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EFFECT OF SIMULATED ACID RAIN ON SOIL MICROORGANISMS

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INTRODUCTION

It is assumed that acidic deposition especially at high loading rates, affects soil organisms and biological processes in the soil. These effects may be positive or negative, depending upon environmental conditions and the organism or process involved [13]. Some artificial acidification experiments have shown reductions in total bacterial populations in the forest floor [5, 6]. Other workers have shown no significant changes in total numbers, but a significant decrease in metabolically active bacteria with high levels of acidic inputs [4].

Most soil bacteria grow best at pH values near neutrality. Decreased pH can affect membrane permeability and enzyme activity [15] resulting in lower growth and activity. Not all bacteria react negatively to low pH. Among bacteria many acidophilic species are known. Also microorganisms have the capability to adapt to altered soil pH [16].

The objective of our study was to learn how simulated acidic deposition affects microorganisms in three soils. The problem of the effect of acidic deposition on soil microorganisms is important and actual because plant growth, development and health are connected with the biochemical activity of saprophytic both symbiotic and non symbiotic soil microorganisms and because the soil receives more and more acidic depositions.

MATERIALS AND METHODS

DESCRIPTIONS OF THE OBJECT OF STUDY

The studies were carried out in the northern part of Tuchola Forests, Czersk Forest District. This area is situated far from greater

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industrial centers and is relatively free of air pollution (mainly sulphur and nitrogen oxides).

The experimental plot (42 × 52 m) was set up on clear cutting area in a lichen pine forest (*Cladonio-Pinetum*) with transition to a poor form of the suboceanic forest (*Leucobryo-Pinetum cladonietosum*). The soil was classified among rusty soils, with humus of moder type (xeromoder). It is formed from well drainage fluvioglacial sands. The signs of former ploughing are visible in a profile.

The selected plot was cleared of stubs and 15 parallel belts (37 m long) were traced out. Ten ditches (0.5 m deep, 0.5 m wide) were dug on the belts. Five of them were filled with marly sandy loam and five — with mucky soil. The remaining five belts were lacking of forest floor and the natural rusty soil was left.

Some physical and chemical properties of the soils used are given in Tables 1 and 2.

One year old pine seedlings (*Pinus sylvestris* L.) were planted on the belts between 22—24 of April 1987. During 1987—1989 the soils were watered monthly (from April to November) with solutions of sulphuric and nitric acids or with water from the deep-water well (control). Any contact of the seedlings with the acids was avoided. 1 l of

Table 1

Some physico-chemical properties of the soils (Ahp horizon, depth 0—15 cm)

Bulk density g/cm^3	Ignition losses %	C %	N %	C : N
1.48	Rusty soil			
	2.96	1.65	0.052	31.7
	Mucky soil			
	34.26	20.11	0.723	27.8
	Marly sandy loam	(1.5% $CaCO_3$)		
	1.11	0.25	0.014	17.9

Table 2

Dynamics of pH changes (pH-H_2O) of experimental soils measured always in November (mean values; n = 25)

Soil	Combination	1987	1988	1989
Rusty soil	control	4,72	4.74	4.60
	acidified soil	3.70	3.57	3.54
Mucky soil	control	5.20	5.14	5.13
	acidified soil	3.89	3.55	3.29
Marly sandy loam	control	8.10	8.25	8.20
	acidified soil	7.53	7.60	6.79

0.225 M H_2SO_4 and 0.5 l of 0.225 M HNO_3 were applied each time to 1 m^2 — this corresponds to 1.5 mm of rainfall.

Detailed data on the experimental area are given by Prusinkiewicz, Kwiatkowska and Pokojska [17].

MICROBIOLOGICAL STUDIES

The samples of three soils for microbiological studies — from control and acidified area — were collected on spring, summer, autumn during 1987—1988 and on spring 1989. The surface layer of the soil was removed and samples were taken to a depth of 5 cm.

The following analyses were performed:

1. Total number of bacteria on soil extract agar [12]. Nystatin and actidion in amount of 50 mg/l were added to the autoclaved medium.

2. Number of fungi on Martin's medium with rose bengal (33.3 mg/l) and aureomycin (30 mg/l).

3. Identification of fungi was performed according to Gilman [11] and Barnett [8] manuals.

4. For enumeration of numbers of microorganisms belonging to different physiological groups: ammonifiers, denitryfires, microorganisms reducting of sulphates and decomposing of cellulose, the most probable number method has been applied [18]. The media used for growing the above microorganisms were the same as recommended by Allen [3].

RESULTS

Results on total number of bacteria and fungi in control and acidified soils are presented in Table 3. Depending on the season of the year, soil type and the degree of acidification big differences in the quantitative occurrences of these microorganisms were noted. In general the marly sandy loam and mucky soil contained more bacteria than the rusty one, but the number of fungi was the smalest in the marly sandy loam (Table 3).

Acidification of the soil affected stronger the bacteria than fungi. The numerosity of bacteria in control and acid treated soils differed statistically (Student's test) when examined at different seasons of the year (Tabela 3). In the rusty and mucky soils acidification caused a significant decrease in the number of bacteria but an increase in the marly sandy loam. Differences in number of fungi in control and acidified soils in successive seasons were often statistically not significant. In the rusty soil acidification caused a decrease of the number of

Table 3

Numbers of bacteria and fungi in different soils (in thousands per 1.0 g of dry soil)

Date of sampling		Rusty soil		Mucky soil		Marly sandy loam	
		control	acidified soil	control	acidified soil	control	acidified soil
1987 3 VI	*a*	356*	110*	8 562*	3 190*	4 029⁻	4 894⁻
	b	271⁻	251⁻	219⁻	144⁻	100⁻	75⁻
5 VIII	*a*	1 686⁻	2 006⁻	10 734*	3 713*	68 639⁻	85 727⁻
	b	346⁻	334⁻	177⁻	162⁻	34*	104*
7 X	*a*	2 244*	1 367*	7 410⁻	6 840⁻	7 405*	12 159*
	b	271*	144*	69⁻	96⁻	45⁻	45⁻
1988 19 IV	*a*	633*	283*	10 236*	909*	3 789⁻	5 179⁻
	b	301⁻	235⁻	72⁻	165⁻	44⁻	38⁻
1 VII	*a*	456⁻	257⁻	9 210*	3 451*	20 866*	37 668*
	b	176*	70*	194*	350*	50⁻	60⁻
4 X	*a*	1 465*	157*	11 686*	1 070*	5 762*	10 797*
	b	201⁻	195⁻	257⁻	278*	76*	184*
5 V	*a*	1 361*	44*	2 441*	602*	3 710*	9 052*
	b	195*	61*	115*	215*	40*	84*

a — numbers of bacteria, *b* — numbers of fungi.
* significant differences at $P \leqslant 0.05$.
— no significant differences at $P \leqslant 0.05$.

Table 4

Two-factor analysis of variance (ANOVA) comparing the effects of seasons and acidification on numbers of bacteria and fungi in different soils

Effects of:	Degrees of freedom	Rusty soil		Mucky soil		Marly sandy loam	
		F	*P*	*F*	*P*	*F*	*P*
				Bacteria			
Seasons	6	81.79	0.00000	11.13	0.00002	214.02	0.00000
Acidification	1	102.81	0.00000	172.31	0.00000	28.28	0.00006
Seasons x acidification	6	17.31	0.00000	9.84	0.00004	3.49	0.01067
Error	28						
				Fungi			
Seasons	6	10.94	0.00002	11.38	0.00002	12.51	0.00001
Acidification	1	14.10	0.00111	7.76	0.00925	19.30	0.00031
Seasons x acidification	6	1.38	0.25625	3.50	0.01053	7.51	0.00017
Error	28						

F — Fischer-Snedecor parameter.
P — significance level.

fungi. On the other hand in the mucky soil and marly sandy loam increasing acidification affected the numerosity of fungi.

Two-factor analysis of variance (ANOVA) has shown a significant effect of the season of the year and acidification on numbers of fungi and especially on bacteria in particular soils (Table 4). Usually the effect of the season was statistically more significant than acidification.

No significant differences were noted in kind composition of fungi among the control and acidified soils (Table 5). Such differences were connected rather with the soil type than with acidification.

Tables 6—8 show numbers of ammonifires, denitryfires, sulphate reducers and cellulose decomposers. Numbers of denitryfiers and ammonifiers differed considerable in different seasons. In the soils under study cellulose decomposers and sulphate reducers were not numerous. Statistical evaluations (Mann-Whitney *U* test) have shown that the effect of simulated acid rain on different physiological groups of microorganisms was most often unsignificant.

Table 5

Predominant kinds of fungi in the soils under study

Soil	Control	Acidified soil
Rusty soil	*Penicillium* sp.	*Penicillium* sp. *Mortierella* sp. *Trichoderma* sp.
Mucky soil	*Penicillium* sp. *Trichoderma* sp. *Mucor* sp. *Pullularia* sp. *Cladosporium* sp.	*Mortierella* sp. *Trichoderma* sp. *Penicillium* sp. *Mucor* sp.
Marly sandy loam	*Penicillium* sp. *Mortierella* sp. *Trichoderma* sp. *Mucor* sp.	*Penicillium* sp. *Trichoderma* sp. *Hyalopus* sp. *Pullularia* sp. *Mucor* sp.

DISCUSSION

Reviews on the effects of acidic deposition on soil organisms or nutrient cycling have been published by Alexander [1, 2], Coleman [10], Olson [14]. An excellent review on the effects of acidic deposition on soil organisms has been published by Myrold [13]. Many of the studies on the effect of atmospheric deposition on bacteria have also measured fungal populations. In general moderate to high acidic inputs

Table 6

Numbers of microorganisms belonging to different physiological groups in the rusty soil (per 1.0 g of dry soil)

Date of sampling	Denitryfiers		Ammonifiers		Microorganisms reducing of sulphates		Microorganisms decomposing of cellulose	
	control	acidified soil	control	acidified soli	control	acidified soil	control	acidified soil
1987								
3 VI	7 900	26 000	10 000	98 000	0	0	0	4
5 VIII	108 400	106 700	5 100	168 000	8	3	5	0
7 X	1 000	490	4 800	500	0	0	10	0
1988								
19 IV	11 800	46 000	260	4 600	4	4	4	4
1 VII	260 000	21 000	260	40	0	26	0	4
4 X	800	50	260	25	0	9	46	0
1989								
5 V	455 000	150	4 500	40	9	0	4	0
Mann-Whitney *U* test (significance level)	$P = 0.28$		$P = 0.66$		$P = 0.66$		$P = 0.14$	

Table 7

Numbers of microorganisms belonging to different physiological group in the mucky soil (per. 1.0 g of dry soil)

Date of sampling	Denitryfiers		Ammonifiers		Microorganisms reducing of sulphates		Microorganisms decomposing of cellulose	
	control	acidified soil	control	acidified soil	control	acidified soil	control	acidified soil
1987								
3 VI	150 000	6 700	70 500	44 800	0	10	150	220
5 VIII	4 570 000	350 000	820 000	260 000	7	12	820	12
7 X	40 000	2 400	18 400	24 000	0	0	150	10
1988								
19 IV	790 000	41 300	3 900	24 800	89	10	390	120
1 VII	1 860 000	147 000	15 400	4 000	32	62	650	310
4 X	4 500	1 500	3 700	3 100	67	17	1 100	46
1989								
5 V	99 500	2 600	12 600	5 800	33	19	12	0
Mann-Whitney *U* test *U* test (significance level)	$P = 0.06$		$P = 0.91$		$P = 0.66$		$P = 0.04$	

Table 8

Numbers of microorganisms belonging to different physiological groups in the marly sandy loam (per 1.0 g of dry soil)

Date of sampling	Denitryfiers		Ammonifiers		Microorganisms reducing of sulphates		Microorganisms decomposing of cellulose	
	control	acidified soil	control	acidified soil	control	acidified soil	control	acidified soil
1987								
3 VI	25 800	9 700	46 500	30 600	9	0	0	15
5 VIII	10 600	8 400	28 000	16 700	0	0	280	280
7 X	2 000	78 000	11 900	4 700	0	0	2 800	4 700
1988								
19 IV	460 000	1 000 000	46 000	21 000	3	0	97	16
1 VII	483 000	451 000	2 700	250	4	0	8	95
4 X	26 000	46 000	2 600	1 500	4	0	26	97
5 V	30 000	45 000	750	4 000	4	0	0	11
Mann-Whitney *U* test (significance level)	$P = 0.57$		$P = 0.57$		$P = 0.02$		$P = 0.49$	

have not been found to effect fungal populations in organic or mineral soil orizons [7, 9]. The same phenomenon was stated in our studies. Soil acidification affected stronger bacteria than fungi. In rusty and mucky soils acidification caused a decrease in number of bacteria but an increase in marly sandy loam soil.

Acidic deposition may affect soil organisms directly or indirectly. The greatest attention received acidity. The acidity is however complicated by association of H^+ with various anions, especially NO_3^- and SO_4^-. Indirest effects of atmospheric inputs on soil organisms may be concerned with alternation of the soil chemical environment and altered organic matter inputs [13]. Thus it seems that both the direct and indirect impact of acidic deposition on soil microorganisms requires futher studies.

It is suggested by Myrold [13] that futher research should be done on the effects of realistic chronic inputs, because much work on the effect of acidic deposition on soil organisms has involved unrealistically high inputs over short time periods.

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ВЛИЯНИЕ ИСКУССТВЕННЫХ КИСЛЫХ ДОЖДЕЙ НА ПОЧВЕННУЮ МИКРОФЛОРУ

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Резюме

В течение трех лет исследовали изменения микробиологического состояния 3 почв: песчаной, суглинистой с примесью $CaCO_3$ и муршевой. Все три почвы (кроме контрольных вариантов) ежемесячно подкисляли смесью растворов H_2SO_4 и HNO_3.

Выявлено, что подкисление почв оказывало гораздо больше влияние на количество бактерий, чем на количество грибов. В результате подкисления песчаной и муршевой почв, обнаружено существенное уменьшение количества бактерии, однако в суглинистой почве выявилась обратная тенденция.

Влияние сезонов года на количество почвенных бактерии и грибов проявлялось гораздо более чётко, чем влияние подкисления почв.

Исследование влияния искусственных кислых дождей на выбранные физиологические группы почвенной микрофлоры не привело к статистически достоверным результатам.

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WPŁYW SYMULOWANYCH KWAŚNYCH DESZCZÓW NA DROBNOUSTROJE GLEBOWE

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Streszczenie

Przeprowadzono analizy mikrobiologiczne trzech gleb (piaszczysta, murszowa i gliniasta) kontrolnych i zakwaszonych. Stwierdzono, że zakwaszenie gleb wpływało silniej na liczebność bakterii niż grzybów. W glebach piaszczystej i murszowej notowano istotny spadek liczebności bakterii pod wpływem zakwaszania, a w glebie gliniastej — wzrost ich liczebności.

Wpływ pór roku na liczebność bakterii i grzybów był najczęściej bardziej istotny statystycznie niż wpływ zakwaszania.

Wpływ symulowanego kwaśnego deszczu na różne grupy fizjologiczne drobnoustrojów był nieistotny statystycznie.

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