystems, which accounts for the given type of transformations but prevents the generalization and formulation of proper conclusions on the basis of results obtained in other experimental objects, and on the other, by the varied effects of different pollutants on the environment (Badura 1988).

The studies presented here concerned the determination of the quantitative level and activity of destruents in the soil of experimental plots established in the Niepołomice Forest in a mixed forest habitat (Greszta et al. 1987).

The chief aim of the present study was to investigate the effect of long and short periods of action of the dusts, conventionally called cadmium and zinc dusts, on the development and activity of the microflora in forest soils.

2. Study area

The Niepołomice Forest is a lowland forest complex, chiefly composed of mixed pine forests of the *Pino-Quercetum* type (Kozl. 1925 em. Matuszkiewicz, Polakowska 1955) and to a lower degree, of broadleaf oak-hornbeam forest of the *Tilio-Carpinetum* type (Traczyk 1962). The vast forest (11 000 ha) lies in southern Poland in the fork of the

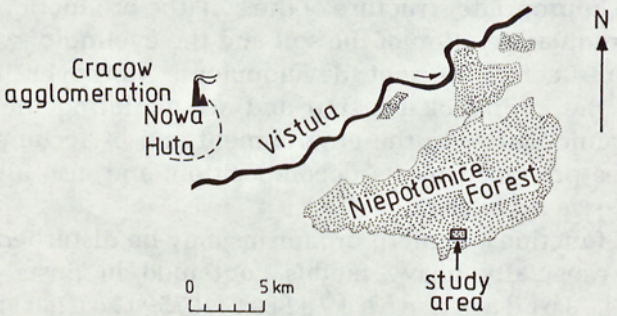

Fig. 1. Map of the study area in the Niepołomice Forest

valleys of the Rivers' Vistula and Raba, about 20 km east of Kraków (50° 07'N, 20° 23'E). For more than 30 years the forest has been polluted by industrial gases and dusts emitted to the atmosphere by various boiler houses and metallurgic plants in the region of Kraków and Katowice.

The Kraków urban agglomeration includes several industrial plants. The largest of them, the metallurgic complex in the Nowa Huta quarter, is the source of 64% of dusts containing heavy metals (Pb, Zn, Cd, and Fe) and 44—68% of different gases (SO_2, CO, and CO_2) emitted by the entire urban agglomeration (Garścia et al. 1984). The average annual deposit on 1 km² of the Niepołomice Forest area is about 100 t of dusts and about 6 t of sulphur (in the form of SO_2) (Manecki, Skowroński 1984).

The stands of the southern part of the Niepołomice Forest (*Pino-Quercetum)* grow on grey-brown poor podsol formed on pleistocene fluvoglacial sands. These soils are characterized by a well-developed fermentation-humus layer (A_{oF+H}) which passes at a depth of about 5—6 cm into the mineral horizon (A_1) composed of loose sands.

In 1981 in this part of the forest experimental plots of 2.4 ares each were established (fig. 1) and treated with cadmium and zinc dusts containing different amounts of heavy metals in the form of oxides (Greszta et al. 1987) (Table I). The dusts, collected from various technological lines of the

Table I. Chemical composition of dust (in %) from zinc and cadmium plants (according to Greszta et al. 1987). n.d. - not determined; tr - traces

Element	Kind of dusts		Element	Kind of dusts	
	zinc	cadmium		zinc	cadmium
Na_2O	0.48	0.04	CdO	0.63	3.02
K_2O	0.71	0.42	CoO	0.01	0.01
MgO	0.72	0.02	NiO	0.02	tr
CaO	7.94	5.67	Fe_2O_3	4.58	0.20
MnO	0.12	0.01	SiO_2	43.74	45.30
ZnO	22.06	1.75	Al_2O_3	8.13	21.83
CuO	0.08	0.01	S	1.16	0.42
PbO	3.08	4.07	F	n.d.	n.d.

zinc and lead smelters at Miasteczko Śląskie, were applied at doses of O, 100, 500, 1000, and 2000 t km^{-2} $year^{-1}$ (this corresponding to 177.2—3544.0 kg Zn and 26.4—528 kg Cd ha^{-1}) (Table II) and 5000 t km^{-2} $year^{-1}$ (an equivalent of 8860 kg Zn and 1320 kg Cd ha^{-1}).

Table II. Content of zinc and cadmium in dusts and in the A_{oF+H} horizon of soil in the experimental and "mini" plots in 1987. x - data according to Greszta unpubl.

Dose of zinc or cadmium dusts	Zn dose in zinc dusts	Cd dose in cadmium dusts	mg Zn kg^{-1} of soil d.w.		mg Cd kg^{-1} d.w. of soil					
					non-fertilized		fertilized		from "mini" plots	
t km^{-2} $year^{-1}$	kg ha^{-1}	kg ha^{-1}	total	soluble	total	soluble	total	soluble	total	soluble
0	-	-	162	136	29	0.7	29	0.7	14	0.3
100	172.2	26.4	867	739	430	10.2	328	1.8	114	3.2
500	886.0	132.0	1148	829	473	6.7	478	2.4	723	20.2
1000	1772.0	264.0	3690	1052	1870	35.5	1600	16.0	1510	21.1
2000	3544.0	528.0	4747	971	2210	36.6	4740	45.6	3360	41.5

3. Material and methods

The investigation of soils from the experimental plots (the control and plots treated with zinc and cadmium dusts at doses of 100, 500, 1000, and 2000 t km^{-2} $year^{-1}$) (Table II) was carried out in 1986 and 1987, i.e. within 5 and 6 years of the introduction of dusts to the soil.

The study did not include the plots with 5000 t km^{-2} $year^{-1}$ since in those treated with 2000 t km^{-2} $year^{-1}$ of the respective dusts, analogical changes in higher plants were already observed.

Initially, i.e., in 1986, analyses concerned the control plot and those treated with zinc and cadmium dusts. The samples of soil were taken from the fermentation-humus layer (A_{oF+H}) and from the mineral horizon (A_1) in early summer (24 June) and towards its end (1 September), and from the A_{oF+H} layer also in autumn (22 October).

In 1986 in the mineral soil horizon (A_1) the activity of micro-organisms was poor and since no significant effect of zinc dusts on the respiration of

micro-organisms in the fermentation-humus layer (A_{oF+H}) was detected, the investigation of 1987 was limited to the A_{oF+H} horizon of the control plot and of the plots treated with cadmium dusts.

At the same time, an investigation was carried out on those plots treated with cadmium dusts where in 1986—1987 recultivation measures were applied. They consisted of mineral fertilization (NPK at a dose of 720 kg ha^{-1}) and liming (magnesium-dolomite lime at a dose of 3000 kg ha^{-1}) applied on half of each plot. The samples were taken in spring (22 May), early in summer (23 June) and towards its end (1 September), and in autumn (20 October).

To illustrate the development of bacteria in fertilized soils a logarithmic comparative coefficient (LCC) was applied determining the ratio of the logarithm of the number of bacteria in these soils to the logarithm of their number in non-fertilized ones (Starzecka 1977). The positive value of the coefficient shows larger numbers of bacteria in the fertilized and the negative one in the non-fertilized soil.

An additional field experiment consisted in subdividing small plots of 1 m^2 each (hereafter called the "mini" plots) on one of the control plots. The "mini" plots were separated with thin plastic foil dug in to a depth of 40 cm below the soil surface. On the surface of these plots cadmium dusts were applied at doses proportional to those used on 2.4 are plots 6 years earlier. The aim of the experiment was to obtain information concerning the effect of the cadmium dusts on the number and activity of micro-organisms in the first year of its action on the soil. The investigation of soil from the "mini" plots was carried out on the same dates as that from the 2.4-are plots, i.e., on 25 May, 23 June, 1 September, and 20 October, 1987.

The soil samples were taken with a device constructed by the author; it allowed cylindrical soil cores 5 cm in diameter and 20 cm in lenght to be cut out. The determinations were made on the surface soil layer from the horizons A_{oF+H} and A_1, which were carefully separated with a knife blade. The average sample from a given layer was obtained by mixing 8—10 parts of soil cores taken from different sites of each plot.

The determinations made on the basis of soil samples included mineral composition, using Tokarski's method (Oleksynowa et al. 1983), physical properties, using Kopecky's method (Oleksynowa et al. 1983), pH, using the potentiometric method, content of macroelements and nutrients, according to methods used in agricultural-chemical stations (Czerwińska et al. 1983), and the concentration of heavy metals, using the atomic absorption method.

The coefficient of soil humidity was given as the ratio of wet weight of the soil (W) to its dry weight (D) $\left(\frac{W}{D}\right)$.

The dry weight was determined by drying samples at 105°C and the content of organic matter by the Tokarski thermic method at 570°C (Oleksynowa et al. 1983).

In microbiological analyses the total numbers of heterotrophic bacteria (enriched agar), fungi (Bacto Czapek Dox Broth), and Actinomycetes (Gaus medium) were determined using the plate method.

Among the microflora of varied biochemical properties, the numbers of bacteria taking part in the conversion of nitrogen, nitrogen-free, phosphorus, and sulphuric compounds, were determined.

Proteolytic (15% gelatine medium), amylolytic (Pochon's medium), and phosphorous (medium with glucose and $Ca_3/PO_4/_2$) bacteria were identified using the plate method. The titre method was used in identifying ammonifying bacteria (medium with peptone water), nitrifying 1st phase bacteria (Winogradski medium with an addition of $CaCo_3$ in the sediment), denitrifying bacteria (medium with glucose and KNO_3), anaerobic nitrogen fixing bacteria (Winogradski medium with yeast extract and ascorbic acid), cellulolytic bacteria (Winogradski method in the presence of paper strips), and bacteria decomposing protein compounds with H_2S released (broth medium in the presence of paper strips saturated with lead acetate).

The titre values were converted to numbers using McCrady's statistical tables (Rodina 1968, Collins, Lyne 1980). The composition of the applied media was given by Rodina (1968) and Starzecka (1979). All determinations were carried out in 3 replications and the results represent arithmetical means calculated per 1 g of soil dry weight.

The cultures were incubated at 20°C $\pm 1°$. The determinations were made after a 7-day incubation, with the exception of proteolytic and cellulolytic bacteria, and nitrifying ones which were determined after a 48 h and 30-day incubation respectively.

The total activity of micro-organisms was determined using an Infralyt IV infrared gas analyser with a system of feeding CO_2 to a prescribed concentration in the air (Starzecki 1979) and a standard 6-channel measuring unit of the gas analyser (fig. 2).

A system of exposing soil samples was developed: it was composed of a tank with a regulated water temperature, maintained by a thermostat controlling a refrigerating unit and a heater. An electric mixer ensured the maintainance of an even temperature in the whole water mass (fig. 2). Respiration chambers composed of perforated basket with the soil samples, closed in an airtight container, were designed and constructed (fig. 3). The air introduced to the chamber did not pass through the soil sample but flowed around the basket, this allowing the diffusion of CO_2 from the soil to the measuring unit. The above device to a certain degree ensured conditions similar to natural ones. The samples were equal with regard to volume but their weight varied. For soils from the A_{oF+H} horizon the weighed portions amounted to 150 g and from A_1 horizon 250 g fresh weight. The respiration of soil micro-organisms was measured at $20 \pm 1°C$, with a content of 330 cm^3 CO_2 m^{-3} and rate of air flow of 50 dm^3 h^{-1}.

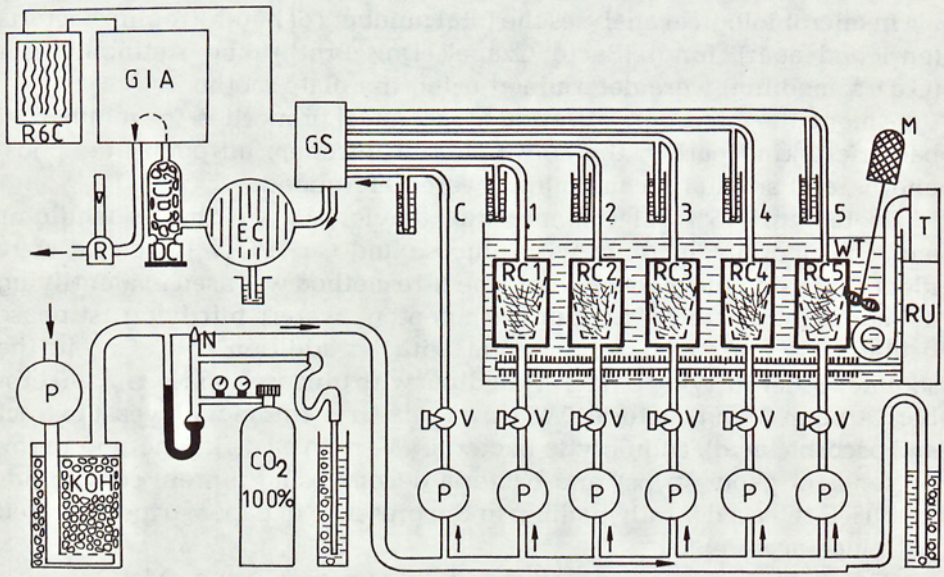

Fig. 2. Diagram of the system for measuring soil respiration. P — pump; N — nozzle; V — needle valve; C — channel for controlling CO_2 in air; RC — respiration chamber; WT — water tank; T — thermostat; RU — refrigeration unit; M — mixer; GS — gas switch; EC — electric cooler; DC — drying column; GIA — Infralyt IV gas infrared analyser; R6C — 6-channel recorder; R — rotameter; 1, 2, 3, 4, 5, — measuring channels

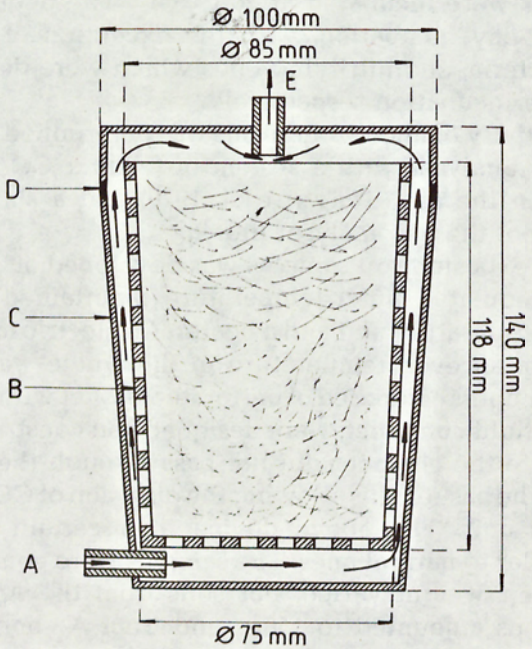

Fig. 3. Diagram of the respiration chamber. A — air inlet; B — basket with soil sample; C — hermetic housing; D — packing; E — air outlet

The respiration of bacteria was measured using the Clark oxygen electrode Digitalmeter DIGI 610. The bacterial fraction was isolated from the soil supernatant (100 g of fresh soil in 1 dm^3 H_2O, shaken for 20 min, and decanted after a 10-minute settling), using the method of membrane filters produced by Sartorius. In this fraction oxygen losses were determined after 12—16 h culture at 20 ± 1°C. The respiration of one cell in mg O_2 $24\ h^{-1}$ and then the value of bacterial respiration per 1 g of soil dry mass in mg O_2 $24\ h^{-1}$ were calculated from the number of bacteria in the culture and the amount of oxygen consumed by the bacteria during a given time.

The amounts of released CO_2 and assimilated O_2 were converted to nergy units and given in joules (J) g^{-1} of soil dry weight 24 h^{-1}.

Dependences concerning the total respiration of micro-organisms in soils with various doses of dusts were subjected to statistical analysis. The regression lines (traits y — the amount of energy released from the environment due to the respiration of micro-organisms, in relation to traits x — different doses of dusts) were plotted in the linear, power, exponential, and logarithmic form, and the coefficients of non-linear correlation (Krysicki et al. 1986) were calculated.

The proteolytic and cellulolytic activity of soils and the activity of acidic and alkaline phosphatases were determined in samples collected in 1987.

The proteolytic activity was determined using the viscosimetric method and quoted as a percentage decrease in gelatine viscosity at 30 °C, after a 48 h incubation of the soil with a medium at 37°C (Russel 1972).

The cellulolytic activity was determined using the Samogyi-Nelson method of reducing sugars produced by enzymatic hydrolysis of MC cellulose mixed with the soil and incubated at 37°C during 24 h in the presence of toluene. The results are given as the amount of reducing sugars in mg 100 g^{-1} of soil (Russel 1972).

The activity of acidic and alkaline phosphatases was colorimetrically determined on the basis of hydrolysis of sodium phenylophosphate and given as the amount of released phenol in mg 100 g^{-1} of soil (Russel 1972).

4. Results

4.1. Physical and chemical characteristics of soils

The thermal analysis of soils shows that the A_{oF+H} horizon contains poorly humified organic matter (mor). Its average content in the control soil was 41%; in the soil with a dose of 100 t km^{-2} $year^{-1}$ the content of organic matter was larger than in the control, amounting to 63%, and in soils with the successive doses of dusts (500, 1000, and 2000 t km^{-2} $year^{-1}$) it was 31% on the average. In the soil with the smallest dose of cadmium dusts

(100 t km^{-2} $year^{-1}$) the content of organic matter also exceeded that in the control, amounting to 47%, while in soils treated with the other doses of these dusts it was about 33% on the average.

In the A_1 layer there mainly occurs strongly dried muck of great hydrophobicity. The content of organic matter was much smaller than in the A_{oF+H} layer and amounted on average to 7% in the control soil, 9% in soils with zinc dusts, and 11.5% in soils with cadmium dusts.

Quartz is the chief mineral component of the investigated soils. Its average content was 67% in the control soil in the A_{oF+H} layer and over 92% in the A_1 layer. A similar content of quartz was characteristic for soils with dusts, though it was larger by 3% in layer A_{oF+H} and smaller by 1% in layer A_1.

In all soils calcite was absent, while trace amounts of kaolinite were found.

The determined physical properties showed a low volumetric weight (0.87%), great porosity (61.2%) and a great capillary capacity (59.8%) of the control soil. In plots with dusts a deterioration of the physical properties of the soil was observed. With the largest doses of dusts (2000 t km^{-2} $year^{-1}$, the volumetric weight of the soil distinctly increased (1.23%), while porosity and water capacity decreased (45% and 45.6%, respectively).

The reaction of the control soil varied from 3.5—4.6 in the A_{oF+H} horizon and from 4.2—4.3 in the A_1 layer. In soils with dusts the values of pH were slightly higher: from 4.5—6.3 in the A_{oF+H} horizon and 4.9 and 5.5 in the A_1 layer. In fertilized soils (A_{oF+H} layer) treated with cadmium dusts the pH values were usually slightly higher, i.e. 4.5 and 4.6.

With an increase in the doses of zinc or cadmium dusts, the content of total zinc increased from 162 to 4747 and of soluble zinc from 136 to 1052 mg kg^{-1} of soil dry weight and of total cadmium from 14 to 4740 and of soluble cadmium from 0.3 to 45.6 mg kg^{-1} of soil dry weight in the A_{oF+H} layer of the soil (Table II).

Trace amounts of nitrogen varied from 0.021—0.036 and from 0.001—0.008 mg g^{-1} of soil dry weight for the nitrate and ammonia forms, respectively.

The greatest content of organic carbon (220 mg g^{-1} d.w. of soil) was found in the non-fertilized soil treated with the smallest dose of dusts (100 t km^{-2} $year^{-1}$) and in the fertilized soil (207.6 mg g^{-1} d.w.) with the largest dose (2000 t km^{-2} $year^{-1}$). In the control plot the content of carbon was smaller, amounting on the average to 184 mg g^{-1} of soil dry weight.

In the soil from the „mini" plots the content of organic carbon was distinctly greater in combinations containing larger doses of dusts (1000 and 2000 t km^{-2} $year^{-1}$) than in the control soil and that treated with smaller doses. The amounts of phosphorus, calcium, and magnesium were low and varied around 0.20—0.58 P, 0.41—0.76 Ca, and 0.11—0.73 Mg mg g^{-1} d.w. of soil. Potassium occurred in increased amounts in the range

Table III. Characteristics of soils from experimental plots exposed to the action of different doses of cadmium dusts (in 10^2 t km^{-2} $year^{-1}$): non-fertilized soils, mineral fertilized soils, and soil from"mini" plots, on the basis of selected chemical factors (in mg g^{-1} d.w. of soil) in 1987. W/D - coefficient of humidity, W - wet weight, D - dry weight

Factors	Plots													
	non-fertilized					mineral fertilized				"mini"				
	0	1	5	10	20	1	5	10	20	0	1	5	10	20
pH	4.2	3.9	3.9	4.1	4.3	4.5	4.1	4.3	4.6	4.4	4.1	3.9	4.0	3.5
W/D	1.87	2.14	1.77	1.93	1.79	1.98	1.73	1.99	1.83	1.45	1.54	1.70	1.66	1.85
C-org.	184.000	220.000	218.200	175.000	189.000	198.000	158.700	198.800	207.600	141.000	135.100	138.000	218.100	210.700
$N\text{-}NO_3$	0.021	0.056	0.025	0.036	0.023	0.041	0.023	0.026	0.022	0.013	0.014	0.025	0.017	0.020
$N\text{-}NH_4$	0.002	0.006	0.003	0.005	0.002	0.008	0.005	0.005	0.005	0.001	0.003	0.003	0.003	0.003
S-org.	2.187	6.185	24.453	28.119	26.511	4.682	11.376	19.849	32.320	0.884	1.310	14.831	11.061	11.135
$S\text{-}SO_4$	1.125	2.811	9.038	22.495	16.790	2.861	8.191	17.339	30.499	0.252	0.910	11.123	10.176	25.450
P	0.250	0.587	0.268	0.396	0.260	0.489	0.245	0.252	0.224	0.155	0.156	0.304	0.201	0.249
K	0.457	0.781	0.268	0.111	0.104	0.312	0.213	0.205	0.455	0.207	0.237	0.421	0.452	0.398
Ca	0.939	1.500	0.469	1.511	0.416	1.769	0.455	0.629	0.884	0.397	0.456	1.035	0.678	0.954
Mg	0.214	0.369	0.157	0.325	0.112	0.728	0.227	0.230	0.213	0.097	0.114	0.217	0.156	0.172
Pb	3.600	12.596	8.160	19.172	34.243	8.739	11.431	17.115	35.279	0.148	2.911	9.032	8.263	33.509
Fe	4.941	6.748	5.563	6.937	4.678	8.115	5.597	4.782	4.998	3.335	3.377	5.108	4.429	5.044
Zn	0.162	0.506	1.101	2.372	3.089	0.468	1.352	1.679	3.678	0.072	0.153	1.481	1.889	4.454
Cd	0.029	0.430	0.473	1.870	2.210	0.328	0.478	1.600	4.740	0.014	0.144	0.723	1.510	3.360

0.10—1.11 mg g^{-1} d.w. of soil. Also the content of sulphur in the soil was increased, the greatest amounts of both the organic and sulphate forms being found in the soils with the largest dose of dusts (28.1—32.3 S-org. and 22.5—30.5 S—SO_4 mg g^{-1} d.w. of soil). The smallest content of sulphur occurred in the control soil (0.9—2.2 S-org. and 0.2—1.1 S—SO_4 mg g^{-1} d.w. of soil). Of heavy metals determined in the soils total lead occurred in the greatest quantities (0.1 — 34.2 mg g^{-1} d.w.), followed by iron (3.3—8.1), zinc (0.1—4.4), and cadmium (0.03—4.7). In general, the content of these metals increased with increasing doses of dusts. This was particularly pronounced in the case of lead and especially of cadmium, whose content in the control soil constituted only 1% of the amount found in the fertilized and non-fertilized soils with the greatest dose of dusts (Table III).

The humidity of soils hardly varied during the period of the investigation. The mean annual value of the W/D coefficient showed a slightly greater humidity in soils treated with industrial dusts, this being most distinct in the soils of the "mini" plots (Table III).

4.2. Effect of long-term action of zinc and cadmium dusts on the development and activity of soil microflora

As compared with fungi and Actinomycetes, heterotrophic bacteria constituted the most numerous group at the A_{oF+H} horizon both in the control plot and in soils with cadmium or zinc dusts. The difference in

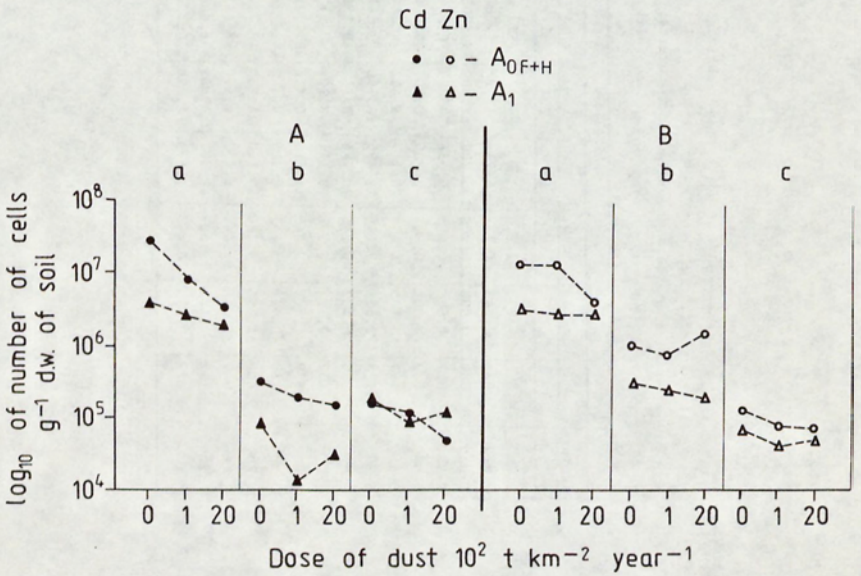

Fig. 4. Number of micro-organisms in two soil horizons — A_{oF+H} and A_1 — on non-fertilized plots affected with dusts for a long time. A — cadmium dusts; B — zinc dusts; a — total number of heterotrophic bacteria; b — fungi; c — Actinomycetes

numbers in favour of bacteria was considerable, reaching 1—2 orders of magnitude compared with fungi and 2—3 orders of magnitude compared with Actinomycetes. Heterotrophic bacteria were also characterized by greater differences in numbers between the control soil and soils with the two kinds of dust (fig. 4 A, B).

In the A_1 layer of the investigated soils there were similar quantitative relations between the groups of identified micro-organisms. The quantitative predominance of bacteria was also observed here, while fungi occurred in smaller numbers than Actinomycetes in soils with cadmium dusts, contrary to soils treated with zinc dusts (fig. 4 A, B).

In soils with cadmium dusts, of the group of bacteria transforming nitrogen compounds ammonifying (10^7—10^8 cells g^{-1} d.w. of soil) and denitrifying (10^6—10^7 cells g^{-1} d.w. of soil) bacteria were most numerous. In the control soil and in that with the smallest dose of cadmium dusts (100 t km^{-2} $year^{-1}$) the number of proteolytic and nitrifying 1-st phase bacteria was reduced and uniform (10^4 cells g^{-1} d.w. of soil), while in the soil treated with 2000 t km^{-2} $year^{-1}$ of these dusts the number of proteolytic, and especially of 1-st phase nitrifiers was decisively reduced, reaching the order of 10^3 cells g^{-1} d.w. of soil (fig. 5A). Considerable

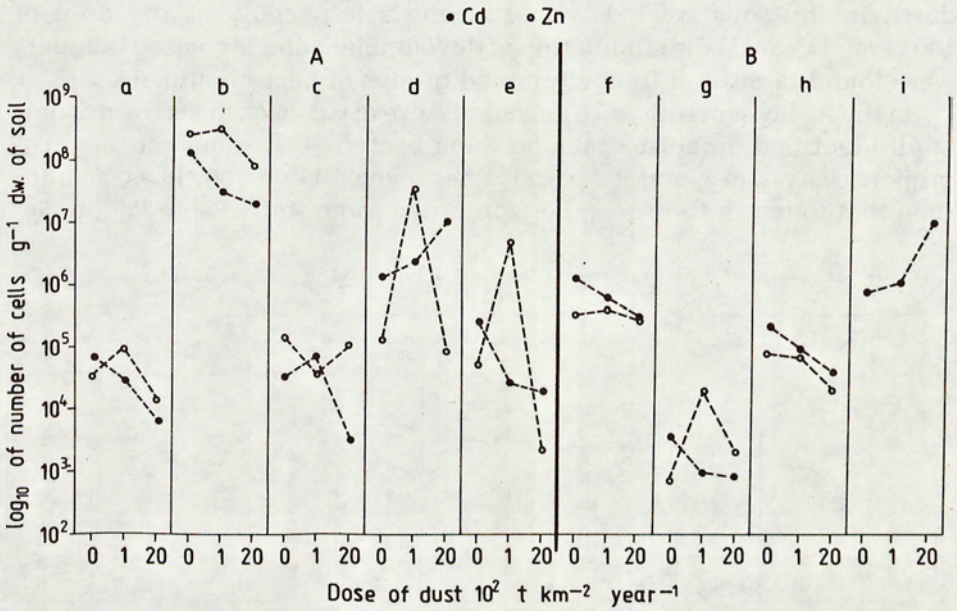

Fig. 5. Number of bacteria in the A_{oF+H} horizon of non-fertilized soils exposed to long-term action of cadmium and zinc dusts. A — bacteria transforming nitrogen compounds; a — proteolytic bacteria; b — ammonifying bacteria; c — nitrifying 1st phase bacteria; d —denitrifying bacteria; e— anaerobic nitrogen-fixing bacteria. B — bacteria transforming nitrogen-free compounds: f — amylolytic bacteria; g — cellulolytic bacteria; h — phosphorous bacteria; i — H_2S-releasing bacteria

differences, of almost one order of magnitude, were also found in the group of anaerobic nitrogen-fixing bacteria between the control soil and soils with 100 and 2000 t km^{-2} $year^{-1}$ of cadmium dusts (this corresponding to 26.4 and 528 kg of total Cd ha^{-1}) (fig. 5A).

Of bacteria converting nitrogen-free compounds (amylolytic and cellulolytic bacteria) and phosphorous and sulphuric compounds, the bacteria decomposing protein compounds with a release of H_2S, prevailed (10^5—10^7 cells g^{-1} d.w. of soil) (fig. 5B).

In the A_{oF+H} layer of soils treated with zinc dusts similar quantitative relations were observed among the groups of bacteria with different biochemical properties. However, as compared with soils treated with cadmium dusts, the number of ammonifying and cellulolytic bacteria was much larger in soils with the two doses of zinc dusts (100 and 2000 t km^{-2} $year^{-1}$, corresponding with 177.2 and 3544.0 kg Zn_{tot} ha^{-1}), while the denitrifying and anaerobic nitrogen-fixing bacteria prevailed in the variant with the dose of 100 t km^{-2} $year^{-1}$ (fig. 5A, B) of these dusts.

In general, the largest numbers of different groups of bacteria were found in the control soil and decreased in those with increasing doses of cadmium dusts. An exception was constituted by denitrifying and H_2S--releasing bacteria whose numbers increased with the doses of cadmium dusts. In the soils treated with zinc dusts (especially at the dose of 100 t km^{-2} $year^{-1}$) the stimulation of development and increased numbers were found in most of the determined groups of bacteria (fig. 5A, B).

In the A_1 layer of soils with and admixture of Cd or Zn dusts the number of all identified organisms, among them bacteria of various biochemical properties, was in general several times, even 10 and 100 times, smaller then that found in the A_{oF+H} horizon of the same soils (Table IV, fig. 5).

Table IV. Average numbers of micro-organisms in the mineral layer (A_1) of soils with cadmium and zinc dusts in 1986

Kind of dust	Dose of dust 10^2 t km^{-2} $year^{-1}$	Total number of			Bacteria decomposing nitrogen compounds					Bacteria decomposing nitrogen-free compounds			
		heterotrophic bacteria	fungi	Actinomycetes	proteolytic	ammonifying	nitrifying - 1st phase	denitrifying	fixing nitrogen	amylolytic	cellulolytic	phosphorous	realising H_2S
Cd	0	$4.0 \cdot 10^6$	$8.5 \cdot 10^4$	$1.9 \cdot 10^5$	$1.0 \cdot 10^4$	$2.4 \cdot 10^7$	$1.1 \cdot 10^3$	$2.0 \cdot 10^5$	$2.9 \cdot 10^4$	$1.6 \cdot 10^5$	$4.7 \cdot 10^2$	$1.2 \cdot 10^5$	0
	1	$2.3 \cdot 10^6$	$1.3 \cdot 10^4$	$9.9 \cdot 10^4$	$5.4 \cdot 10^3$	$3.3 \cdot 10^6$	$2.5 \cdot 10$	$3.1 \cdot 10^4$	$1.7 \cdot 10^3$	$2.4 \cdot 10^5$	$2.7 \cdot 10^2$	$1.0 \cdot 10^5$	0
	20	$2.1 \cdot 10^6$	$3.2 \cdot 10^4$	$1.3 \cdot 10^5$	$5.5 \cdot 10^3$	$6.8 \cdot 10^6$	$2.8 \cdot 10$	$1.3 \cdot 10^5$	$1.3 \cdot 10^3$	$3.3 \cdot 10^5$	$1.4 \cdot 10^2$	$7.4 \cdot 10^4$	0
Zn	0	$3.5 \cdot 10^6$	$3.1 \cdot 10^5$	$6.9 \cdot 10^4$	$5.1 \cdot 10^7$	$1.9 \cdot 10^7$	$1.9 \cdot 10^2$	$9.0 \cdot 10^4$	$2.0 \cdot 10^3$	$1.8 \cdot 10^5$	$5.1 \cdot 10^2$	$8.7 \cdot 10^3$	0
	1	$3.1 \cdot 10^6$	$2.4 \cdot 10^5$	$3.8 \cdot 10^4$	$1.0 \cdot 10^4$	$1.6 \cdot 10^7$	$1.9 \cdot 10^4$	$2.3 \cdot 10^5$	$3.0 \cdot 10^4$	$1.7 \cdot 10^5$	$4.1 \cdot 10^2$	$9.8 \cdot 10^3$	0
	20	$3.8 \cdot 10^6$	$2.2 \cdot 10^5$	$5.1 \cdot 10^4$	$3.5 \cdot 10^3$	$1.9 \cdot 10^7$	$1.2 \cdot 10^4$	$2.3 \cdot 10^5$	$1.9 \cdot 10^3$	$1.1 \cdot 10^5$	$1.2 \cdot 10^3$	$1.1 \cdot 10^4$	0

The total microbiological activity, expressed by the amount of energy released by the fermentative-humus layer (the A_{oF+H} horizon) on account of the metabolic action of micro-organisms, varied in the control soil from 9.4—18.8 J g^{-1} d.w. of soil 24 h^{-1} (fig. 6 A, B).

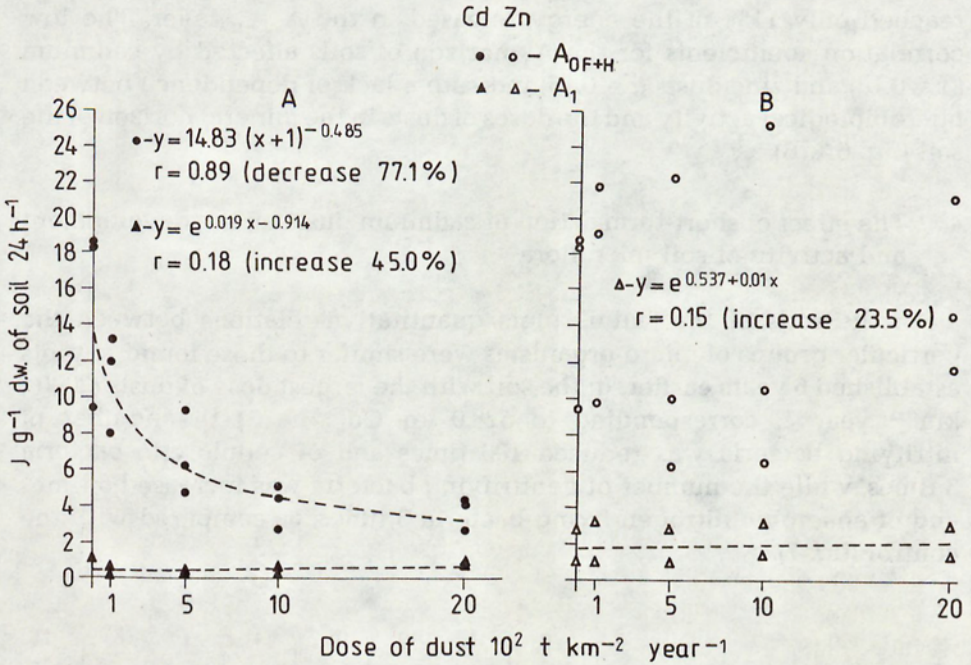

Fig. 6. Total microbiological activity in layers A_{oF+H} and A_1 of non-fertilized soils exposed to long-term action of dusts. A — cadmium dusts; B — zinc dusts

In the case of soils affected by cadmium dusts the activity of micro-organisms in the same layer was several times poorer. The regression curve indicates a distinct decrease in the respiration of micro-organisms with the growing doses of these dusts (fig. 6A). In the soil treated with the largest dose of cadmium dusts (2000 t km^{-2} $year^{-1}$) the respiration was only 2.6—4.4 J g^{-1} d.w. of soil 24 h^{-1}, this showing a decrease in the decomposition of organic matter by about 77% of the control.

In the A_{oF+H} horizon of soils with zinc dusts the amount of energy released from the environment varied greatly, from 6.3—21.2 J g^{-1} d.w. of soil 24 h^{-1} (fig. 6B). This suggests that there a periodical stimulation or inhibition of microbiological processes occurred as compared with the control soil (fig. 6B).

The dependence between the activity of micro-organisms and the dose of zinc dusts cannot be described by a regression equation, showing a lack of correlation between these values. In the case of cadmium dusts, the regression curve is characterized by a high correlation coefficient, r = 0.89,

distinctly confirming the inhibition of microbial activity and, hence, of the decomposition of organic matter in soils treated with these dusts (fig. 6A, B).

In the A_1 layer of the control soil and the soils with an admixture of cadmium or zinc dusts the energy release due to microbiological processes reached only 11% of the energy released in the A_{oF+H} layer. The low correlation coefficients for the A_1 horizon of soils affected by cadmium ($r = 0.18$) and zinc dusts ($r = 0.15$) indicate a lack of dependence between microbiological activity and the doses of dusts in the mineral horizon of the soil (fig. 6A, B).

4.3. The effect of short-term action of cadmium dusts on the development and activity of soil microflora

In the soils of the "mini" plots quantitative relations between the particular groups of micro-organisms were similar to those found in plots established 6 years earlier. In the soil with the largest dose of dusts (2000 t km^{-2} $year^{-1}$, corresponding to 528.0 kg Cd_{tot} ha^{-1}) the number of nitrifying bacteria was reduced 100 times and of cellulolytic bacteria 3 times, while the number of denitrifying bacteria was increased 5 times and of anaerobic nitrogen-fixing bacteria 3 times, as compared with the control (fig. 7).

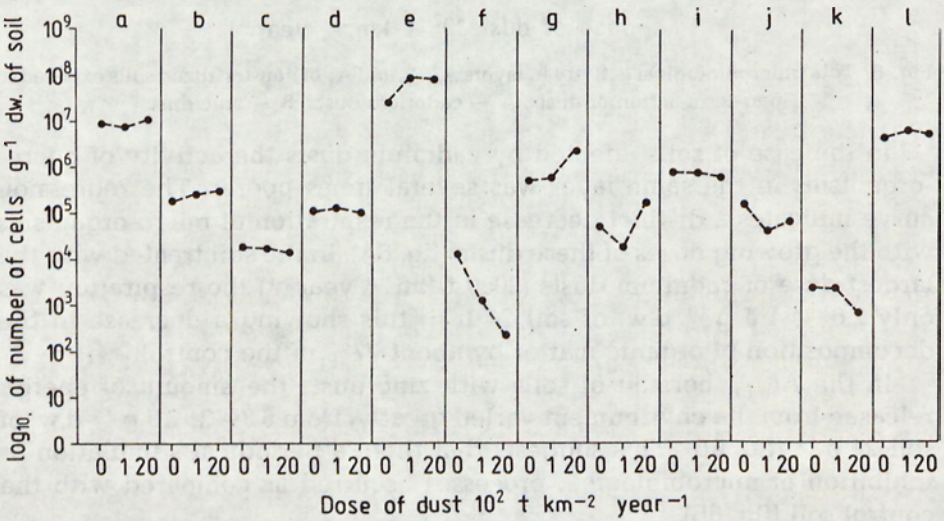

Fig. 7. Total number of micro-organisms in soil of "mini" plots exposed to short-term action of cadmium dusts. a — total number of heterotrophic bacteria; b — fungi; c — Actinomycetes. Bacteria: d — proteolytic; e — ammonifying; f — nitrifying 1st phase; g — denitrifying; h — anaerobic nitrogen fixing; i — amylolytic; j — cellulolytic; k — phosphorous; l — H_2S-releasing

On the other hand, microbial activity was stimulated and increased with increasing doses of dusts. On the first sampling date (23 June, 1987), i.e. within 53 days of the introduction of dusts into the soils, the obtained dependences approximated to the probability limit (r = 0.56) and in the soil with the largest dose of dusts the microbial activity exceeded that found in the control soil by more than 35%. In the same variant of the experiment the smallest increase in the activity (by 28%) was found towards the end of summer (1 September, 1987), i.e. within 5 months of the dust treatment, a much higher correlation coefficient, r = 0.85 being obtained there. The greatest increase in the activity of micro-organisms, by 139% with r = 0.97, was found in autumn (20 October, 1987), i.e. six months after initiation of the experiment (fig. 8). As the period of the action of dusts was prolonged, the

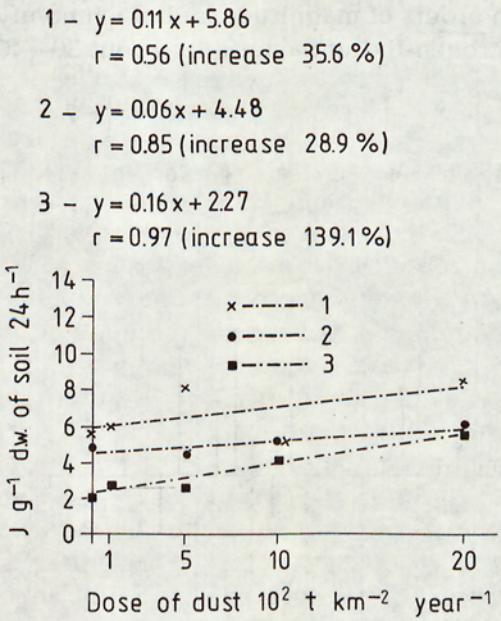

Fig. 8. Total microbial activity of soils from "mini" plots exposed to short-term action of cadmium dusts, on three dates of sampling: 1—26 June, 2—1 September, and 3—20 October, 1987

amount of the diel release of energy from the fermentation-humus layer (A_{oF+H}) decreased from the value of 5.57 to 2.14 J g^{-1} d.w. 24 h^{-1} in the control soil and from 8.49 to 5.50 J g^{-1} d.w. 24 h^{-1} in that with the largest dose of dusts (fig. 8).

4.4. The effect of mineral fertilization of soils degraded by the long-term action of cadmium dusts on the development and activity of microflora

The fertilization (with NPK and magnesium-dolomite lime) of plots treated with cadmium dusts brought about an increase in the numbers of microflora in the soils. The favourable effect of fertilization was more distinct in the soil with the smallest dose of dusts (100 t km^{-2} $year^{-1}$), where the numbers of fungi and Actinomycetes, and of bacteria of different physiological properties, increased from a few to 10 times. Nevertheless, it should be stressed that in the soils where the applied dose of dusts was 20 times greater (2000 t km^{-2} $year^{-1}$) the numbers of nitrifying and cellulolytic bacteria distinctly rose (5 and 7 times, respectively). The numbers of bacteria decomposing protein compounds with a release of H_2S, however, decreased by two orders of magnitude and of ammonifying, denitrifying, and anaerobic nitrogen-fixing bacteria by about 30—40% (fig. 9).

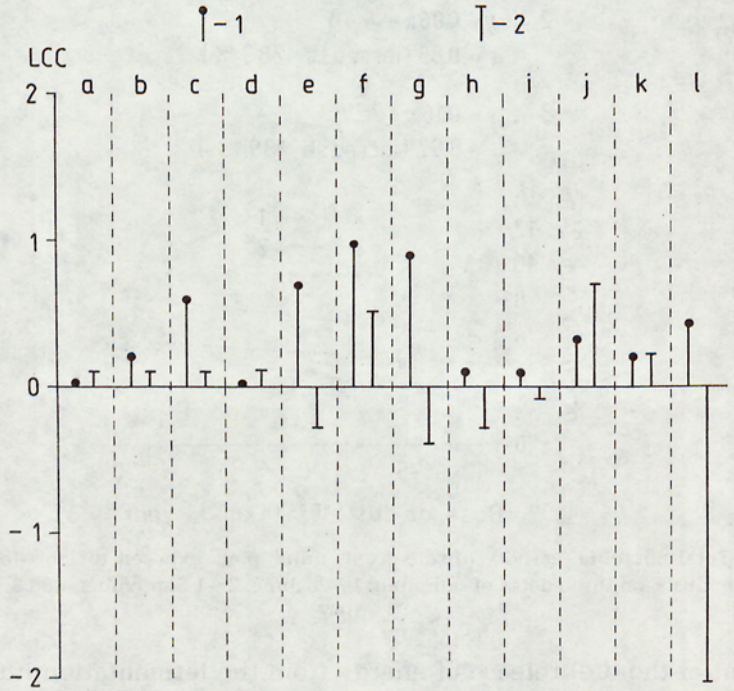

Fig. 9. Comparison of numbers of micro-organisms in fertilized and non-fertilized soils exposed to long-term action of cadmium dusts. 1 — dose of $1 \cdot 10^2$ t km^{-2} $year^{-1}$, 2 — dose of $20 \cdot 10^2$ t km^{-2} $year^{-1}$. LCC — logarithmic coefficient of comparison. Explanations of symbols a—l as in fig. 7

In the control soil the activity of micro-organisms was slightly less intense than in the preceding year, as was shown by the amount of energy released, varying from 8.4—12.9 J g^{-1} d.w. of soil 24 h^{-1} (fig. 10).

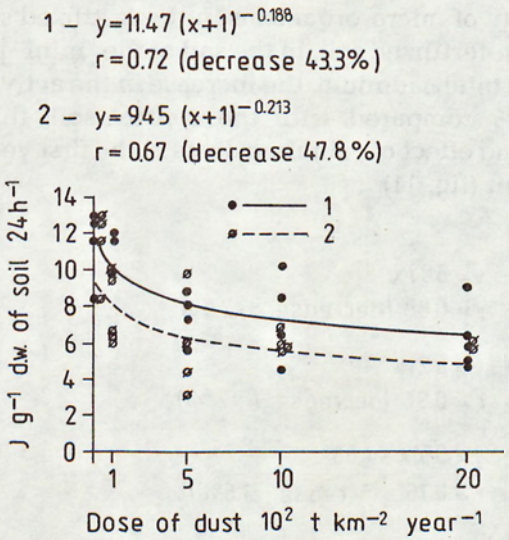

Fig. 10. Total microbial activity in soils exposed to long-term action of cadmium dusts. 1 — fertilized soils; 2 — non-fertilized soils

With larger doses of dusts the microbial activity of soils decreased and the amount of energy released from 1 g d.w. of soil during 24 h varied from 3.1—9.8 J in the non-fertilized soil and from 4.6—12.0 J in the fertilized one. With the largest dose of dusts (2000 t km^{-2} $year^{-1}$) the decrease of respiration of micro-organisms amount to 47.8% with the correlation coefficient r = 0.67 in the non-fertilized and 43.3% (r = 0.72) in the fertilized soil (fig. 10).

4.5. Comparison of microbial activity in fertilized and non-fertilized soils subjected to the short and long-term action of cadmium dusts

In order to compare the activity of micro-organisms in the fertilized and non-fertilized soils and in the soil from the „mini" plots with the same content of total cadmium, the regression curves were plotted for the range of concentrations of this element varying from 0—2.0 mg g^{-1} d.w. of soil. The correlation coefficients illustrating these dependences were high, reaching r = 0.96 for non-fertilized soils, r = 0.88 for fertilized ones, and r = 0.75 for the soil from the „mini" plots. With a cadmium concentration of 2.0 mg g^{-1} d.w. of soil (corresponding to the dose of dusts of 498.0 t km^{-2} $year^{-1}$, and in this amount 131.5 Cd kg ha^{-1} in the non-fertilized soil, 668.9 t km^{-2} $year^{-1}$ and 176.6 Cd kg ha^{-1} in the fertilized soil, and 2000 t km^{-2} $year^{-1}$ and 528.0 Cd kg ha^{-1} in the soil of the "mini" plot) the amount of energy released from the fertilized soil exceeded by about 27% that from the non-fertilized soil. However, in relation to the control soil the

decrease in the activity of micro-organisms in the fertilized soil was 12% smaller than in the non-fertilized soil. In the soil of the "mini" plot with the same concentration of total cadmium, the increase in the activity of micro-organisms was 21.6% compared with the control soil, this distinctly showing the stimulating effect of cadmium dusts in the first year after their introduction to the soil (fig. 11).

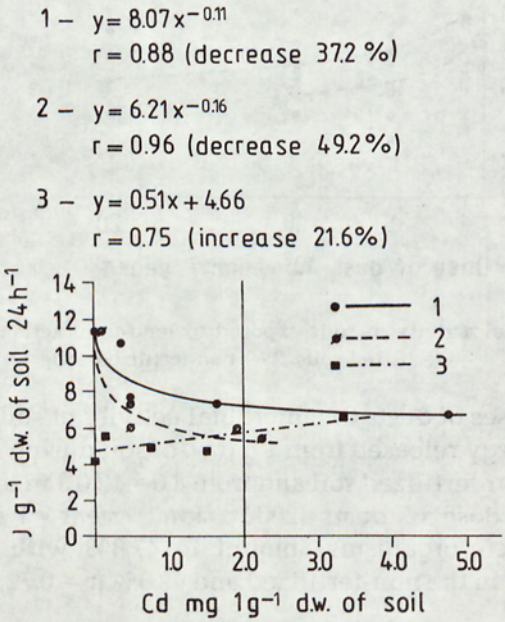

Fig. 11. Total microbial activity in soils at a total cadmium concentration of 0—2 mg g^{-1} of soil dry weight. 1 — fertilized soils; 2 — non-fertilized soils; 3 — soils from "mini" plots

4.6. The activity of the bacterial fraction isolated from soils after long-term action of cadmium dusts

The bacterial decomposition of organic matter in the soil was determined in the period June 1986-October 1987. It was found that the cellular activity of bacteria increased with larger doses of cadmium dusts. As compared with the control soil, cellular activity increased 4 times in the soil with the largest dose of dusts (2000 t km^{-2} $year^{-1}$), the content of organic matter in the soil being reduced almost 1.5 times and the amount of energy released from the soil as a result of the bacterial metabolism being decreased almost twofold (fig. 12).

During the entire period of the investigation (1986—1987) the role of bacteria in the total microbiological decomposition of organic matter was reduced by 4.6% in the control soil and in that treated with the smallest

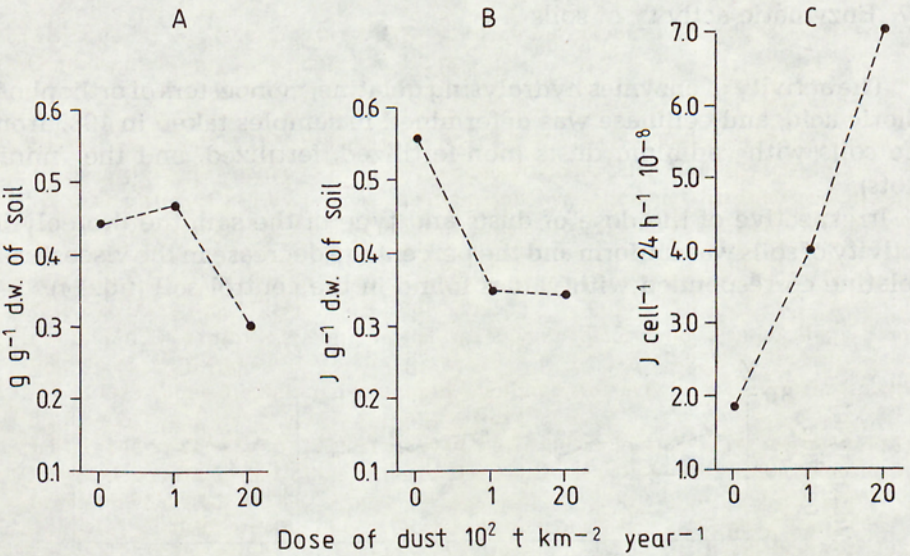

Fig. 12. Content of organic matter — A, bacterial activity — B, and bacterial cellular activity — C in non-fertilized soils exposed to long-term action of cadmium dusts

dose of cadmium dusts (100 t km^{-2} year^{-1}) but considerably increased (7.2%) in the soil with the dose of 2000 t km^{-2} year^{-1} of these dusts (fig. 13).

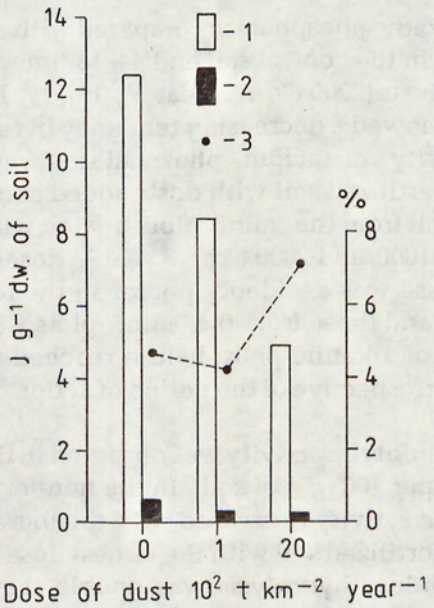

Fig. 13. Share of bacteria in total microbial activity in non-fertilized soils exposed to long-term action of cadmium dusts. 1 — total activity of soil micro-organisms; 2 — activity of bacteria; 3 — percentage share of bacterial activity in total activity of soil micro-organisms

4.7. Enzymatic activity of soils

The activity of enzymes hydrolysing gelatine, monoesters of orthophosphoric acid, and cellulase was determined in samples taken in 1987 from the soils with cadmium dusts (non-fertilized, fertilized, and the "mini" plots).

Irrespective of the dose of dusts and type of the soil, the proteolytic activity of soils was uniform and the percentage decrease in the viscosity of gelatine corresponded with values found in the control soil (fig. 14).

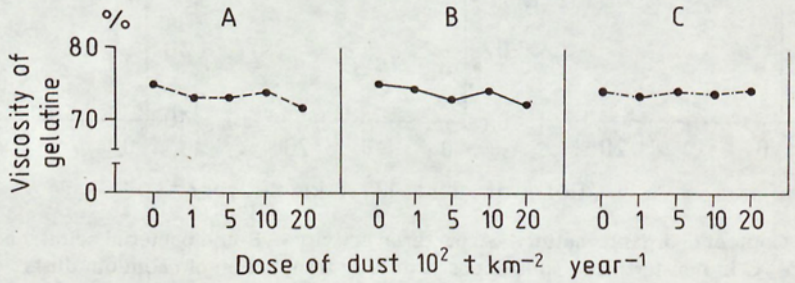

Fig. 14. Proteolytic activity in non-fertilized soils — A, and fertilized soils — B exposed to long-term action of cadmium dusts, and in soils from "mini" plots exposed to short-term action of these dusts

The activity of acidic phosphatase compared with alkaline phosphatase was 5 times greater in the control soil and 4—18 times greater in soils with the largest dose of dusts (2000 t km^{-2} year^{-1}) (fig. 15). In all kinds of soil the two phosphatases showed a decreasing tendency in relation to the control. The poorest activity of acidic phosphatase was observed in the non-fertilized and fertilized soil with dusts added at a dose of 1000 t km^{-2} year^{-1} and in the soil from the „mini" plots treated with the largest dose of dusts. With the 500, 1000, and 2000 t km^{-2} year^{-1} doses of dusts the activity of acidic phosphatase was decidedly poorer in the fertilized soil than in non-fertilized ones and those from the "mini" plots with the same doses of dusts. The activity of alkaline phosphatase reached a similar level in all three kinds of soil, irrespective of the period of action of the cadmium dusts (fig. 15).

The greatest cellulolytic activity was observed in the control soil (42 mg of reducing sugars per 100 g^{-1} of soil). In the non-fertilized and fertilized soils the cellulolytic activity decreased with the increasing doses of dusts (fig. 16 A, B). In the fertilized soil with the largest dose (2000 t km^{-2} year^{-1}) the activity of cellulose hydrolysis was double that found in the non-fertilized soil treated with the same dose (fig. 16 A, B). In the soil from the "mini" plots a tendency to increase cellulolytic activity appeared with the increasing doses of cadmium dusts (fig. 16 C).

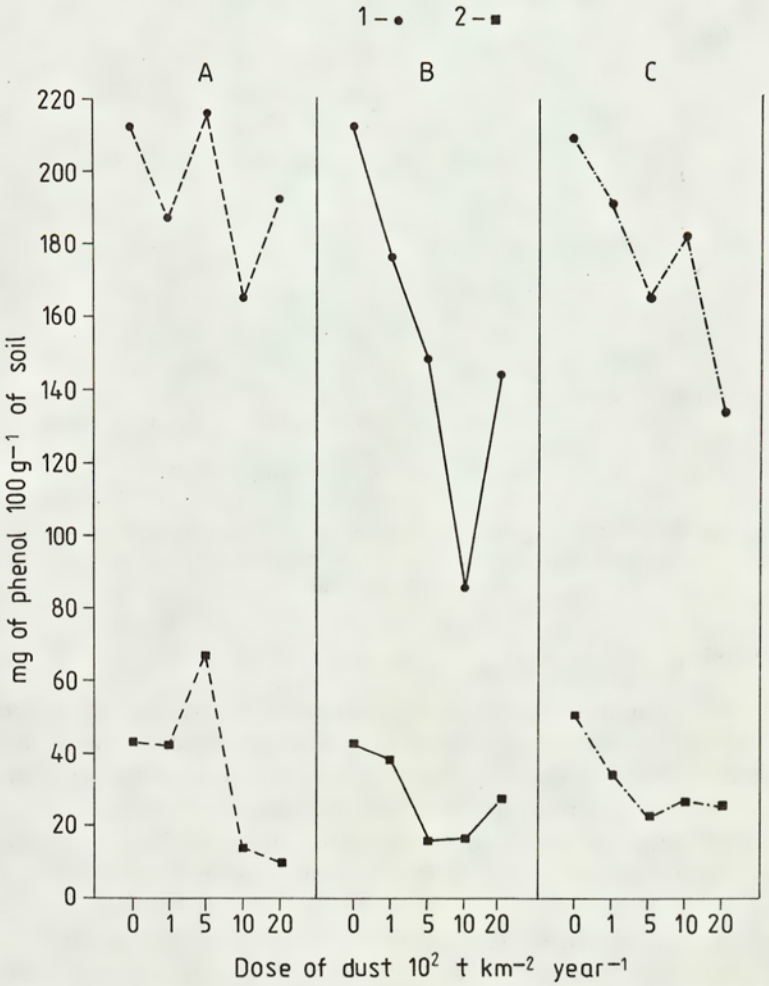

Fig. 15. Activity of acidic phosphatase — 1 and alkaline phosphatase — 2, in bon-fertilized soils — A, fertilized soils — B, exposed to long-term action of cadmium dusts, and in soil from "mini" plots exposed to short-term action of these dusts

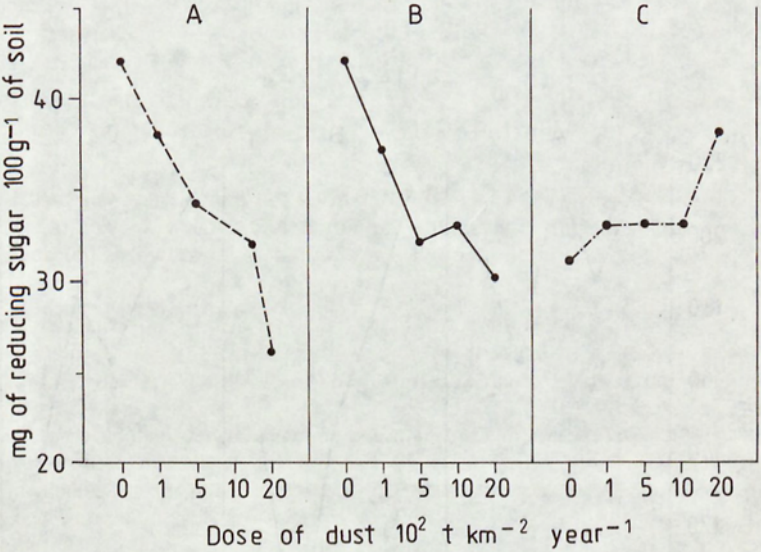

Fig. 16. Cellulolytic activity in non-fertilized soils — A, fertilized soils — B, exposed to long-term action of cadmium dusts, and in soil from "mini" plots exposed to short-term action of these dusts

5. Discussion

The aim of the microbiological studies of soils carried out in conditions of model field experiments in the Niepołomice Forest, was to determine the effect of industrial dusts containing heavy metals on the structure of the populations and the function of micro-organisms.

Micro-organisms are an important link in the cycling and transport of nutrients and pollutants and in the energy flow in the environment. The important function of micro-organisms, which constitute only 2—3% of the total biomass of the forest ecosystem (Richards 1979), is associated with their enormous metabolic activity. This activity forms the balance between the production and destruction of organic matter and is decisive for the proper functioning of the ecosystem.

The degree of noxiousness of heavy metals for micro-organisms depends on the physical and chemical properties of the soil and on the sorptive capacity of the soil environment connected with them. The sorptive capacity depends on strong hydrogen bonds or poor Van der Waals's forces occurring between mineral particles of the soil and also on the occurrence there of organic substances, especially humic ones. The latter contain carboxyl (—COOH) and hydroxyl (—OH) groups which readily react with ions of heavy metals (Schnitzer, Kerndorff 1981), this favourably affecting quantitative and qualitative changes of micro-organisms and their activity in soils contaminated with heavy metals

(Babich, Stotzky 1980, Sterrit, Lesler 1980, Badura, Pacha 1984, Badura et al. 1984a, 1984c).

The occurrence of mor in the fermentative-humus horizon (A_{oF+H}) of the investigated soils showed that in this horizon processes of organic matter humification were taking place. This horizon was distinctly separated from the lower mineral layer (A_1) where the chief component was muck of great hydrophobicity, strongly dried and therefore little subject to microbiological decomposition. This was reflected in the larger numbers of micro-organisms in the A_{oF+H} horizon than in the A_1 layer (fig. 4, Table IV). The observed reduction in numbers of bacteria, fungi, and Actinomycetes in the soil with increasing doses of cadmium and zinc dusts, and hence of total cadmium from 26.4—528.0 kg ha^{-1} or total zinc from 177.2—3544.0 kg ha^{-1} (Table II), confirmed the reports of other authors about the quantitative reduction of micro-organisms as well as their selection under the influence of heavy metals (Bisessar 1982, Badura et al. 1983a, 1983c, Badura 1985). However, it should be stressed that the basic part of results of the present work to a certain degree concerns the remnants of deformations and disturbances brought about by the introduction of large doses of industrial dusts with a great content of cadmium or zinc 6 years earlier. Thus, the obtained data above all permit estimation of the degree to which the changes were maintained or compensated but not the direct response to the dust treatment. This part of the study was complemented by the experiment with "mini" plots.

The quantitative predominance of heterotrophic bacteria over fungi and Actinomycetes in both soil layers (A_{oF+H} and A_1), treated either with cadmium or zinc dusts, indicated that bacteria prevailed in these soils (fig. 4). On the other hand, the larger numbers of micro-organisms in the fermentation-humus layer than in the mineral horizon resulted from the considerable accessibility of nutritive substrates in the upper soil layers, which were characterized by greater resources of organic substances than the deeper horizons (fig. 4). Similar relations in the occurrence of micro-organisms in forest soils, as depending on depth and associated with the presence of organic compounds, were observed by Badura et al. (1976), Burges and Raw (1971), Krzemieniewska and Badura (1954).

As compared with bacteria and fungi, the number of Actinomycetes was more stable in the two soil layers (A_{oF+H}, A_1), smaller quantitative differences in these organisms being found in the soil treated with cadmium dusts (fig. 4). It is difficult to explain this phenomenon since the action of industrial emissions containing heavy metals on phytocoenoses and micro-organisms depends on numerous factors of the physical and chemical constitution of soils (Babich, Stotzky 1979, Badura et al. 1979a, 1979b, Strojan 1978). A significant role is also played by the adaptability of micro-organisms to abiotic factors of the environment,

associated with the mechanisms of resistance in the particular species or strains (Silver et al. 1976) and with the ability to immobilize heavy metals inside the cell (Jones et al. 1976). The smallest variation in the number of Actinomycetes in the investigated soils may also result from the delayed settling of organic materials by these organisms in comparison with other micro-organisms, and also from dependences of the succession type occurring amoung them (Smyłła 1982).

In general, the numbers of bacteria carrying out the conversion of nitrogen compounds in the fermentation-humus layer of soils with cadmium or zinc dusts decreased with increasing doses of dusts (fig. 5A). The only exception were the denitrifying bacteria, whose numbers increased with increasing concentrations of cadmium dusts. As compared with the control soil, the numbers of denitrifiers and anaerobic nitrogen-fixing bacteria were much larger in soils with the smallest dose of zinc dusts (100 t km^{-2} $year^{-1}$) (fig. 5A). In soils with cadmium dusts the improved development of denitrifying bacteria suggested deterioration of the oxygen conditions there and, hence, more favourable conditions for the reduction processes of nitrates. As was shown, the introduction of dusts in the soils brought about an increase in their bulk density associated with a decrease in porosity and water capacity. This results in the destruction of the structure and texture of soils and, in consequence, in their poorer aeration. Besides, cadmium causes coagulation of the coloidal fraction of the soil, this impeding the access of oxygen to spaces between soil lumps (Hattori T., R. Hattori 1976). The distinct stimulation of development of denitrifying and anaerobic nitrogen-fixing bacteria in soils with the smallest dose of zinc dusts (100 t km^{-2} $year^{-1}$) may be associated with the varied resistance of particular micro-organisms or their groups not only to different heavy metals but also to their different concentrations. Badura et al. (1984c) observed a good adaptation of bacteria isolated from forest soils to increased doses of zinc and copper (within the range 10^{-5}M——10^{-1}M). The mentioned authors also found that most bacteria investigated by them were more readily adapted to zinc than to copper and that these abilities were associated with individual species or strains of bacteria. Moreover, contrary to the very toxic cadmium, while zinc is a pollutant at greater concentrations in trace amounts it is a microelement indispensable for the normal functioning of micro-organisms.

In the group of nitric bacteria, ammonifiers occurred in the largest numbers (fig. 5A). This is not surprising, since numerous bacteria have the ability to deaminate amino acids, commonly occurring in the environment (Hobbie et al. 1968, Hall et al. 1970, Lichtfild, Prescott 1970) and utilized by bacteria as a source of nitrogen of carbon and nitrogen jointly (Halvorson 1972).

Similarly as nitric bacteria, the numbers of bacteria decomposing nitrogen-free compounds were smaller in soils treated with the two kinds

of dust (fig. 5B) than in the control. The more abundant development of bacteria decomposing protein compounds containing sulphur, observed with the growing concentrations of cadmium dusts, probably resulted from the greater resistance of these organisms to metals. As a consequence of the metabolism of these bacteria, H_2S released by them is bound with ions of metals and produces insoluble sulphides, harmless for the vital activity of these micro-organisms in the micro-habitat (Gadd, Griffiths 1978, Badura, Pacha 1984).

The smallest numbers of nitrifying and cellulolytic bacteria found in the soils are worth stressing (fig. 5A, B). Contrary to the remaining identified groups, these bacteria are highly specialized organisms and require precisely specified substrates for their development. The acidic reaction of soils and poorer oxygen conditions due to the devastated structure and texture of soils, especially those treated with the largest dose of dusts (2000 t km^{-2} $year^{-1}$), did not favour the development of nitrifying bacteria. Moreover, the presence of mor, in which the occurring nitrification process is poorer than in soils containing mull (Richards 1979), might have limited the development of these bacteria. Nor can the poorer competition in the uptake of ammonia nitrogen by nitrifying bacteria than by heterotrophic ones be excluded (Jansson 1958).

The poorer development of cellulolytic bacteria might have resulted from the very nature of exogeneous cellulolytic, bacterial, and fungal enzymes. The last named enzymes pass more readily to the solution and begin to decompose the substrate at a certain distance from the hyphae of the producer. And, reversely, bacterial cellulases are strongly adsorbed on the surface of the cell, the decomposition of cellulose occurring only with distinct contact of the organisms with the substrate, this limiting its accessibility for these bacteria (Russel 1974).

The measures of soil recultivation by mineral fertilizing and liming to a greater or lesser degree resulted in increased numbers of all groups of the identified micro-organisms in the soil with the smallest dose of cadmium-dusts (100 t km^{-2} $year^{-1}$). In the soil most devastated by the action of dusts (the dose of 2000 t km^{-2} $year^{-1}$) the effect of the fertilization was varied. It was manifested by a distinct increase in the number of nitrifying and cellulolytic bacteria but a decrease in the quantitative level of bacteria decomposing protein substances with the release of H_2S, and also a decrease in the numbers of ammonifying, denitrifying, and anaerobic nitrogen-fixing bacteria (fig. 9). An unequivocal elucidation of all these particular cases is difficult on account of the complexity of the problem conditioned by physical, chemical, and biological factors of microhabitats within the investigated soils (Burns 1977, Gadd, Griffiths 1978). The liming of soils contaminated by heavy metals restores their biological activity by producing better conditions for the development of most micro-organims (Balicka et al. 1977, Badura et al. 1982). It increases

the pH value of soils and enhances their sorptive capacity, and facilitates the exchange of Ca^{2+} ions weakly bound with the sorptive complex and heavy metal ions (Badura et al. 1983d). The favourable effect of liming also consists in the transformation of soluble salts of heavy metals into less soluble carbonates which are not so noxious for micro-organisms (Wolf et al. 1977, Wiklander 1980). On the other hand, different kinds of mineral fertilizers may increase (Roberge, Knowles 1967, Salonius, Mahendrappa 1975, Roberge 1976), decrease, or have no effect (Schalin 1967) on the numbers and activity of micro-organisms. Certain data suggest a decrease in the biomass of soil bacteria owing to nitrogen fertilization which, as was shown, may induce the formation of organic matter more resistant to decomposition and, hence, periodically limit the accessibility of carbon, an indispensable element in the development of micro-organisms (Bääth et al. 1981).

The "mini" experiment, whose aim was to compare the long- and short-term action of cadmium dusts on the development and activity of soil micro-organisms, showed that numbers and system of predominating groups of micro-organisms, and of bacteria alone, were similar to those found in plots treated 6 years earlier (fig. 4A, 5, 7). The only difference consisted in a smaller variation in the numbers of microflora between the control and treated plots (doses of 100 and 2000 t km^{-2} $year^{-1}$). This may be explained by the development of zymogenic microflora (Winogradski 1925) and by a less intense inhibition of the enzymatic activity of micro-organisms in general, owing to the inflow of fresh organic matter (Doelman 1978), as was the case in the first year of the action of dusts on soil, because of the rapid decline of higher plants.

The energy flow in soils, measured by the amount of CO_2 released, is frequently used in determining the magnitude of biological activity in various conditions and ecosystems (Reiners 1968). The release of CO_2 by the particular populations of micro-organisms is difficult to estimate, since its value varies not only throughout the year but also during shorter periods (Witkamp 1966). However, the use of a modified method, in which large soil samples (150 g) could be exposed, permitted increased exactitude of measurements of CO_2 released (owing to the metabolism of the total soil microflora) and comparison of the heterotrophic activity of soils contaminated to a varied degree by heavy metals.

Data concerning the energy flow through the populations of micro-organisms obtained in the first year of the experiment distinctly showed differences in the microbiological activity of soils as depending on the investigated soil layer and the applied cadmium or zinc dusts (fig. 6).

A decrease in the microbiological activity of soils with increasing doses of cadmium dusts and its reduction by about 77%, as compared with the control, in the soil with the largest dose (2000 t km^{-2} $year^{-1}$) demonstrated

a pronounced inhibition of the metabolic activity of micro-organisms affected by these dusts (fig. 6A).

In the soils with zinc dusts both the wide range of energy released in the course of microbial processes and the periodically enhanced decomposition of organic matter, as compared with the control soil, suggested that these dusts were less toxic and less active in relation to micro-organisms and that their effect depended to a greater degree on seasonal changes in environmental factors (fig. 6B).

The cadmium and zinc properties themselves might have accounted for the varied effect of the two kinds of dust on the activity of micro-organisms. Cadmium is an element of no known biological function and its accumulation in living organisms seriously disturbs the processes of cycling of matter and energy flow (Babich, Stotzky 1977a). Unlike cadmium, zinc is an indispensable microelement and plays an important role in various vital functions of micro-organisms. It is found in the composition of some cell enzymes and, in combination with nucleic acids, takes part in the normal functioning of ribosomes and in the processes of cell division. It also affects the maintenance of the proper cell wall structure of micro-organisms, which is an indispensable condition of the transport of different particles and compounds into and outside the cell (Babich, Stotzky 1978, 1983, Beveridge 1981, Badura, Pacha 1984). The toxicity of larger doses of zinc might have been periodically decreased by the action of ions of other metals also occurring in the dusts (McLead, Snell 1950, Bartlett et al. 1974). Finally, the zinc itself (applied at suitable doses) might have directly stimulated certain enzymes of soil bacteria (Searle, Hughes 1977, Badura et al. 1980) and hence favourably affected their activity and the decomposition of organic matter. In spite of great differences in the content of total zinc, correlated with the doses of zinc dusts, differences in the amount of soluble zinc, more toxic for micro-organisms, were slight in the investigated soils (Table II). Apart from other factors, this probably accounted for the lack of dependence between the dose of zinc dusts and the activity of micro-organisms. A similar negligible variation in the intensity of microbiological processes in soils with a large content of total zinc and a small content of soluble zinc was found by Badura et al. (1976). This finding was confirmed in model experiments which showed that increasing concentrations of zinc ions in the soil did not bring about any negative changes (even a stimulated development of microflora being sometimes observed) as long as the concentration of water-soluble ions, did not exceed the value for the given conditions (Badura et al. 1977).

Already in the first year after the introduction of NPK fertilizers and magnesium-dolomite lime in the recultivated soils with cadmium dusts the activity of micro-organisms increased on the average by almost 30%, as compared with non-fertilized soils (fig. 10). This showed that the recultiva-

tion measures improved the physical, chemical, and biological properties of soils degraded by the long-term action of heavy metals introduced with dusts. As already mentioned, liming contributes to an increase in the sorption of heavy metals by humic acids (Wolf et al. 1977). Owing to the decreased toxicity of metals and the action of mineral fertilization, there occures an increase in the development of most groups of micro-organisms and their activity (Balicka et al. 1977, Martin, Focht 1977, Hutchinson, Collins 1978), and hence in the decomposition of organic matter.

The investigation carried out within 6 years of treating the plots with dusts confirms the reports of other authors that metals once introduced into the soil persist for a long time, being toxic for the metabolism of micro-organisms and the structure of populations (Jordan, Lechevalier 1975). They also show that heavy metals differently affect various groups of micro-organisms living in the soil, this being not only associated with the concentration of ions of the given metal and the period of its action but also with the degree of adaptation of the micro-organisms to such unfavourable conditions (De Leval, Demonty 1972, Jordan, Lechevalier 1975, Balicka et al. 1977).

The "mini" plots were established in order to elucidate how the industrial dusts affected micro-organisms in the first year after their introduction, because in earlier studies (Greszta et al. 1979, 1987) no comparable methods were used. It was found in the course of the experiment (May-October 1987) that the dynamics in numbers of the determined groups of micro-organisms was similar to the numbers found in the soils of plots established years ago (figs 4A, 5, 7). The rapid decline of higher plants, particularly intensive in the "mini" plots with the largest of dusts (1000 and 2000 t km^{-2} $year^{-1}$), brought about an inflow of fresh organic matter to the soil, this probably accounting for the stimulated respiration of micro-organisms as compared with the control soils, and its enhancement parallel to the increasing doses of dusts (fig. 8). The inflow of organic matter was also manifested by a 50% increase in the content of organic carbon in the soils of these variants of the experiment as compared with that of the control plot (Table III). The data obtained from the soils of the "mini" plots are consistent with reports in the literature that no inhibition of the enzymatic activity of micro-organisms by heavy metals was detected in soils with a large content of organic matter (Doelman 1978) and that there was a positive correlation between the increasing concentrations of heavy metals and the rate of decomposition of organic matter (Ebregt, Boldewijn 1977). A 30% decrease in the microbial activity found in the last phase of the experiment (October 1987), as compared with the initial phase (June 1987), agrees with the results of other studies which showed a reduction in CO_2 released from forest litter as the readily accessible carbon and other nutrients were depleted (Ausmus 1978).

A different content of total cadmium was determined in the non--fertilized and fertilized soil and in the "mini" plots (Table III) in spite of the same doses of dusts applied. Therefore, a supplementary analysis of the activity of micro-organisms in these soils was related to the same concentration of total cadmium, i.e. in the range 0—2 mg Cd g^{-1} of soil (fig. 11). As the concentration of total cadmium increased, analogical dependences were found as in the case of dust doses, i.e. a decrease in the activity of micro-organisms in the soils of plots affected by the dusts over long periods and an increase in the activity of soils in the "mini" plots (short-term action). With the cadmium dose of 2 mg g^{-1} the microbial activity was more intense in the fertilized soil and the decomposition of organic matter increased by about 30% as compared with the non-fertilized soil. The observed changes in the intensity of respiration of micro-organisms with equal concentrations of total cadmium are consistent with reports in the literature concerning variable toxicity of a given metal depending on the sorptive capacity of the soil (non-fertilized or fertilized soils), the time of action of the metal (experimental plots, "mini" plots), the concomitance of other metals, and the content of readily accessible organic matter. These factors shape the development of more resistant groups of micro-organisms and their metabolic activity (De Leval, Demonty 1972, Wolf et al. 1977, Jordan, Lechevalier 1975, Doelman 1978, Babich, Stotzky 1980, Sterrit, Lesler 1980, Wiklander 1980, Badura et al. 1983b, 1984a, 1984c, 1984d, Badura, Pacha 1984).

In determining the activity of the bacterial fraction isolated from the soil supernatant, a 75% decrease (mean for the investigation period 1986—1987) in the bacterial decomposition of organic matter was found in soils contaminated with cadmium dusts in comparison with the control soil (fig. 12B). A distinct increase in the cellular activity of bacteria was observed with a simultaneous decrease in the content of organic matter, especially in the soil treated with the dusts dose of 2000 t km^{-2} $year^{-1}$ (fig. 12A, C). This suggests that the dusts inhibited primary production, bringing about a decline of herbaceous plants and a dieback of trees, as had already been reported by Greszta (1988), Greszta et al. (1979, 1987) from the same forest. On account of the decrease in primary production and limited inflow of organic matter to the soil (Badura et al. 1984b), there appeared an increase in "the maintenance costs" of bacteria (Odum 1982) and a decrease in the energy released from the environment.

Of the soil hydrolyses investigated, a decrease was found in the activity of acidic and alkaline phosphatases with larger doses of dusts in all the analysed soils (fig. 15) and a varying intensity of C_x cellulase, depending on the time of action of the metals (experimental plots, "mini" plots) (fig. 16). On the other hand, the level of proteolytic activity of soils was uniform, irrespective of the kind of soil (fertilized, non-fertilized, from the "mini"

plots), the applied dose of dusts (0—2000 t km^{-2} $year^{-1}$), and the time of their action (long- or short-term) (fig. 14).

In general, the enzymatic activity of soil, which is the sum of intracellular enzymes and of those released by organisms to the environment and adsorbed on soil colloids (Burns 1978) is inhibited by heavy metals (Bääth et al. 1980, McFee 1980). However, the relations between the effect of heavy metals and the enzymatic activity of the soil are complex and, among other factors, depend on the content of organic and inorganic material in it, its humidity, and its pH, this being associated with the changed valency of metals and their occurrence in the form of soluble or insoluble salts, more or less noxious for enzymatic conversions (Babich, Stotzky 1977a, 1977b, Patrick et al. 1977, Gadd, Griffiths 1978). In the soil investigated the greater activity of acidic phosphatase may be associated with their acidic reaction, which limits the activity of alkaline phosphatase.

The small content of simple forms of nitrogen (Table III) in the soils might have offered favourable conditions for the induction of proteolytic enzymes by the cells of microbes (Badura et al. 1986), irrespective of the concentration of heavy metals in the soil and the time of their action.

The stimulation of cellulolytic activity in the soils of the "mini" plots by increasing doses of dusts (fig. 16), in spite of a simultaneous decrease in the number of cellulolytic bacteria (fig. 7), was probably brought about by a slightly more intense development of fungi, actively releasing cellulolytic enzymes even in soils treated with the largest doses of dusts (1000 and 2000 t km^{-2} $year^{-1}$). A similar phenomenon of more intensive cellulolytic activity in soils treated with copper and zinc compounds was observed by Badura et al. (1980, 1984d).

In spite of liming, which increases the pH value of soil, a slightly more intensive cellulolytic activity (fig. 16), a decrease in the activity of acidic phosphatase, but unchanged activity of protelytic enzymes (figs 14, 15) were found in fertilized soils compared with non-fertilized ones, though it is known that calcium ions activate enzymes. As Andrzejczuk-Hybel (1969) showed, calcium is indispensable in the formation of the intramolecular bridges which stabilize the structure of soil proteases and phosphatases.

In general, it may be postulated that the decrease in the activity of phosphatases and cellulase, observed in non-fertilized and fertilized soils as the doses of dusts increased, was probably caused, among other factors, by a decrease in the number of micro-organisms with specific biochemical properties. On the other hand, the ability of numerous species of bacteria from very abundant and qualitatively differentiated populations to release proteolytic enzymes may account for the uniform level of proteolytic activity of the investigated soils. Nor is it possible to disregard the fact that enzymatic reactions occur on the surface of negatively charged soil

colloids, whose surface charge is decreased by the cations of heavy metals introduced with dusts. In consequence, the positively charged substrates may be reduced in the soil and the rate of formation of the enzymes-substrate complex limited (Hattori T., R. Hattori 1976). Moreover, cadmium alone might have decreased the activity of enzymes by reacting with their acidic groups (Tyler 1980).

The results obtained in the present study are consistent with those reported from Europe and the USA, showing inhibition of the rate of organic matter decomposition and disturbance in the cycling of nutrients in environments exposed to industrial emission containing heavy metals (Ruhling, Tyler 1973, Jackson, Watson 1977). They also confirm the results of earlier research carried out on the same experimental plots in the Niepołomice Forest (Greszta 1988, Greszta et al. 1979, 1987, Grodziński unpubl. data). The above authors showed that in plots treated with dusts at doses of 100—5000 t km^{-2} $year^{-1}$ the accumulation of organic matter increased by 25—275% as compared with the control, the numbers of micro-organisms were distinctly reduced, and the enzymatic activity of soils was inhibited.

The experiment carried out in the Niepołomice Forest illustrates the ecological disaster which may ensure as the consequence of increasing air pollution and excessive accumulation of heavy metals in the soil. In plots with heavy doses of dusts (2000 t km^{-2} $year^{-1}$) applied as a single treatment, degradation of the soil structure and texture followed on account of the inhibition of primary production and a negligible inflow of organic substance to the soil. "Desertization" of the area was also observed on account of the complete decline of herbaceous plants and the dieback of trees. Badura et al. (1984b) found a similar picture of soil degradation brought by a decreased inflow of organic matter, and the desertization of areas in the vicinity of metallurgic works emitting pollution. In the case of the Niepołomice Forest the described changes were more distinct in plots treated with cadmium dusts than in those with zinc dusts. This indicates that the toxicity of cadmium is much greater and maintained for longer periods than that of zinc. Even 6 years after the dust treatment of soils, cadmium has continued drastically to limit the development and activity of macro- and micro-organisms. The disturbance in the processes of decomposition of organic matter and the accumulation of litter has upset the balance in the environment. In the investigated area the balance between the production and decomposition of organic matter may be regained only after 15 or even 20 years, as was shown by Burges (1965) for Californian coniferous forests.

6. Polish summary

Wpływ pyłów przemysłowych na rozwój i aktywność mikroorganizmów w glebach Puszczy Niepołomickiej (południowa Polska)

Badania mikrobiologiczne gleb z poziomów A_{oF+H} i A_1 przeprowadzono w Puszczy Niepołomickiej (ryc. 1), na działkach eksperymentalnych (o pow. 2,4 ara każda), po 5—6 latach od wprowadzenia na nie pyłów przemysłowych (w ilości 100, 500, 1000, 2000 t km^{-2} rok^{-1}, tabela I), zawierających metale ciężkie z przewagą kadmu lub cynku (tabele II, III). Działanie analogicznych dawek pyłów kadmowych w pierwszym roku po wprowadzeniu ich do gleb sprawdzono na poziomie A_{oF+H} gleb „mini" poletek, zlokalizowanych na jednej z działek kontrolnych (bez pyłów).

W poziomie A_{oF+H} niezależnie od długo- i krótkoterminowego działania pyłów, liczebność mikroorganizmów na ogół była większa w glebie kontrolnej, a niższa w glebach z dodatkiem pyłów (ryc. 4, 5, 7). Wyjątek stanowiły bakterie denitryfikacyjne i wydzielające H_2S, których liczebność wzrastała z dawką pyłów kadmowych (ryc. 5A, B). Natomiast w glebach z pyłami cynkowymi, zwłaszcza w ilości 100 t km^{-2} rok^{-1}, obserwowano stymulację rozwoju większości oznaczanych grup bakterii (ryc. 5A, B). Nawożenie mineralne i wapnowanie gleb poddanych długoterminowemu działaniu pyłów kadmowych, spowodowało wzrost liczebności mikroorganizmów, szczególnie w glebie z dodatkiem tych pyłów w ilości 100 t km^{-2} rok^{-1} (ryc. 9).

Aktywność mikroorganizmów w glebach, oznaczona przy zastosowaniu gazowego analizatora podczerwieni — Infralyt IV (ryc. 2, 3), była zróżnicowana zależnie od: 1) rodzaju wprowadzonych pyłów (kadmowe lub cynkowe) (ryc. 6), 2) czasu działania pyłów kadmowych (ryc. 6, 8), i 3) zastosowanych zabiegów nawożeniowych (ryc. 10).

Aktywność mikroorganizmów przy takiej samej zawartości kadmu ogólnego w glebach poddanych długoterminowemu działaniu pyłów kadmowych, była większa w glebie nawożonej w porównaniu z glebą nie nawożoną, lecz mniejsza niż w glebie „mini" poletek (krótkoterminowe działanie pyłów) (ryc. 11).

Pyły kadmowe stymulowały aktywność komórkową bakterii, co wyraźnie zaznaczyło się w glebie z dodatkiem tych pyłów w ilości 2000 t km^{-2} rok^{-1}, przy równocześnie mniejszej zawartości materii organicznej i spadku dekompozycji bakteryjnej (ryc. 12). Udział bakterii w ogólnej dekompozycji materii organicznej wahał się od 4,6% w glebie kontrolnej do 7,2% w glebie z największą dawką pyłów kadmowych (ryc. 13).

Niezależnie od dawki pyłów kadmowych, zastosowanego nawożenia i czasu działania pyłów, nie stwierdzono różnic w aktywności proteolitycznej gleb (ryc. 14). Wykazano natomiast spadek aktywności fosfataz w glebach ze wzrastającym stężeniem pyłów kadmowych, przy równoczesnej większej aktywności fosfatazy kwaśnej w porównaniu z fosfatazą alkaliczną (ryc. 15). Aktywność celulolityczna gleb obniżała się ze wzrostem stężenia pyłów kadmowych w glebach nie nawożonych i nawożonych, natomiast w glebach „mini" poletek obserwowano stymulację aktywności celulolitycznej wraz ze wzrostem dawki tych pyłów (ryc. 16).

W poziomie A_1 gleb, stosunki ilościowe pomiędzy oznaczanymi grupami mikroorganizmów układały się podobnie jak w poziomie A_{oF+H}, przy czym liczebność, jak i aktywność mikroorganizmów była zdecydowanie mniejsza (ryc. 4, 6, tabela IV).

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