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Microbial, Chemical and Enzymatic Properties in Spitsbergen Soils

by Teodor Gheorghe Negoita[1], Gheorghe Stefanic[1], Mirela Emilia Irimescu-Orzan[2], Vasilica Palanciuc[2] and Georgeta Oprea[2]

Summary: Chemical analyses showed that on the western coast of Spitsbergen some soils have a very high content of organic matter (2.15-5.63 Ct %) in the top layer while other soils show significantly lower quantities (0.75-0.82 Ct %). With respect to the total nitrogen content (Nt %) a similar situation can be found. Investigations in upper soil horizons, carried out for 5 places in Spitsbergen, showed that one of the soils studied was very little developed with 51,000 colony forming units (cfu) g^{-1} dried soil while four soils were more developed with 233,600-695,000 cfu g^{-1} dry wt. Heterotrophic, aerobic, non-sporogenous and Gram-negative bacteria are typical for the rhizosphere. Micromycetes, however, were very scarce. Soil respiration and cellulolytic potentials were similar to those found in acid and cold soils of Romania (temperate climate). The five soils were enzymatically tested for: catalase, saccharase, urease and total phosphatase potentials. It is important to underline that the biotic and enzymatic potentials of the four more developed soils from Spitsbergen are comparable with the poorer soils from Romania. In Spitsbergen the period with a climate favorable for biological processes is too short (2-3 months) and consequently the organic layer is only 9-12 cm thick.

Zusammenfassung: Chemische Analysen zeigen, dass entlang der Westküste von Spitzbergen manche Böden einen sehr hohen Gehalt an organischem Material (2,15-5,63 Ct %) aufweisen, andere hingegen einen deutlich niedrigeren Gehalt (0,75-0,82 Ct %). Dasselbe gilt für den Gehalt an Gesamtstickstoff (Nt %). Untersuchungen zu Bodenhorizonten, die in fünf Gebieten auf Spitzbergen durchgeführt wurden, haben gezeigt, dass einer der untersuchten Böden sehr schwach mit Bakterien 51.000 cfu g^{-1} TG entwickelt war, während die Böden an vier Standorten besser entwickelt waren (233.600-695.000 cfu g^{-1} TG). Heterotrophe, aerobe, nicht-sporogene, Gram-negative Bakterien sind typisch für die Rhyzosphäre. Mikromyceten sind dagegen selten. Bodenatmung und cellulolytisches Potential haben ähnliche Werte wie saure und kalte Böden in Rumänien (gemäßigtes Klima). Die fünf Böden von Spitzbergen wurden enzymatisch auf Katalase, Saccharase, Urease und Gesamtphosphatase-Potential getestet. Wichtig ist zu betonen, dass die biotischen und enzymatischen Potentiale bei den vier mehr entwickelten Böden aus Spitzbergen denen der ärmeren Böden aus Rumänien (gemäßigte Zone) vergleichbar sind. Die Vegetationphase in Spitzbergen ist mit 2-3 Monaten nur kurz und die organische Schicht demzufolge nur 9-12 cm dick.

INTRODUCTION

There are only a few papers studying the soil microbiology and enzymology in Spitsbergen. In August 18-25, SOLHEIM (1990) collected soil and vegetation types from 51 locations and, after testing the nitrogenase activity (N_2-fixation), he found that the microorganisms responsible for nitrogen fixation are cyanobacteria, living in or on the moss. Efforts to isolate other nitrogen-fixing bacteria from moss and plant roots were unsuccessful. SOLHEIM et al. (1996) found that in Spitsbergen (79°N, 12°E) the most important source of biologically fixed nitrogen were cyanobacteria either as free living colonies of *Nostoc sp.* in wet unvegetated or sparsely vegetated grounds, or growing as epiphytes on bryophytes. Fixation associated with plant roots or in soil and peat samples had little or no significance for nitrogen fixation and has been greatly influenced by grazing of geese. Under cliffs harbouring colonies of birds, the biological nitrogen fixation has been inhibited by bird droppings. In his opinion, bird droppings in these areas provide a good source of nitrogen and phosphorus available for plant nutrition.

Our investigations were aimed at characterizing the soil microflora, the soil enzymatic potential and some chemical features of the soil in order to get an image of the soil fertility in Spitsbergen.

METHODOLOGY

Site description

The Arctic polar zone of Western Spitsbergen, explored by Negoita in 1996 between 78°13'N, 15°18'E and 78°03'N; 14°18'E, has a climate more moderate than that found in areas of comparable latitudes in Alaska, because it is influenced by the North Atlantic Current (a continuation of the Gulf Stream) which has a branch, the Norwegian Current, flowing towards the West Coast of Svalbard. In the Isford Radio zone, located at 78°04'N, 13°37'E, VIDAR (1985) mentions the temperature record between 1934 and 1975 as follows: for June, -8.2 °C to 12.3 °C; for July, -1.3 °C to 17 °C and for August, -2.3 °C to 14.3 °C. In this area, temperatures range usually between 0 °C and 10 °C.

Details on general properties of soils and plant cover in a similar area have been published by KLIMOWICZ & UZIAK (1996). According to their description, the first 10 m from the fjord form a relatively flat area composed of light silty loam at land surface and heavy silty loam beneath. Soils are initially brown ones, with poorly visible mud boils at the surface. Plant cover occupies, generally, about 30 % of the area and is mainly composed of *Cetraria delisei* and *C. hiascens, Stereocaulon sp., Silene acaulis, Saxifraga oppositifolia.* Towards the river, there is a clearly visible and considerably inclined valley slope, mainly composed of light loam in the bed and of striped soils. Plant cover is more widespread there (65 %) than on the top - the flattened part of the terrace. Among the species there is also *Equisetum variegatum.* The valley bottom is mainly filled with stones and gravel, containing mixtures of small soil particles (<1 mm). Such poor substrate has made soil development difficult and therefore the latter has no plant cover.

[1] Romanian Polar Research Institute, 1 Libertatii, 760161, Bucharest - 4, Romania
[2] Research Institute for Cereals and Industrial Crops, Fundulea, jud. Calarasi, Romania

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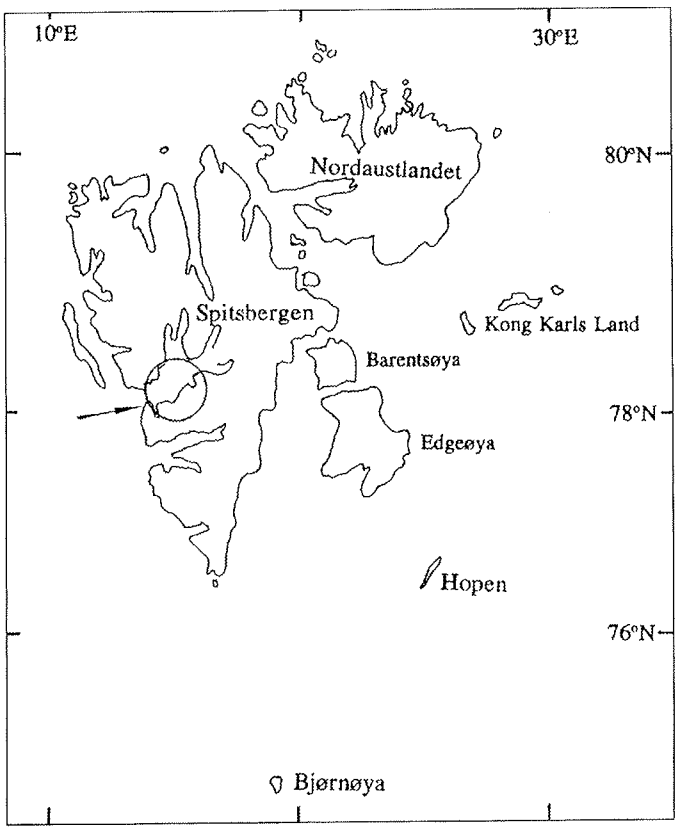

Fig. 1: Soil sampling sites.

Abb. 1: Lage der Probennahmepunkte.

The soil samples from five locations were gathered by Negoita, during the Sixth Romanian Polar Expedition in the Spitsbergen area (Fig. 1). The soils were collected from the following depths: 0-9 cm; 9-12 cm and 12-20 cm, put into plastic bags under aseptic conditions and preserved in frozen condition during the transport and in the laboratory (15 days) during analysis.

Microbial analyses.

The number of bacteria (colony forming units - cfu) was determined by the soil decimal dilution method on soil extract medium with 1.8 % agar (ALLEN 1950, POCHON 1954) and incubated at 15 °C, for seven days. Colonies were counted only in 0-9 cm of soil layer and transferred into tubes on Topping slant medium (yeast extract-peptone-agar, TOPPING 1937) for morphological studies.

With regard to the nitrogen-fixing bacteria, we tested the frequency of bacterial colonies originated from 10 soil granules by the method of WINOGRADSKY (1926) applied on Ashby's nitrogen-free agar medium (ASHBY 1907) in Petri dishes. For morphological studies the colonies that had formed around the soil granules were transferred into tubes with the same slant medium.

Micromycetes were sought only in 0-9 cm of soil layer, quantifying the index of colonization with fungi (as %) by the method of PARKINSON et al. (1969). Micromycetes that grew around the five soil granules on the water-agar medium in Petri dishes (PAPACOSTEA 1976) were expressed as frequency index (%). Their taxonomic affiliation has been made by their specific fructification.

In order to determine soil respiration, cellulolytic and enzymatic potentials, the wet soil samples were gently dried, then passed through a 2.5 mm sieve, and all visible roots and the fragments of weathered rock were removed. We worked with soil samples at 16-18 % humidity. These activities were performed for the three soil layers with a thickness of 0-9, 9-12 and 12-20 cm, respectively.

The soil respiration potential was tested using the Stefanic's respirometer (STEFANIC 1991, 1994) able to complete the loss of O_2 by respiration from H_2O_2 in automatic contact with MnO_2 dust, controlled by the internal pressure/atmosphere equilibrium. The soil respiration was measured by means of CO_2 respired in 24 hours at 15 °C.

The soil cellulolytic potential was determined by the method of VOSTROV & PETROVA (1961): a piece of cotton tissue (4 x 4 cm) with known weight (105 °C) is placed in a Petri dish on a thin soil layer, then another thin layer of soil is put above. Stefanic's improvement to this method consists in using cotton linen with 50 % polyester in order to avoid a possible error that could be produced by mechanical losses of the degraded linen during the washing. After washing and drying (105 °C) the piece of cotton tissue is weighed once more. The difference between the two weights multiplied by two represents the cellulolytic potential of the soil in %.

Enzymatic analyses.

Several papers cited by SKUJINS (1978) lead to the conclusion that the accumulated enzymes in the soil have their sources in the microbial cells, in superior plant and in animal remains. This enables a more comprehensive evaluation of the soil vital level. With this purpose in view, we considered it necessary to extend the information on the cryogenic soil biology from western zones of Spitsbergen by testing the level of the enzymatic potential involved in the main links of the trophic and energetic chains in the evolution of the soil forming process.

The soil catalase potential was tested by an original device (STEFANIC et al. 1984), by determining the O_2 as cm^3 evolved by 4 g soil referred to 100 g soil / minute, at 26 °C. The soil saccharase potential was determined spectrometrically (STEFANIC 1972) by means of hydrolyzed saccharose (glucose + fructose) referred to 100 g soil / 24 hours, at 28 °C. The total phosphatase potential was determined by a new method using glucose as a trap for PO_4^{3-} (IRIMESCU & STEFANIC 1999). Generally, in soil enzymology all methods have been borrowed from general biochemistry applied to different extracts from animal, inferior and superior plant organs or tissues, using a specific substrate in the enzymatic mixture. Thus, for phosphomonoesterase activity assessment a phosphomonoester is utilized; for phosphodiesterase activity a phosphodiester is utilized; for pyrophosphatase or metaphosphatase a pyrophosphate or metaphosphate is utilized etc. The authors of this method had another conception. Under laboratory conditions all phosphatases

accumulated in soil act specifically on all specific substrates with phosphorus originating in soil. The ability of glucose to enter into combination with o-phosphoric acid is well known. This method is based on these properties of glucose and phosphoric acid. Thus, 5 g of soil are mixed with 10 ml sodium azide solution (0.015 %) which contain 0.025 g glucose and 40 ml of potassium alum (0.3 %). This mixture is stirred for 15 minutes and then it is filtered. This is to be considered as the inactive phosphatase mixture. The active phosphatase mixture is prepared as above, but the K alum is added after 24 hours of incubation at 28 °C, during which the mixture is stirred and then filtered. The rest of non combined glucose from each soil mixture is quantified with dinitrosalycylic acid reagent. The difference between inactive and active phosphatase mixtures is the glucose combined with the enzymatically released PO_4^{3-}. This glucose quantity is multiplied by 0.04 (the quotient of combining between glucose and H_3PO_4, determined by the authors within the limits of real proportions in the phosphatase mixtures), for expressing the combined glucose by phosphorus (P). Data are expressed as mg P to 100 g soil.

Chemical analyses

Soil organic matter has been analyzed by sulpho-chrome oxidation determined as total organic carbon (C_t%) by a spectrometric method (SALFELD 1974). Soil extractable carbon (Ec %) has been obtained by alkaline extraction (KONONOVA & BELCHIKOVA 1961, SALFELD 1974). Soil organic phosphorus (P) has been analyzed by the method of LEGG & BLACK (1955). Soil chemical reaction was determined as pH by the electrometric method.

RESULTS AND DISCUSSION

A short description of the analyzed soils is given in Table 1. The heterotrophic bacteria ranged between 28 and 2336 x 10^3 cfu / g of soil. The highest cfu found in the soil samples obtained from Bjørndalen and Grøndalen and presented in Table 1 are surely a rhizosphere effect, due to a dense perennial graminea - *Poa alpina.* In Bjørndalen there are scarce roots of *Mertensia maritima,* but in Colesbukta there is a weak rhizosphere of *Papaver dahlianum, Saxifraga oppositifolia* and *Dryas octopetala*, in microzones with a low density and the lowest cfu. All heterotrophic bacteria were non-spore forming, Gram-negative, short rods (1 µ / 2-3 µ) or coccoids and were classified as belonging to the first nutritional group (LOCHHEAD 1939).

We did not find cyanobacteria which have frequently been found by SOLHEIM (1990) and OLSEN (1995), but we identified some bacterial cells of various length on the nitrogen free agar medium, with shapes ranging from rods to coccoid, which grow well in 24 hours in hyaline colonies like *Azomonas* described by NEDWELL & RUTTER (1940) for Antarctic soils and Bergey's Manual of Determinative Bacteriology (HOLT et al. 1994). We did not find any *Azotobacter sp.*. SOLHEIM et al. (1996) showed that bacterial N_2-fixation in Svalbard soils had only little significance for nitrogen input to the ecosystem.

Gathering zones of soil samples A (factor)	Short description of soil layers B (factor)	Bacteria cfu x 10^3 g^{-1} soil	Micromycetes observed around soil granules
a.I. Grøndalen zone 10 m from Atlantic coast; loamy-sandy soil	b.1. 0-9 cm, blackish soil; dense roots b.2. 9-12 cm, yellowish soil, scarce roots b.3. 12-20 cm, yellowish soil, without roots	695	*Zygorhincus* *Alternaria* *Penicillium*
a.II. Grøndalen zone sea cliff; sandy soil; scarce rock debris	b.1. 0-9 cm, yellowish soil, without roots b.2. 9-12 cm, yellowish soil, without roots b.3. 12-20 cm, yellowish soil, without roots	51	*Zygorhincus* *Alternaria* *Penicillium* *Mucor, Mortierella*
a.III. Bjørndalen zone, end of Bear Valley; loamy sandy soil; scarce rock debris	b.1. 0-9 cm, yellowish soil, scarce roots b.2. 9-12 cm, yellowish soil, without roots b.3. 12-20 cm, yellowish soil, without roots	361	*Zygorhincus* *Alternaria* *Penicillium*
a.IV. Bjørndalen zone, entrance of Bear Valley; loamy-sandy soil; near sea cliff	b.1. 0-9 cm, blackish soil; dense roots b.2. 9-12 cm, blackish soil; dense roots b.3. 12-20 cm, yellowish blackish soil, scarce roots	2336	*Zygorhincus* *Alternaria* *Penicillium* *Fusarium, Polyscytalum*
a.V. Colesbukta zone; sea cliff; sandy soil	b.1. 0-9 cm, blackish soil; dense roots b.2. 9-12 cm, yellowish-blackish soil; scarce roots b.3. 12-20 cm, yellowish soil; scarce roots	28	*Zygorhincus* *Alternaria* *Penicillium*

Tab. 1: Some pedological and microbial features of the soils from the west coast zone of Spitsbergen.

Tab. 1: Pedologische und mikrobielle Eigenschaften der Böden an der Westküste Spitzbergens.

On water-agar medium, different mycelia of micromycetes grew from soil granules. The colonization index was 100 %. Among the studied micromycetes the following have been identified by their fructiferous bodies: *Zygorhincus, Alternaria, Penicillium, Mucor, Mortierella, Polyscytalum, Fusarium.* As sporogenous bacteria were absent and micromycetes were scarce we concluded that the bacteria in these soils belong to the rhizosphere of bryophytes.

The biotic and enzymatic activity of the soils determined here are always potential activities, because they are tested in the laboratory, under optimum conditions, not *"in situ"* under uncontrolled conditions, where the different enzymes may exist, but the specific substrates may fail, or inversely.

The respiration potential (Tab. 2) is higher in the soils I and IV and significantly lower in the other soils. The analysis of soil layers shows a very high potential of the soil samples from the shallow soil (0-9 cm) with a dense root system. In Tables 1 and 3 it can be noticed that the soils I and IV accumulated most organic carbon and more dense heterotrophic bacteria (695 and 2336 cfu). By comparison, the soils II, III and V are significantly inferior as far as vitality is concerned. The soils have been classified by means of the LD 5 % into class "a" with the higher averages of the respiration potential, and class "b" with a lower respiration potential, respectively. The differentiation of the respiration potentials between soils and soil layers has been made by means of Duncan test (SENDER 1965) showing in alphabetical order the decrease of the potential activities or of the chemical accumulations.

The reduction of the respiration potential with the soil depth may by explained in connection with the root mass diminution. Results obtained by FISCHER (1995) concerning the intensity of bioenergetical processes in three soils differently covered with vegetation pointed out that in Svalbard the structured

Soils (A)	Depth cm (B)	Respiration (mg CO_2 to 100 g soil)	Celulolyse (% lost to 100 g cotton tissue)	Catalase (cm^3 evolved O_2 min^{-1} to 100 g soil)	Saccharase (mg hydrolyzed saccharose to 100 g soil)	Total phosphatase (mg P released to 100 g soil)
a.I	b.1	a 32.9	a 34.9	a 229	a 671	c 4.00
	b.2	b 25.4	b 27.4	b 51	b 73	b 4.83
	b 3	c 15.5	c 21.7	b 43	b 129	a 5.67
Average (A)		a 24.6	c 28,0	c 131	d 291	d 4.83
a.II	b.1	a 11.4	b 29.6	a 1.4	a 5	a 6.24
	b.2	b 8.0	a 38.6	a 0.0	a 6	a 6.60
	b.3	b 8.7	a 36.3	a 0.0	a 3	b 5.60
Average (A)		b 9.4	b 34.9	e 1.3	e 4.7	b 6.15
a.III	b.1	b 6.9	b 25.8	b 27	b 55	a 9.79
	b.2	a 11.3	a 34.1	a 65	b 70	b 8.44
	b.3	a 12.6	a 34.6	a 46	a 1438	c 3.81
Average (A)		a 10.3	b 31.5	d 46	c 521	a 7.34
a.IV	b.1	b 17.4	a 50.2	a 257	b 403	b 4.29
	b.2	a 29.1	b 35.6	a 228	a 1601	a 6.07
	b.3	b 19.5	b 35.7	b 85	a 1507	c 2.93
Average (A)		a 22.0	a 40.5	a 191	a 1170	e 4.43
a.V	b.1	a 16.4	a 26.8	a 408	a 1150	b 5.30
	b.2	b 9.8	a 27.4	a 47	b 779	a 6.74
	b.3	b 7.3	a 29.1	b 12	c 590	c 4.27
Average (A)		b 11.2	c 27.8	b 156	b 840	c 5.48
LDP 5% (A)		2.6	43	35	1.22	0.55
LDP 5% (BxA)		2.4	1.9	18	1.73	0.20
For comparison with soil activity potentials from Spitsbergen						
Albic luvisol (Romania)		30	71	182	753	0.40
Chenozem (Romania)		55	591	2130	2700	17.00

Tab. 2: Potential of biotic and enzymatic activities in different soils from Spitsbergen. The letter before the number points out the size of potential activity, statistically provided by Limit Difference (LD P 5 %) separately for A factor (average for each soil) and for interactions between A and B factors (A x B).

Tab. 2: Potentielle biologische und enzymatische Aktivität in verschiedenen Böden auf Spitzbergen.

soil had a higher respiration potential than those covered with moss or willow.

To get an image of the size of the respiration potential of the soils from western coast of Spitsbergen and of the evolution of soil fertility, we mentioned the characteristic data for the chernozem and the albic luvisols from Romania for each test below the tables. We noticed that the respiration potential is very low in the west of Spitsbergen compared with the chernozem and even with albic luvisol from temperate climate in Romania, which benefits from about 8 months of positive temperatures. The very short summer in Western Spitsbergen makes the humidification very difficult and is the reason for a low efficiency of biomass synthesis in soil.

The soil cellulolytic potential in the shallow layers (Tab. 2) is highest in soil IV where the soil pH is 4.47. In the other soils (I and V) with more organic matter (C_t % and N_t %) the cellulolytic potentials are lower probably because of the pH which ranges between 3.80 and 4.20. The cellulolytic potential of Western Spitsbergen soils is within the limits of the Romanian soils, but the short summer in Spitsbergen makes the transformation of the plant cellulose into a material sufficiently energetic for the metabolism of the soil microflora impossible. Quantitatively, the root exudates are the most abundant sources of energy, being immediately used by the heterotrophic microflora.

Catalase, saccharase and phosphatase activities, as indicated in Table 2, show levels somewhat related with bacterial number, respiration potential, organic carbon, nitrogen and phosphorus contents (Tab. 3). It can be noticed that the yellowish soil (aII Grøndalen) without roots has the worst biological and enzymatic activities at all depths of profile and also the lowest contents of organic carbon, nitrogen and phosphorus. It is clear that the vegetal cover is the main source of energetic and trophical material for microbiological and enzymatic activity.

Although in the top layers of the soils I, IV and V, there are some important quantities of organic carbon and nitrogen and there is a certain biotic potential for transforming them in humus, this process is very slow. DZIADOWIEC et al. (1994) who

Factors A	B	Organic carbon (C_t%) Interaction BA	Total nitrogen (N_t%) interaction BA	Organic phosphorus (P%) interaction BA	Soil chemical reaction (pH) interaction BA
a.I	b.1	a 5.63	c 0.177	a 124	a 4.80
	b.2	b 5.08	a 0.398	c 77	b 3.90
	b.3	b 2.38	b 0.207	b 97	c 2.69
Average (A)		a 3.36	c 0.261	a 99	c 3.80
a.II	b.1	a 0.82	a 0.084	a 53	b 3.74
	b.2	a 0.75	a 0.084	a 60	c 3.36
	b.3	a 0.86	b 0.077	a 64	a 4.01
Average (A)		c 0.81	e 0.082	c 59	c 3.70
a.III	b.1	b 1.37	b 0.117	a 64	b 2.60
	b.2	a 2.14	a 0.159	a 66	a 3.37
	b.3	a 2.16	b 0.115	a 69	a 3.45
Average (A)		b 1.89	d 0.130	c 66	d 3.14
a.IV	b.1	c 1.94	c 0.166	b 46	b 4.44
	b.2	b 3.60	a 0.432	a 67	c 4.09
	b.3	a 4.39	b 0.294	a 57	a 4.87
Average (A)		a 3.31	b 0.298	d 57	a 4.47
a.V	b.1	a 2.96	a 0.168	a 108	a 5.32
	b.2	b 1.45	b 0.106	b 57	c 3.21
	b.3	b 1.18	b 0.100	b 67	b 4.08
Average (A)		b 1.86	a 0.358	b 77	b 4.20
LDP 5% (A)		0.23	0.031	7	0.23
LDP 5% (BxA)		0.36	0.006	13	0.33

Tab. 3: Chemical features of the soils from northwest coast zone of Spitsbergen. The letter before the number points out the size of potential activity, statistically provided by Limit Difference (LD P 5%), separately for A factor (average for each soil) and for interactions between A and B factors.

Tab. 3: Chemische Eigenschaften der Böden an der nordwestlichen Küste Spitzbergens.

studied the properties of humic acids in tundra soils from Spitsbergen, stated that a weak synthesis of humic acids occurs because of low temperatures, high moisture content in soils, frost-thaw alteration and organic components of plant remains, predominantly poor in lignin,.

The highest differences between the soils from temperate climate (Romania) and cryogenic soils consist in the lack of clay, in a very reduced quantity of organic material input and in a too short period with positive temperatures in Spitsbergen soils and, consequently, in a very slow soil forming process. It is obvious that the vegetal cover is the main source of energetic and tropical material for microbiological and enzymatic activity.

CONCLUSIONS

Being favourably influenced by the North Atlantic Current, the soils from the western coast of Spitsbergen with perennial herbs in microzones developed in different modes, depending on the vegetal cover. The best vital and enzymatic potentials of these cryogenic soils are comparable with the acid and cold soils from Romania (temperate climate). However, they differ by a too weak efficiency in chemical element recycling and humus accumulation which is caused by a too short summer and the lack of clay.

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