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MICROBIOLOGICAL CHARACTERISTICS OF QUATERNARY SEDIMENTS AT STARUNIA PALAEONTOLOGICAL SITE AND VICINITY (CARPATHIAN REGION, UKRAINE)

Wiesław BARABASZ, Maria J. CHMIEL & Marek OSTAFIN

Department of Microbiology, University of Agriculture, Al. Mickiewicza 24/28, 30-059, Kraków, Poland; e-mail: rrbaraba@cyf-kr.edu.pl

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Abstract: The microbiological research on the area of the palaeontological site in Starunia (Ukraine) reveals the details of biological activity of the near-surface layers and Quaternary sediments. In Starunia area remnants of a mammoth and three woolly rhinoceroses, and one almost completely preserved rhinoceros carcass were found in 1907 and 1929. The gained quantitative results regarding the occurrence of different physiological groups of microorganisms show that their number varied significantly depending on the sampling place, sampling depth, pH, humidity and the organic matter content. The amount and differentiation of the tested groups of microorganisms typically decreased with the depth. In several deep-sampling locations there was increase in the microorganisms, especially with methanogens and methanotrophs. The metanogens occurred mainly in Pleistocene sediments, comprised of clayey mud and peat, while saprophytic microorganisms (bacteria, fungi and actinomycetes) occur in Holocene sediments comprised of clayey mud, peat and peat mud. The quantity of microorganisms in selected boreholes was related to high concentration of the organic matter (mainly peat and peat mud) and correlated with methane occurrence.

Key words: microorganisms, methanogens, Holocene, Pleistocene, Starunia palaeontological site, Carpathian region, Ukraine.

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INTRODUCTION

The area of the abandoned ozokerite mine in Starunia near Ivano-Frankivsk is one of the most interesting places in Ukraine regarding the geological structure and occurrence of oil and ozokerite as well as specific Pleistocene flora and fauna (Kotarba, 2009). In the Starunia area remnants of a mammoth and three woolly rhinoceroses, and one almost completely preserved rhinoceros carcass were found in 1907 and 1929. Scientists consider the possibility of finding more fauna examples and conduct research to locate occurrences of another rhinoceros or other representatives of Quaternary fauna. General information covering the history of the area and details about geology and results of the earlier research works conducted in the area are described in a special monograph devoted to Starunia (Kotarba, ed., 2005) and more recent works (Kotarba *et al.*, 2008; Kotarba & Stachowicz-Rybka, 2008; Sokołowski *et al.*, 2009; Sokołowski & Stachowicz-Rybka, 2009; Stachowicz-Rybka *et al.*, 2009).

Special attention should be paid to microbiological indicators as one of the parameters which may indicate the presence of a large amount of organic matter and their biotransformation products in the soil environment.

All biological growth and development processes are related to the most important biogenic compounds, carbon, nitrogen, sulphur and phosphorus use. The transformation of these elements mostly regulated by the biotic processes (Alexander, 1975; Barabasz, 1991; Barabasz, 1992; Barabasz & Vořišek, 2002; Smyk, 1995). Microorganisms with a high metabolic rate, influence the dynamics of many biochemical and biogeochemical processes in soils. Biochemical processes related to the transformation of organic matter and synthesis of inorganic and organic compounds as well as biologically active substances depend on the microorganisms (Baran *et al.*, 1993; Lynch & Poole, 1979; Metting, 1993; Mc Arthur, 2006; Stewart & Carlton, 1986; Trevors & van Elsas, 1995).

Microbes, take part in many biogeochemical processes and generate many characteristic products, and while do so, greatly increase their own numbers. Microbiological methods can be used to detect specific groups of microorgan-

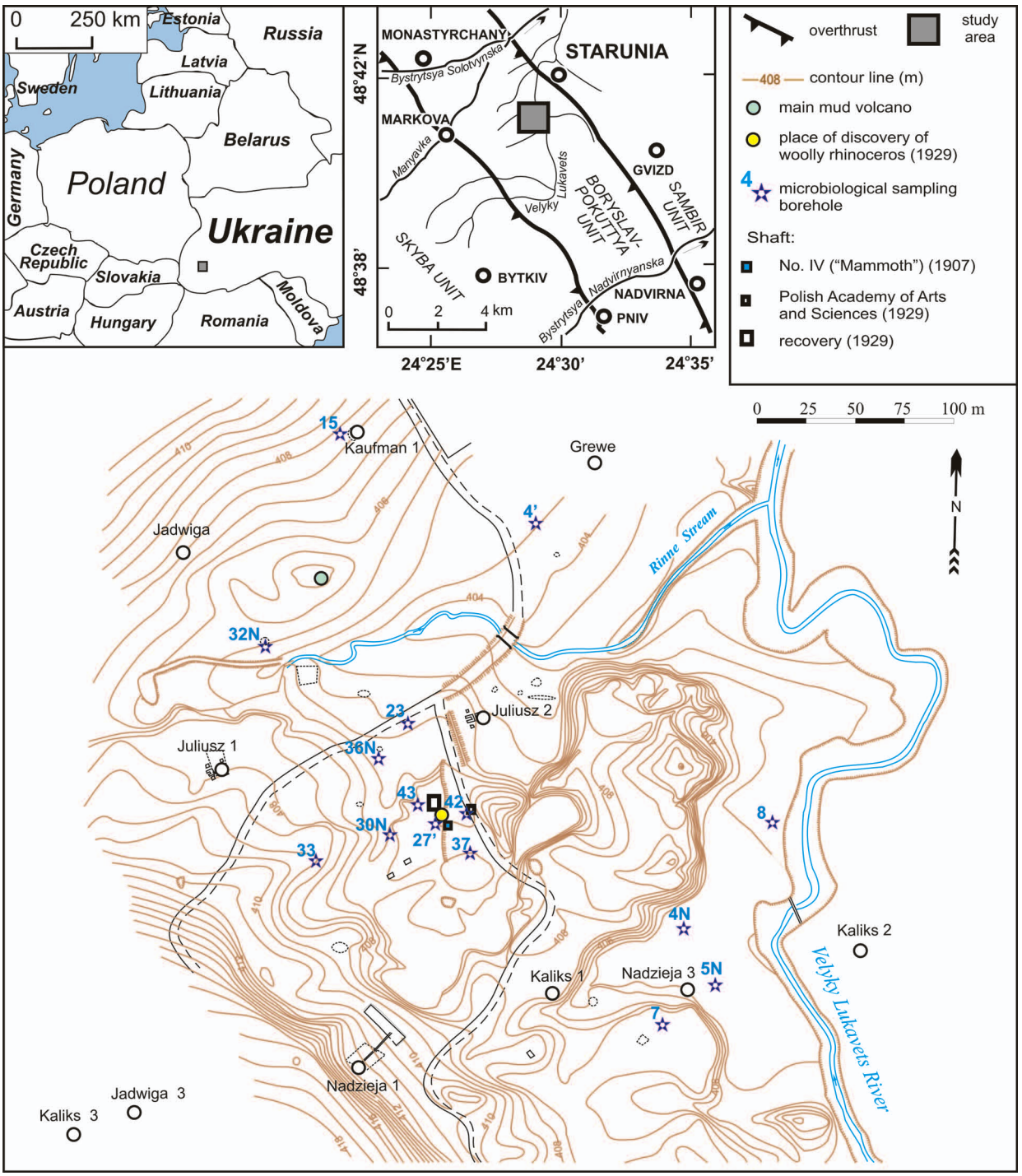

Fig. 1. Sketch map of the Starunia palaeontological site and surrounding area (Carpathian region, Ukraine) showing the location of sampling boreholes for microbiological study

isms, which will suggest certain processes. By estimating the quantity of microorganisms or the activity of some processes, the places of the highest intensity of these changes can be defined (Alexander, 1975; Buckley & Schmidt, 2002; Degenes *et al.*, 2001; Tate, 1986; Torsvik *et al.*, 1990; Torsvik & Øvreeås, 2002). Such places, in which large groups of specific microorganisms occur as the typical ones, or as anomalies, should receive special attention. High amounts of microorganisms, active as the organic carbon (cellulolytic bacteria, methanogenic and methanotrophic), nitrogen (ammonifiers, nitrifiers, denitrifiers) and phosphorus compounds bio-transformers, will show the processes of organic matter mineralization which occur in aerobic as well as in anaerobic conditions. Microbiological organic matter mineralization may result in the occurrence of some different products depending on the environmental conditions. Anaerobic conditions, which occurred in the near-surface layers of the Quaternary sediments, will promote the

fermentation processes, dry rotting and methanogenesis, whereas the aerobic conditions will promote the oxidation and rotting processes (Fenchel *et al.*, 1998; Kirk *et al.*, 2004; Stewart & Carlton, 1986).

Groups of microorganisms change their biomass, metabolic activity and the microbiocenotic composition (biodiversity) as a response to numerous stress factors and stimulators (Ehrlich, 1996; Metting, 1993; Tate, 1986). Microorganisms play an important role in the altering of various substances in the environment as well as in the soil. The result of their activity is not only the decay and mineralization of the organic compounds, but also the activation of many minerals, which play a fundamental role in higher plants' growth. Numerous soil and pedogenesis processes like decomposition of silicates, aluminosilicates, apatites and other mineral compounds of soils, soil structure creation, synthesis and decomposition of humus, free nitrogen fixation etc. are highly dependent on higher plants (Barabasz & Vořišek, 2002; Smyk, 1984). It may be easily suggested that interrelations between populations of soil microorganisms and the microbiological succession influences the entire soil metabolism. Elimination or limitation of activity of any of the microbiological chain in the soil environment breaks or at least disrupts the whole food chain (Pepper *et al.*, 1995; Mc Arthur, 2006).

The main aim of the research was the characterization of the microbiological activity in the general environment of Pleistocene and Holocene sediments and in the mine dump. In addition an objective was locating any anomalies related to the quantity or biodiversity of the so-called microbiocenotic composition of groups of microorganisms, which had been selecteded as indicator.

SAMPLING AND METHODS

Sediment core samples were taken from the boreholes in 2007 and 2008. Fourty-six samples from various depths of 15 boreholes were used for microbiological analyses (Fig. 1). The boreholes were made in places in which natural anomalies had been identified during the previous analyses. Microbiological analyses were focused on microorganisms associated with carbon transformations. These microbes were chosen as indicators to evaluate microbiocenotic transformations influenced by natural environmental changes and by human activity e.g. during ozokerite exploitation.

About 500 grams of sediment from each sample were collected during drilling and put into containers, preserving the microbiological sterility. Immediately after sampling the pH was evaluated in the field (pH in H_2O). The samples were stored at 4^oC until ready for analysis. Microbiological analyses were carried out using the serial dilutions method to determine the quantity of bacteria, spore forming bacteria, actinomyces, fungi and metylotrophic as well as methanogenic bacteria on several specific media, commonly used in microbiological laboratory.

Because of huge differences between the humidity of samples, dry mass was also determined; the results were calculated and presented as colony forming units (cfu) per 1 gram of the soils' dry mass (Alef & Nannipieri, 1995; Atlas & Parks, 1997; Tunlid & White, 1991; Paul & Clark, 2000). All microbiological analyses were carried out at the laboratory of Department of Microbiology, University of Agriculture in Kraków.

RESULTS AND DICUSSION

The microbiological part of the analyses of sediment samples aimed at showing the probable changes in the quantity of soil microorganisms in the boreholes on each geological level. The results, presented in (Tab. 1, Fig. 2) show very large differences in the population of the tested groups of microbes. The graphs (Fig. 2) show quantitative changes in the microorganisms' composition depending on the depth and the pH reaction.

The results, related to the occurrence of different physiological groups of microbes (Table 1), show that their quantity varied considerably, depending on the sampling site, sampling depth and organic matter content. The detailed microbiological analyses indicated that together with the sampling depth, the quantity and diversity of microorganisms are lower. A definitely higher quantity of the tested microbes, especially bacteria and actinomyces and a very high number of fungi which take part in cellulose decomposition, suggesting that the near-surface level with aerobic conditions, promotes the processes of organic matter mineralization in the tested Quaternary sediments.

The pH of the tested samples was mostly equal to about 6, frequently ranging between 6.5 and 7.5. This signifies that the samples were neutral or slightly acidic. The borehole No. 5N with pH from 6.36 to 7.54 and 33 in which pH was alkaline, from 8.07 to 9.4 were minimal and maximal (Table 2, Fig. 2). Similarly, in the tested material high differences in humidity were observed (30 to 55%), sometimes it was even over 80%, e.g. samples from boreholes Nos 8, 23, 30N, 32N, 33, 42 and 43 (Table 2).

The near-surface level, with intense organic matter biotransformation processes, supplied the sediment with much nutrient-rich substances which are favourable to physicochemical conditions. These processes also improved the environmental conditions for the growth and development of different groups of microorganisms including the tested groups of microbes. The microbiological analyses of the biodiversity suggest that this location has specific soil microflora, typical for grassland ecosystems, rich in organic matter and with neutral or slightly acidic pH. After analysing the microbiocenotic composition in each testing point it may be ascertained that the tested groups underwent a high differentiation depending on the sampling depth, especially for bacteria, actinomyces and fungi.

However in some boreholes at great depths where less organic matter occurred, the number of microorganisms was observed to increase. This was particularly in the case of the samples containing organic mud at greater depths. For example, in the Holocene profiles of boreholes Nos 5N, 8 and 32N (Fig. 2) where peat predominated, a significant increase of the fungi – especially yeasts – count was observed, most likely because they cope with the anaerobic

Table 1

Number of selected groups of microorganisms in core samples

Bore-hole	Depth (m)	Dry mass (%)	pH	Strati-graphy	Number of bacteria 10^3 (cfu g^{-1})	Number of sporulating bacteria 10^3 (cfu g^{-1})	Number of fungi 10^3 (cfu g^{-1})	Number of actinomycetes 10^3 (cfu g^{-1})	Number of methylotrofic bacteria (cfu g^{-1})	Number of methanogenic bacteria (cfu g^{-1})
4'	2.0	45.9	6.98	H	3050	261	17	1702	120	0
	4.0	62.6	6.92	P	2891	48	38	1240	350	170
4N	1.4	73.3	8.00	H	392906	82	6	68921	0	0
	2.5	67.1	7.21	H	125186	63	1.5	51431	0	0
	4.15	70.5	7.07	H	34043	79	1.1	790	0	0
	6.3	69.5	7.41	P	3108	46	1.0	9	0	0
5N	2.1	72.8	7.54	H	6923	41	5	4321	0	0
	2.7	67.3	6.64	H	6315	1204	4	1405	0	0
	4.7	60.9	6.36	H	77	805	3	1.2	30	60
	6.5	78.9	6.57	H	5678	253	0.4	0.1	30	40
7	5.2	66.5	7.31	H	481	0.3	0.9	320	0	0
	6.55	64.2	6.63	H	436	0.3	0.6	281	20	40
8	0.5	83.9	7.61	H	38141	13	5	12387	0	0
	1.5	77.2	8.04	H	43523	39	0.8	3609	0	0
	2.5	78.0	7.82	H	14564	3	6	2808	0	0
	3.5	71.8	6.80	H	42507	0.5	91	5201	0	0
	3.8	72.6	7.78	H	26446	1.4	0.3	490	0	0
15	2.0	61.8	7.36	P	4693	178	11	2030	0	0
	4.0	72.5	7.37	P	566	856	0.6	43	0	20
	6.0	73.8	7.45	P	1.5	163	0.4	0.07	0	0
23	2.0	84.0	7.95	P	4524	30	0.7	3302	0	0
	4.0	74.0	7.09	P	25730	23	0.2	2100	0	10
27'	8.0	76.9	6.96	MD	23927	26	10	4270	0	0
30N	2.9	73.8	6.39	P	126	7	0.5	491	40	0
	5.0	75.0	6.85	P	5.3	27	0	54	210	360
	7.3	84.1	8.25	P	108	6	0	2	150	270
	8.2	77.8	7.90	P	129	9	0.2	57	80	60
32N	1.0	82.0	7.27	H	11098	73	6	9644	0	0
	2.0	68.7	6.87	H	34798	2169	0.3	8632	20	0
	3.0	69.3	7.16	H	7619	22	0.5	8501	0	20
	4.0	76.5	7.41	H	10876	16	0	3470	60	70
	5.0	86.0	7.58	H	12047	34	0	6322	80	80
33	1.8	77.3	9.36	H	298	16	0.02	980	0	0
	4.0	67.9	9.29	P	943	6	0.2	410	0	0
	6.0	83.4	8.80	P	432	8	0.08	1.0	0	20
	8.1	70.9	8.07	P	17	2	0.05	0.2	0	0
36N	0.6	64.1	7.23	H	468	17	0.2	320	0	10
	4.0	76.1	7.58	P	946	53	0.4	307	110	170
	6.0	78.0	7.71	P	179	38	0.1	4	120	90
37	6.0	73.8	7.23	P	47	1.5	0.2	50	0	0
42	3.8	76.1	6.74	H	118	0.5	0.9	890	20	30
	4.6	82.5	7.07	H	1091	5	1.1	62	60	40
	6.45	84.4	7.90	P	341	2	0.5	0.3	30	40
43	3.3	70.8	7.33	MD	33898	0.7	0.2	1279	0	0
	4.5	73.8	6.60	MD	23848	0.9	0.08	1310	0	0
	6.7	82.3	7.80	MD	5103	194	0.04	16	0	10

H – Holocene, P – Pleistocene, MD – mine dump, cfu – colony forming units

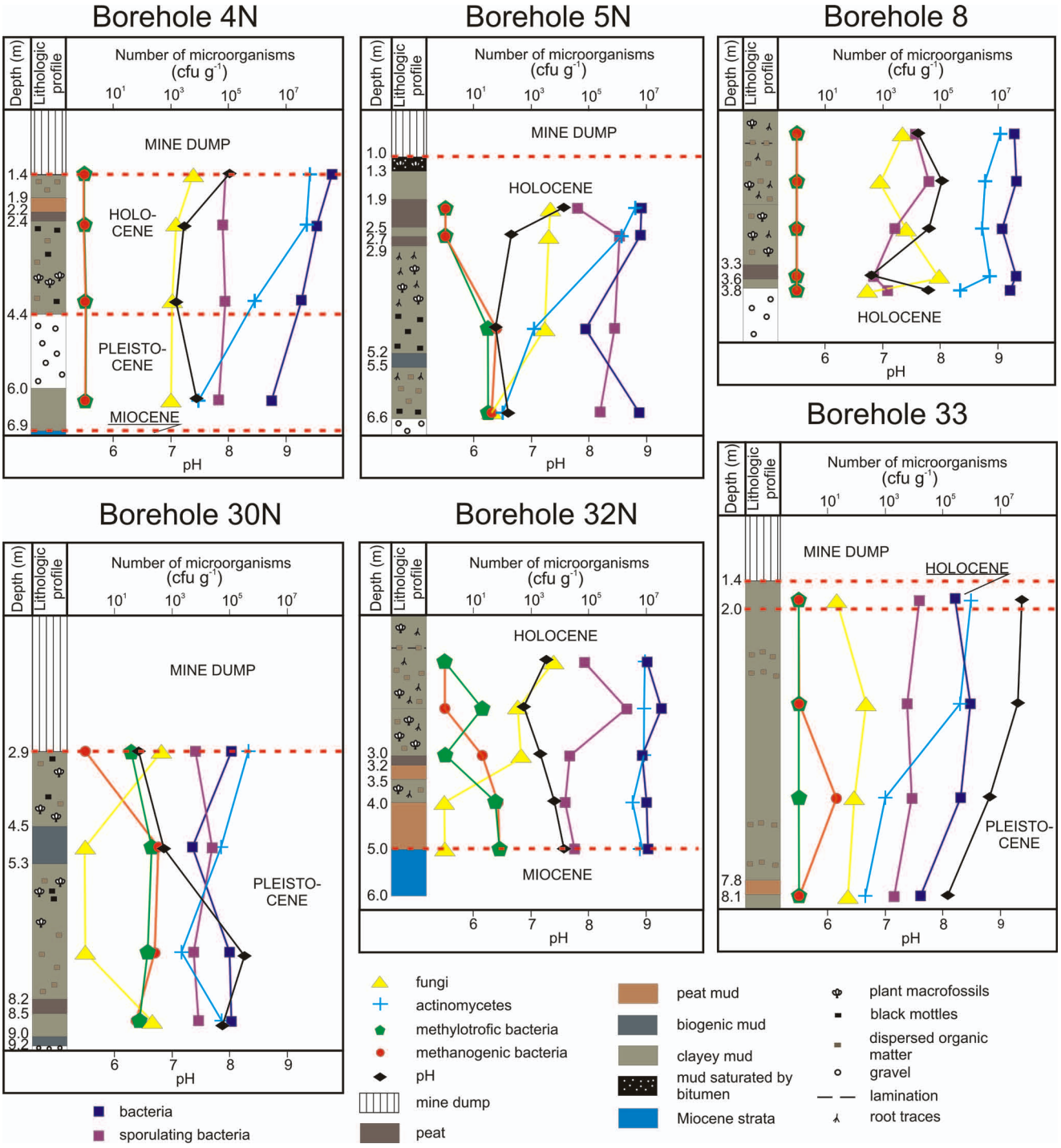

Fig. 2. Distribution selective groups of microorganisms and pH value in profiles of boreholes Nos 4N, 5N, 8, 30N, 32N and 30

conditions due to their fermentative metabolism. A higher number of actinomyces was also observed. These constitute the most numerous microorganisms in Pleistocene (profile No. 23) and Holocene (profiles Nos 5N, 8 and 32N) peats (Fig. 2). Our observations indicate that the main saprophytic microorganisms (bacteria, fungi and actinomycetes) occurred in Holocene sediments containing clayey mud, peat and peat mud.

On the other hand a great number of methanogens were observed in boreholes in which encountered Pleistocene sediments (Table 2). The highest amount of methanogenic bacteria was found in boreholes Nos 30N, 4' and 36N, which contain clayey mud and peat.

From the ecological point of view the isolation of methanogenic and methylotrophic bacteria from the tested sediments especially from the Pleistocene layers was very interesting. These bacteria use CO_2 and acetates which originated from anaerobic fermentation of the organic matter. The abundance of methanogenic bacteria at greater depths of the tested boreholes may indicate the microbial methane

Table 2

Boreholes with occurrence of metanogens in various lithostratigraphic units

Borehole	Depth (m)	Dry mass (%)	pH	Stratigraphy	Lithology	Number of methylotrofic bacteria (cfu g^{-1})	Number of methanogenic bacteria (cfu g^{-1})
4'	2.0	45.9	6.98	H	clayey mud	120	0
	4.0	62.6	6.92	P	clayey mud	350	170
5N	4.7	60.9	6.36	H	clayey mud	30	60
	6.5	78.9	6.57	H	clayey mud	30	40
7	6.55	64.2	6.63	H	biogenic mud	20	40
15	4.0	72.5	7.37	P	clayey mud	0	20
30N	2.9	73.8	6.39	P	clayey mud	40	0
	5.0	75.0	6.85	P	clayey mud	210	360
	7.3	84.1	8.25	P	clayey mud	150	270
	8.2	77.8	7.90	P	peat	80	60
32N	3.0	69.3	7.16	H	peat	0	20
	4.0	76.5	7.41	H	peat mud	60	70
	5.0	86.0	7.58	H	peat mud	80	80
33	6.0	83.4	8.80	P	clayey mud	0	20
36N	0.6	64.1	7.23	H	clayey mud	0	10
	4.0	76.1	7.58	P	clayey mud	110	170
	6.0	78.0	7.71	P	clayey mud	120	90
42	3.8	76.1	6.74	H	clayey mud	20	30
	4.6	82.5	7.07	H	clayey mud	60	40
	6.45	84.4	7.90	P	clayey mud	30	40
43	6.7	82.3	7.80	MD	mine dump	0	10

H – Holocene, P – Pleistocene, MD – mine dump, cfu – colony forming units

origination process. The data show that boreholes Nos 30N, 36N, 5N, 7, 32N and 42 (Table 2) had the highest amounts of methanogenic bacteria. Occasionally methanogens were found in the samples from boreholes Nos 15, 33, and 43 (Table 2), which contained large amounts of organic matter at greater depths. The place where the number of methanogenic bacteria occurred in varying amounts is marked with a circle in Fig. 3. Trying to estimate the quantity, it can be stated that amounts of methanogenic bacteria in the selected boreholes were related to high concentration of the organic matter and were correlated with methane occurrence, which is marked in Fig. 3. This was documented in the near-surface gas research Dzieniewicz *et al.* (2009), Kotarba *et al.* (2005, 2009) and Sechman *et al.* (2009).

CONCLUSIONS

Microbiological analyses of the Holocene and Pleistocene sediment samples indicate high diversity of the microorganisms, depending on the location of the borehole, sampling depth, pH reaction and sample humidity as well as lithology.

Microbiological characteristic of the tested Quaternary sediments showed that they are examples of typical soil microflora of the grassland ecosystems rich in organic matter and with slightly acid to neutral reaction.

Methanogens occurring in places are indicative of anaerobic processes of the organic matter decomposition. Metanogens occurred mainly in Pleistocene layer which containing mainly clayey mud and peat.

The quantity of microorganisms in selected boreholes was related to high concentration of the organic matter (mainly peat and peat mud) and correlated with methane occurrence.

Main groups of saprophytic microorganisms (bacteria, fungi and actinomycetes) occurred in Holocene layers containing clayey muds, peats and peat muds.

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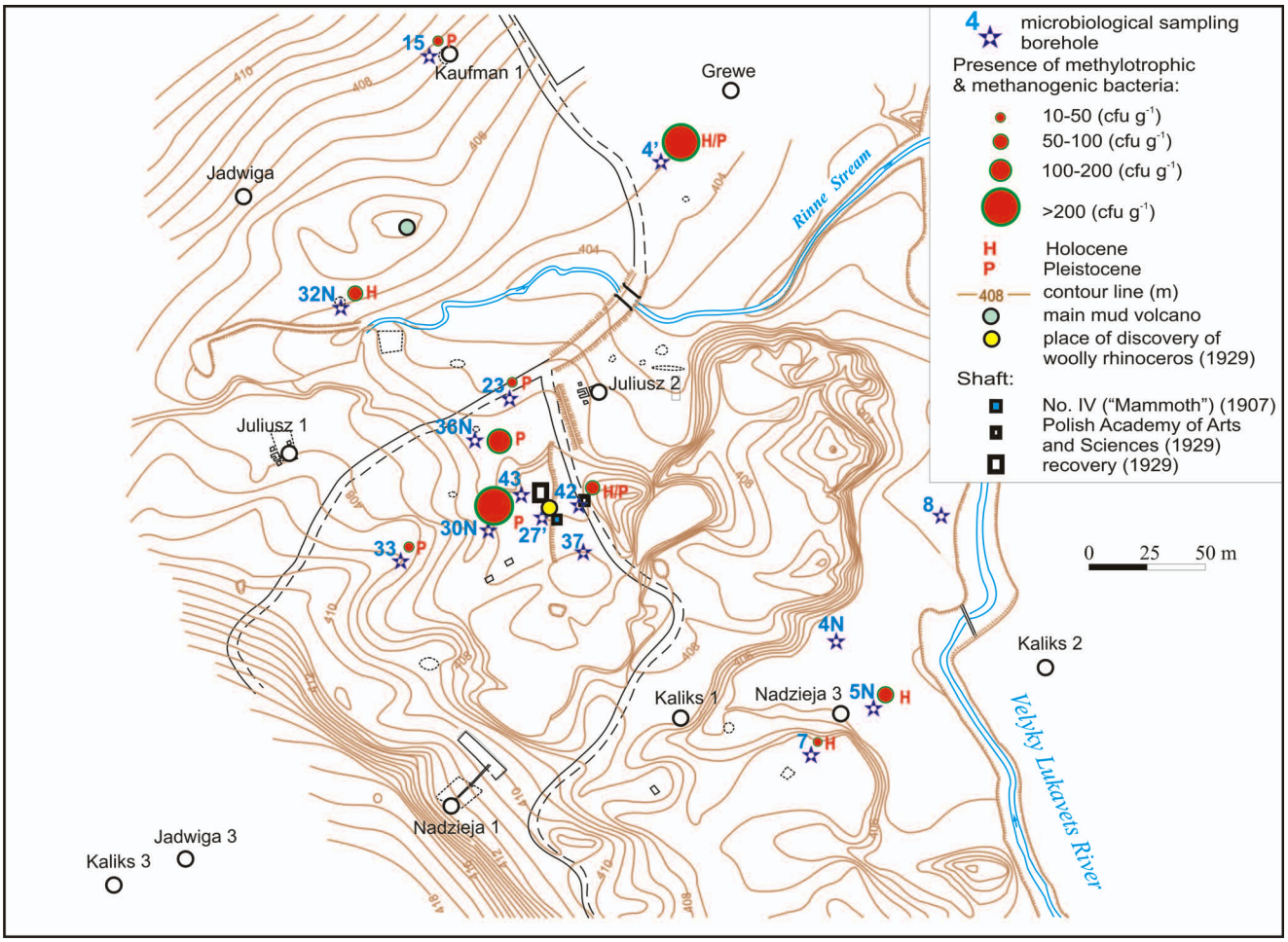

Fig. 3. Variability of concentrations of methylotrophic and methanogenic bacteria in Pleistocene and Holocene sediments in Starunia area

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