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BACTERIA ACTIVE IN THE CONVERSION OF NITROGEN AND SULPHUR COMPOUNDS IN RAINFALL COLLECTED UNDER CROWNS AND FLOWING DOWN TREE TRUNKS OF PINES AND SPRUCES IN WIGRY NATIONAL PARK

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BAKTERIE CZYNNE W PRZEMIANACH ZWIĄZKÓW AZOTU I SIARKI W OPADACH PODKORONOWYCH I SPŁYWIE PO PNIACH SOSEN I ŚWIERKÓW W WIGIERSKIM PARKU NARODOWYM

W pracy przedstawiono wyniki badań liczebności bakterii amonifikacyjnych, nitryfikacyjnych i denitryfikacyjnych, dane dotyczące bakterii utleniających siarkę i tiosiarczany oraz redukujących siarczany w wodach deszczowych stanowiących okap i spływ po pniu sosen i świerków. Próby opadu pobierano wiosną i jesienią równolegle z 3 kolektorów odpowiednio pod okapem świerka pospolitego i sosny zwyczajnej i z kolektorów spiralnych zainstalowanych na pniach tych drzew. Wody podokapowe zawierały w przybliżeniu jednakowo niskie liczebności bakterii amonifikacyjnych i nitryfikacyjnych I fazy oraz okresowo większe liczebności bakterii nitryfikacyjnych II fazy i redukujących NO_3^- do NO_2^- oraz wyjątkowo duże liczebności bakterii denitryfikacyjnych. Żadna z badanych prób wody nie zawierała bakterii utleniających siarkę i jej związki oraz bakterii redukujących siarczany. Różnice w liczebności badanych bakterii cyklu azotowego w wodach opadowych zbieranych z kolektorów umieszczonych pod okapem sosny i świerka oraz z kolektorów zbierających wody spływające po pniach tych gatunków drzew były niejednoznaczne zależne od grupy fizjologicznej bakterii, gatunku drzew i okresu badawczego.

Summary

The paper contains the results of numbers of ammonifying, nitrifying and denitrifying bacteria, the data on bacteria which oxidize sulphur and thiosulphates as well as those reducing sulphates in rainwater that has flown from crowns and trunks of pines and spruces. Rainfall samples were collected in the spring and autumn of 1999. On each occasion samples were taken from three collectors under common spruce (*Picea excelsa*) and common pine (*Pinus silevstris*) canopy and from spiral collectors installed in tree trunks of spruces and pines. Rainwater collected under crowns of pine and spruce trees contain approximately the same, low numbers of ammonifying bacteria and I phase nitrifying bacteria; periodically, these rainwater samples contained elevated counts of II phase nitrifying bacteria and bacteria reducing NO_3^- to NO_2^- and exceptionally high numbers of denitrifying bacteria. None of the rainwater samples analyzed contained bacteria oxidizing sulphur or sulphur compounds or sulphate reducing bacteria. Differences in the counts of the assayed nitrogen cycle bacteria in the rainwater samples taken from the collectors under pine and spruce canopy and from the collectors catching water flowing down trunks of these two tree species were inconsistent and depended on a physiological group of bacteria, tree species and the time of sampling.

INTRODUCTION

Rainfall water plays a special role in dispersion of microorganisms in nature. Passing through all environments in which microorganisms are present, water transports them from one place to another. Together with rainfall, microorganisms migrate from the atmospheric air to the earth's surface, where they fall on plants, penetrate soil, reach surface waters and, through soil infiltration, are transported to groundwater. Rain also carries soluble and insoluble dust and gases absorbed from the atmosphere. Some precipitation will first fall on the surface of plants; in ecosystems dominated by forests nearly all rainfall passes through tree crowns before reaching soil. By doing so it washes away various substances deposited on the surface of trees. Composition of water collected under tree crowns ('subcanopy water') and flowing down tree trunks ('water flow down tree trunks') is much richer in dusts, microorganisms or substances excreted by living organisms than water directly falling on the ground. Rainwater has specific composition, which depends on a route raindrops have passed. Consequently, rainwater can be a major source of non-point pollution, especially in industrialized areas [12]. In the past, thought to be very clean (close to distilled water), at present rainwater is frequently classified as acid rains. Although microbial activity does not play any role whatsoever in chemical transformations occurring in rain while it is falling from the atmosphere towards the earth's surface, it is true that microbial processes involving nitrogen and sulphur circulation can possibly occur in rainwater stored for household use. Such processes influence water pH and content of nitrate, sulphate and other ions. Declining pH noticed in stored rainwater changes drastically habitat conditions for various microorganisms, and to most of them is life-threatening. The present paper contains the results of numbers of ammonifying, nitrifying and denitrifying bacteria, the data on bacteria which oxidize sulphur and thiosulphates as well as those reducing sulphates in rainwater that has flown from crowns and trunks of pines and spruces. Parallel study of rainfall water [4], was carried on pH and concentrations of SO_4^{2-}, NO_3^- and NH_4^+. The study was part of a larger programme, the Integrated Monitoring of Natural Environment, conducted at the Base Station in Wigry National Park. The aim of this project was to trace quantitative and qualitative changes in atmospheric precipitation reaching the soil through tree canopy. The project constitutes an important cognitive component in the research on causes and mechanisms of surface and ground water pollution from non-point pollution sources in this region.

MATERIAL AND METHODS

Study area. The study was conducted on the area of an experimental catchments of the Wigry Base Station (Wigry National Park), under the climatic conditions typical of the region of Suwałki. This territory is characterized by the lowest (in Poland's lowlands) air temperature during winter months, the highest number of days with an average temperature below 0°C (114 days) and over three-month-long period of snow cover. According to the data collected by Krzysztofiak [5], the long-term mean monthly temperatures vary from -6.7°C in January to 16.6°C in July, with the annual mean of 5.3°C; the average annual precipitation is 593 mm. This geographical region of Poland is situated in the subboreal sphere of mixed forests, with permanent presence of spruce (*Picea excelsa*) among trees [9]. The surface of the experimental catchments of the Wigry Base Station is dominated by spruce, spruce-pine and pine forest stands. Most of the forests in Wigry National Park are under the forest management system [1].

Sampling. Samples of rainwater were collected under crowns and flowing down trunks of spruces and pines near the meteorological garden located on the area of the experimental catchments under the Integrated Monitoring of Natural Environment, which involves studies on chemism of atmospheric precipitation. Rainfall samples were collected in the spring and autumn of 1999. On each occasion samples were taken from three collectors under common spruce (*Picea excelsa*) and common pine (*Pinus silevstris*) canopy and from spiral collectors installed in tree trunks of spruces and pines. Both tree species dominate in the forest stand of Wigry National Park. The dates of rainfall collection are specified in Table 1. Rainfall was poured once a week into 300 cm^3 glass bottles with adjusted corks and kept at 4°C. The samples were transported in ice-boxes to a laboratory, where they underwent microbiological assays.

Table 1. Design of rainwater sample collection under crowns and flowing down tree trunks of pines and spruces at the experimental catchments at the Base Station in Wigry National Park in Sobolewo

Number of analyses	Time of analyses (1999 year)	Kind of collector			
		Pine		Spruce	
		under crown	tree trunks	under crown	tree trunks
		time of samples collections (days)			
1	14.05	7	7	7	7
2	20.05	6	6	6	6
3	1.06	31	31	31	31
4	7.10	7	7	7	7
5	14.10	7	7	7	7
6	29.10	30	30	30	30

Microbiological assays. The microbiological assays comprised determination of numbers of the following bacteria: ammonifying, I phase (oxidizing NH_4^+ to NO_2^-) and II phase (oxidizing NO_2^- to NO_3^-) nitrifying, reducing NO_3^- to NO_2^- and denitrifying (reducing NO_3^- to N_2O/N_2), oxidizing sulphur (*Thiobacillus thiooxidans*) and thiosulphates (*Thiobacillus thioparus*) or reducing sulphates (*Desulfovibrio desulphuricans*) on media and substrates, under the conditions specified in Table 2. The methods applied in the research adhered to the protocols described by Rodina [8] and Spandowska *et al.* [10].

Physiological solution of NaCl (0.85%) was used for dilutions. All determinations were made in three analogous replications on the same rainwater sample. The results of the assays on counts of ammonifying bacteria were given in cfu cm^{-3}. The most probable numbers (MPN cm^{-3}) of the remaining physiological groups of bacteria were read out from McCrady's tables [7].

Table 2. Nutrient medium, temperature and incubation time of bacteria active in nitrogen cycle in rainwater collected under pine and spruce canopy and from the collectors catching water flowing down trunks of these trees

Microorganisms	Media used to enumerate bacteria	Incubation	
		temp. °C	days
Ammonifying bacteria	broth-agar + 3% pepton proteose	25	$3^{1)}$
NH_4^+ – oxidizers (*Nitrosomonas*)	mineral medium with $(NH_4)SO_4$ acc. Winogradsky	30	$28^{1)}$
NO_2^- – oxidizers (*Nitrobacter*)	mineral medium with $NaNO_2$ acc. Winogradsky	30	$28^{1)}$
NO_3^- reducers to NO_2^-	Giltay's medium with Durham's tubes	30	$5–7^{1)}$
Denitrifyng bacteria reducers NO_3^- to N_2, NO/N_2O		30	$5–7^{1)}$
Bacteria (*Thiobacillus thiooxidans*) – oxidizers of sulfur	Waksman's medium	30	$14^{2)}$
Bacteria (*Thiobacillus thioparus*) – oxidizers of thiosulfate	Starkey's medium	30	$14^{2)}$
Bacteria (*Desulfovibrio desulphuricans*) – reducers of sufate	Tauson's medium modified by Szturm + Difco agar	30	$2–3^{2)}$

1) according [8]
2) according [10]

RESULTS

Nitrogen cycle bacteria. Among the bacteria active in the conversion of nitrogen compounds in the rainwater collected under crowns and from trunks of spruces and pines, number of I phase nitrifying bacteria was the smallest; bacteria reducing NO_3^- to NO_2^- and denitrifying bacteria (reducing NO_3^- to N_2O/N_2) were the most numerous (Table 3, 4). A number of ammonifying bacteria in the water collected under the canopy of pine trees varied from 120 to 242 cfu cm^{-3}, and in the water flowing down tree trunks ranged from 54 to 276 cfu cm^{-3}. Approximately the same counts of these bacteria were determined in the rainwater under the canopy flowing down the trunks of spruce trees. As a rule, the lowest

Table 3. The number of bacteria active in the conversion of nitrogen compounds in rainfall collected under crowns of pines and spruces in Wigry Base Station in Wigry National Park in Sobolewo (cfu cm^{-3}; mpn cm^{-3})

Date (1999)	Pine					Spruce				
	ammonifiers	NO_2^- oxidizers	NH_4^+ oxidizers	NO_3^- reducers to NO_2^-	NO_3^- reducers to N_2, NO/N_2O	ammonifiers	NO_2^- oxidizers	NH_4^+ oxidizers	NO_3^- reducers to NO_2^-	NO_3^- reducers to N_2, NO/N_2O
14.05	242	< 3	4	4	8	300	5	8	< 3	< 3
20.05	222	< 3	140	< 3	4	265	4	140	< 3	< 3
1.06	185	< 3	140	40	300	62	< 3	< 3	1400	1400
7.10	140	< 3	< 3	< 3	1400	125	< 3	15	140	1400
14.10	120	20	450	4	150	85	12	1100	< 3	1500
29.10	125	11	8	4	400	110	< 3	< 3	4	400
average	172	5	123	4	377	158	3	210	257	780

Table 4. The number of bacteria active in the conversion of nitrogen compounds in rainfall flowing down tree trunks of pines and spruces in Wigry Base Station in Wigry National Park in Sobolewo (cfu cm^{-3}; mpn cm^{-3})

Date (1999)	Pine					Spruce				
	ammonifiers	NO_2^- oxidizers	NH_4^+ oxidizers	NO_3^- reducers to NO_2^-	NO_3^- reducers to N_2, NO/N_2O	ammonifiers	NO_2^- oxidizers	NH_4^+ oxidizers	NO_3^- reducers to NO_2^-	NO_3^- reducers to N_2, NO/N_2O
14.05	275	15	3	<3	7	215	<3	<3	<3	140
20.05	265	45	4	<3	<3	222	<3	30	140	<3
1.06	55	5	4	<3	1400	40	<3	4	<3	<3
7.10	105	10	<3	110	1400	138	<3	10	110	1400
14.10	145	20	35	40	30	100	20	1400	9	4500
29.10	105	3	3	9	14000	120	30	<3	30	300
average	160	15	7	25	2805	140	8	240	48	1033

numbers of ammonifying bacteria were found in June and the highest in May; except the rainwater collected under the pine canopy, when the maximum counts appeared in October.

The count of I phase nitrifying bacteria (oxidizing NH_4^+ to NO_2^-) in the analyzed samples of rainwater was low and did not exceed 45 cfu cm^{-3} (in the water flowing down pine tree trunks). Quite frequently no presence of these bacteria was detected in the analyzed volumes of rainwater. II phase nitrifying bacteria occurred in larger numbers. Their counts reached maximum values in the rainwater collected under tree crowns and flowing down tree trunks of pines and spruces. In the rainwater under spruce crowns and on tree trunks the number of these bacteria reached the values of 1400 cfu cm^{-3}, being several-fold higher than in the rainwater collected under pines and 50 to 100-fold higher than in the rainwater flowing down pine tree trunks.

The counts of bacteria reducing NO_3^- to NO_2^- in rainwater collected under pine tree crowns did not exceed 40 cfu cm^{-3}, and in that sampled under spruce tree crowns was below 1400 cfu cm^{-3}. In the rainwater flowing down tree trunks of these two species the counts of these bacteria did not exceed 110 and 140 cfu cm^{-3} respectively. In a few cases NO_3^- to NO_2^- reducing bacteria were not detected in the water volumes analyzed. The maximum counts of these bacteria (1100 cfu cm^{-3}) were determined in the subcrown rainwater under spruce trees in early June. The number of denitrifying bacteria (reducing NO_3^- to N_2O/N_2) reached the maximum values in the rainwater flowing down pine (up to 14000 cfu cm^{-3}) and spruce tree trunks (up to 4500 cfu cm^{-3}) in October. In pine and spruce subcrown rainwater the counts of these bacteria were below 1400 and 15000 cfu cm^{-3}, respectively (in the pine subcrown rainwater in early October and in the spruce subcrown rainwater in June and in the first half of October).

Bacteria active in the conversion of sulphur compounds. No sulphur and sulphur compounds oxidizing bacteria, and anaerobic bacteria reducing sulphates in the rainwater collected under crowns and on trunks of pines and spruces were detected.

DISCUSSION

The presence of ammonifying bacteria in the rainwater collected under crowns of pines and spruces or flowing down the trunks of these trees is related to the content of organic nitrogen substance in the rainwater. This organic matter can be composed of microbial cells carried by raindrops from the atmospheric air or washed away from tree crowns and trunks as well as some organic residues carried by winds. The assay of the counts of these bacteria in the water samples taken from the rainwater collectors (pine – canopy, spruce – canopy, pine – trunk flow, spruce – trunk flow) in subsequent months and days does not show any clear relationship with the type of rainwater collected. Mean counts of these bacteria in the rainwater samples taken from different collectors did not differ significantly, although the analysis of chemical composition of rainwater [4] would suggest that there was an increase in ammonium nitrate in the order: pine-canopy, spruce-canopy, pine-flow, and spruce-flow (Table 4, 5). Low counts of ammonium-oxidizing bacteria (oxidizing NH_4^+ to NO_2^-) must have been related to the small quantity of ammonium nitrate released by ammonifying bacteria, and, even more strongly, to the low pH (3.0–3.4 for rainwater flowing down tree trunks and 4.0 to 4.4 for subcrown rainwater). At such pH values the development of ammonium-oxidizing bacteria and their oxidation of NH_4^+ to NO_2^- are completely inhibited. As a result there were no differences in numbers of these bacteria in rainwater collected under tree crowns versus the rainwater flowing down tree trunks. Higher numbers of II phase nitrifying bacteria (oxidizing NO_2^- to NO_3^-) in the rainwater collected under crowns of pines and spruces or flowing down the trunks of these trees observed on one occasion in October may have been caused by that fact that some of these bacteria were brought with the dust carried by winds during the rainy weather. The same event could have affected the number of denitrifying bacteria. Although no larger differences were found in the numbers of denitrifying bacteria (reducing NO_3^- to N_2O/N_2) in rainwater flowing down pine and spruce tree trunks, the much lower counts of these microorganisms in the rainwater collected under the canopy of these tree species (from 2-

to 30-fold fewer bacteria) were reflected in the quantitative composition of main anions (SO_4^{2-}, NO_3^-) and cations NH_4^+ as well as in the water pH. Among the outcomes of the activity of these bacteria are the following observations: a slight increase in pH in June (to 5.40 in the pine and 4.92 in spruce subcrown rainwater), a decrease in the content of N-NO_3 (to 0.70 and 1.20 mg dm^{-3}, respectively) and N-NH_4 (to 2.04 and 3.66 mg dm^{-3}, respectively). Such relationships could not be observed in the case of the rainwater flowing down tree trunks due to very small numbers of denitrifying bacteria and a much higher amount of SO_4, which may have had some influence on higher acidity of the rainwater. The presence of SO_4^{2-} ions in the analyzed samples of rainwater is a result of SO_2 oxidizing processes occurring in the atmosphere [2], in which microorganisms did not participate. No typical thionic bacteria (*Thiobacillus thiooxidans*, *Thiobacillus thrioparus*) were detected in the samples of rainwater collected under crowns and on trunks of pine and spruce trees, although the oxygen conditions and low pH should have favored development of these bacteria. Both oxygen conditions and low pH, however, may have been responsible for the absence of sulphate-reducing bacteria in the samples of rainwater.

Table 5. Reaction (pH) and concentration of chosen ions of rainfall under crown and flowing down tree trunks according Krzysztofiak [4]

Date (1999)	pH	N-NH_4	N-NO_3	S-SO_4	pH	N-NH_4	N-NO_3	S-SO_4
		mg dm^{-3}				mg dm^{-3}		
	pine				spruce			
rainfall under crown								
IV	9.47	6.50	2.80	7.34	3.67	9.50	3.50	16.36
V	4.41	3.25	1.90	2.34	4.00	5.05	3.10	8.68
VI	5.40	2.04	0.70	0.00	4.92	3.66	1.20	0.00
VII	5.13	4.25	2.30	0.00	5.72	9.90	2.10	0.00
VIII	5.23	4.10	2.00	0.00	5.33	5.00	1.70	0.00
IX	5.41	1.40	3.70	0.00	5.43	2.33	5.60	0.00
X	4.42	3.20	2.40	2.67	4.38	5.40	2.80	0.33
average	4.85	3.53	2.25	–	4.73	5.40	2.85	--
Rainfall flowing down tree trunks								
IV	3.16	9.25	4.90	23.37	3.14	11.30	4.10	50.07
V	3.23	10.25	4.50	26.70	3.42	10.60	2.10	36.72
VI	3.58	6.20	2.00	11.35	3.12	10.92	3.20	32.71
VII	3.62	8.55	2.40	4.67	3.97	15.30	2.50	32.04
VIII	3.32	8.40	2.30	9.01	3.45	16.20	2.30	13.35
IX	3.19	17.20	5.10	15.69	3.78	25.25	3.90	11.68
X	2.98	10.60	3.90	14.02	3.00	18.50	5.10	52.07
average	3.29	10.06	3.58	–	3.41	15.43	3.31	–

CONCLUSIONS

1. Rainwater collected under crowns of pine and spruce trees contains approximately the same, low counts of ammonifying bacteria (typical for unpolluted waters) and I phase nitrifying bacteria (*Nitrosomonas*); periodically, these rainwater samples contained elevated counts of II phase nitrifying bacteria (*Nitrobacter*) and bacteria reducing NO_3^- to NO_2^- and exceptionally high numbers of denitrifying bacteria (reducing NO_3^- to N_2O/N_2). None of the rainwater samples analyzed contained bacteria oxidizing sulphur or sulphur compounds or sulphate reducing bacteria.
2. Differences in the counts of the assayed nitrogen cycle bacteria in the rainwater samples taken from the collectors under pine and spruce canopy and from the collectors catching water flowing down trunks of these two tree species were inconsistent and depended on a physiological group of bacteria, tree species and the time of sampling. The mean counts of ammonifying and I phase nitrifying bacteria were approximately the same in both types of water samples, while II phase nitrifying bacteria were more numerous in the rainwater collected under tree crowns of either tree species. Bacteria reducing NO_3^- to NO_2^- were more abundant in the water under spruce canopy and denitrifying bacteria had the highest counts in the water flowing down spruce tree trunks.
3. No obvious relationships were observed between the concentration of NO_3-N or NH_4-N and the numbers of respective nitrogen cycle bacteria in the analyzed samples of rainwater collected under the canopy of pine or spruce trees as well as the rainwater flowing down the tree trunks of these two species. The presence of SO_4^{2-} ions in the rainwater samples subjected to analyses was most probably due to the process of SO_2^- oxidation in atmosphere, without any participation of microorganisms.

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