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Electrophoretic Evaluation of Initial Humification in Organic Horizons of Soils of Western Antarctica

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Abstract: Humification aspects were studied for selected soils of the Antarctic with a special reference for electrophoretic indexes of organic matter transformation. Humus formation and humification usually appears in soils of the Antarctic under mono-species plant communities, and this provides an opportunity to investigate the humification process in relatively simple models of pedogenesis. Organic horizons and organo-mineral solum horizons below graminoid, lichen, or moss vegetation or guano material were studied on the Sub-Antarctic King George Island as well as in several soils on the continental Antarctic. Electrophoresis was used for the assessment of the degree of soil organic matter transformation in a soil horizon sequence: from initial, weakly transformed organic matter to partially decomposed or humified ones. The complex investigation of the soil profiles' morphology, carbon and nitrogen contents and their ratio, humic acids (HAs) and fulvic acids (FAs) portions with the relation to optical indexes and electrophoretic properties show that there is current transformation and humification of soil organic matter (SOM) in the studied soils. There are some common tendencies in changes of soil properties with increasing soil depth: decreasing of C/N ratios due to SOM mineralization and transformation, formation of the fulvic type of humus instead of accumulation of HAs, decline of the E465/E656 absorbance ratio of humic substances in correspondence with increase of the absorbance coefficient (A465) of humic substances and strong differentiation of electrophoretic HAs. The most humified organic matter was typical for the organo-mineral A humus horizons (where a lesser portion of peptides and an increased portion of low molecular size (L-MS) fraction resulted from redistribution of organic fractions in soil profile). The obtained data supports evidence of initial humification in weakly developed soils of the Sub-Antarctic and Antarctic. The portions of high molecular (H-MS) and medium molecular size (M-MS) organic compounds were typically higher in the less decomposed and humified Oi and Oe horizons than in the humified Oh and A horizons.

Zusammenfassung: Für ausgewählte Böden der Antarktis wurden die Prozesse der Humifizierung mit besonderem Fokus auf den elektrophoretischen Indizes der Transformation organischer Substanz untersucht. Humusbildung und Humifizierung finden gewöhnlich in Böden der Antarktis unter monospezifischen Ökosystemen statt. Daher ergibt sich die Möglichkeit die Humifizierung in relativ einfachen Pedogenese-Modellen zu untersuchen. Organische Schichten und organo-mineralische Solum-Horizonte wurden unter Gräsern, Flechten, Moosen und Guano-Materialien auf der subantarktischen König-Georg-Insel sowie in mehreren Böden der kontinentalen Antarktis untersucht. Elektrophorese wurde für die Bewertung des Zersetzungsgrades organischer Bodensubstanz in der Bodenhorizontabfolge verwendet: Von initialer, schwach zersetzter bis teilweise zersetzter oder humifizierter organischer Substanz. Die vielseitige Untersuchung der Bodenmorphologie, der Kohlenstoff- und Stickstoffgehalte, des C/N-Verhältnisses und die Untersuchung der Anteile an Humin- und Fulvosäuren in Bezug auf ihre optischen Indizes und elektrophoretischen Eigenschaften zeigen, dass in den untersuchten Böden Zersetzung und Humifizierung von organischer Bodensubstanz stattfindet.
Es wurden einige allgemeine Tendenzen mit zunehmender Bodentiefe gefunden: Abnahme des C/N-Verhältnisses durch Zersetzung organischer Reste, Bildung von Humus des fulvischen Typs relativ zur Anhäufung der Huminsäuren und die Senkung des E465/E656-Verhältnisses in Übereinstimmung mit der Zunahme des A465-Absorptionskoeffizienten und einer starken Differenzierung der elektrophoretischen Eigenschaften von Huminsäuren. Der größte Teil humifizierter organischer Substanz wurde in organo-mineralischen A-Horizonten gefunden (hier ist der Gehalt von Peptiden am niedrigsten und der Gehalt an niedermolekularen Verbindungen am höchsten) infolge der Umverteilung von organischen Verbindungen im Bodenprofil. Die Daten liefern Hinweise auf initiale Humifizierung in schwach entwickelten Böden der Sub-Antarktis und der kontinentalen Antarktis. Es wurden höhere Anteile an hochmolekularen und mittelmolekularen organischen Verbindungen in den weniger zersetzten und humifizierten Horizonten Oi und Oe als in den stärker humifizierten Horizonten Oh und A gefunden.

INTRODUCTION

The soils of the Antarctic attract the special interests of soil scientists, ecologists and chemists because of the isolation of its landscapes and ecosystems from direct effects from other continents and the low anthropogenic disturbance of the region. The severe environmental conditions result in the formation of weakly developed, shallow soils (VLASOV et al. 2005, IVANOV & AVESSALOVOMA 2012), which can be investigated only by sampling in high vertical resolution (MERGELOV et al. 2012). However, there are features of weak podzolisation of the fine earth or formation of Cambic horizons in some regions (BEYER et al 1997, 2000).

The soils and biosediments of different parts of the Antarctic have been investigated in the following aspects: morphology (GLAZOVSKAYA 1958, BOCKHEIM 2002, MERGELOV et al. 2012, ABAKUMOV & KRYLENKOV 2011, ABAKUMOV 2011, 2012, ABAKUMOV et al. 2008), texture and lithology (BEYER 2000, ABAKUMOV 2010), mineralogy (SHAEFER et al. 2008), geography (CAMPBELL & CLARIDGE 1987, GILICHINSKIY et al. 2010) and micromorphology (KUBIENA 1970, ILIEVA et al. 2003) as well as anthropogenic changes (BALKS et al. 2002). Many works are connected to the accumulation of biogenic compounds, humus formation and humification (CROLL et al. 2005, SIMAS et al. 2007, BLUME et al. 1998). It was shown that accumulation of organic matter in Antarctic terrestrial ecosystems can occur in soils, surface sediments, seasonal puddles, and the benthic and litoral zones of ponds and lakes (BEYER et al. 1995, 1997, 1998, ABAKUMOV & KRYLENKOV 2011). The sources of organic matter in Antarctica are plant remnants of mosses, lichens and some grasses, dead remnants of fungi and algae as well as guano material (SYROESHKOVSKY 1999, SIMAS et al. 2007, ABAKUMOV 2010). The initial composition of humus precursors is quite diverse and provides the possibility of humification as a process of synthesis of humic substances (HS).

The morphological forms and chemical types of humus are more diverse in Sub-Antarctic tundras than in polar deserts and barrens (CAMPBELL & CLARIDGE 1987) due to a higher diversity of humus precursors, greater variety of soil profile thickness and longer periods of above-zero temperatures.

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Thus, well-developed organic profiles (O horizons) of 15-20 cm thickness underlain by A and AC horizons are typical for King George Island and other islands of the maritime Antarctic zone (Abakumov & Andreev 2011, Simas et al. 2008, Elberling et al. 2006). Previously, it was shown that the composition of plant remnants essentially affects the humification degree and the humic acids (HAs) to fulvic acids (FAs) ratios (C_{HA}/C_{FA}) in Antarctic soils (Abakumov 2010). The most humified organic matter was revealed for soils under graminoids vegetation and guano, while the C_{HA}/C_{FA} ratios were essentially lower in soils under mosses, algae and lichens. The prevalence of the FAs fraction over the HAs fraction is recognized as typical for all kinds of Sub-Antarctic and Antarctic soils. This corresponds closely with the low aromatic fraction content in the humic substances, which was revealed by ^{13}C Nuclear Magnetic Resonance (C^{13}MNR) studies (Clace et al. 1995, Aiken 1996, Bonivata et al. 1996), and the short period of biological activity, especially for polar deserts and barrens. Recently, a study of diversity of humus forms under different plants in a wide range of Antarctic climates was carried out (Abakumov 2010). However, the question of gradual changes of organic remnants in vertical stratified sequences of organic – organo-mineral – mineral horizons still remains open. Micro-morphological and chemical investigations show that organic layers are differentiated by colour and composition of humified organic matter (Ilieva & Verzilov 2003). It seems that humification appears not only in mineral soil as a result of polymerization of monomers (Zavarzina 2011) but also in layers of differently decomposed organic remnants. Humification is possible not only in presence of lignin-derived compounds but also in presence of other phenolic substances; the latter is quite usual under bryophytes (Asakawa 1999) and lichens (Zavarzina 2011). Beyer et al (1997, 2000) suggest that organic matter may initiate the podzolisation process in soils of coastal landscapes of continental and maritime Antarctica. Humic materials play an important role in initial podzolisation in Antarctic soils; soil organic matter in ornithogenic soils with spodic horizons is characterized by increased amounts of amino-derivates and high contents of carboxyl units (Beyer et al. 1997). This supports the idea that HSs formed under different types of organic materials are different not only in chemical composition but also in molecular size distribution and electrophoretic mobility. The investigation of initial humification under different types of organic materials is expected to show that the mobility of humic substances can be different in soils formed under different types of organic remnants. Another aspect which should be considered is the investigation of soil organic matter (SOM) accumulation and humification under vascular plants, the population of which has increased in recent decades due to warming, deglaciation and zoogenic spreading (Fowbert & Smith 1994, Day et al. 2008, Vera 2011). As the vascular plants support deeper humification, a SOM stabilization scenario should be predicted in plots of new colonization of the grass species *Deschampsia antarctica*.

We previously developed an original method of electrophoresis in polyacrylamide gel for the separation of soil HS into fractions differing in their electrophoretic mobility, molecular sizes, and physical-chemical properties (Trubetskoj et al. 1992, 1997, Trubetskaya et al. 2008, 2011). This method has previously been successfully used for the investigation of:
(i) soil HAs and artificial model phenolic polymers (Saiz-Jimenez et al. 1999);
(ii) soil HAs before and after acid hydrolysis (Trubetskaya et al. 2001);
(iii) different compost humic-like substances (Trubetskoj et al. 2001);
(iv) soil HAs in podzol chronosequences (Abakumov et al. 2010).
This method gives an opportunity to separate humic acids into three fractions, which are different in their electrophoretic mobility and molecular weight. The profile distribution of these fractions indicates the HS migration process and their role in organo-mineral interactions. Soils of the Antarctic develop in different climatic and geogenic conditions and have different sources of organic HS precursors: there are well-developed organo-mineral stratified soils, different kinds of lithogenic soils and soils developed under guano material. We suppose that the soil organic matter is different in soils of the Sub-Antarctic and coastal Antarctica and that there should be a more pronounced differentiation between the soil horizons' organic matter quality in Sub-Antarctic soils due to the longer and more intense development of the humification process.

Therefore, the aim of this study was to evaluate the humification process in vertical soil profiles with the use of electrophoresis to substantiate that there is ongoing humification in the sequence of soil horizons and that there are differences between the humic substances within the examples of several Sub-Antarctic Lithosols and some original Leptosols of western Antarctica were used to investigate the electrophoretic indexes of the humification processes phenomena and to establish the differences in humic acids composition of soils from different climatic regions. The following tasks were set up:
(i) to determine the C, N, HAs and FAs contents in different horizons of soils formed under mono-species plant communities in Sub-Antarctic tundra as well as in original primary soil of polar barrens and in soils formed under guano with different degrees of mineralization, and
(ii) to assess the humification process within vertical soil gradients with the use of the electrophoretic method, to identify the levels of HSs mobility in soil profiles and the role of humus precursors in the formation of HAs molecular size distribution.

MATERIALS AND METHODS

Description of study sites

The sampling of soils and organic layers were conducted during the 53rd Russian Antarctic expedition from January 14 2008 to February 25 2008 by the scientific vessel "Academician Fedorov", which visited the Russian polar stations Bellingshausen, Leningradskaya, Russkaya as well as Lindsey Island and the Hudson Mountains; all are located in Western Antarctica. Soil descriptions were partly published earlier (Abakumov et al. 2008, 2010). Soils investigated belong mostly to the Sub-Antarctic maritime zone (King George Island, South Shetland archipelago), and a few samples are from the barrens of Lindsey Island and Leningradskaya Station belonging to the coastal continental Antarctic region (Fig. 1). The Bellingshausen Station (Russian scientific and logistics centre on King George Island, 62°12' S, 58°58' W,

Fig. 1: Locations of sampling plots. (1) = South Shetland Islands, King George Island, Bellingshausen Station (62°12' S, 58°58' W); (2) = Lindsey Island, Pacific sector of Antarctica (73°36' S, 103°02' W); (3) = Oats Coast, Victoria Land, Leningradskiy Nunatak near Leningradskaya Station (69°30' S, 159°23' W). Cartography: D. Graffe, AWI based on GEBCO.

Abb. 1: Untersuchungsgebiete. (1) = South Shetland Islands, King George Island, Bellingshausen Station (62°12' S, 58°58' W). (2) = Lindsey Island, Pine Island Bay, pazifischer Sektor (73°36' S, 103°02' W). (3) = Oats Coast, Victoria Land, Leningradskiy Nunatak bei der Leningradskaya Station (69°30' S, 159°23' W). Kartographie: D. Graffe, AWI und GEBCO.

40 m a.s.l.) is found on the Fildes Peninsula of King George Island. The parent materials for soil genesis here are andesite, basalt, and tuffs. The coastal areas are covered by maritime sands and gravels, while the periglacial plots are occupied by moraines and some fluvio-glacial materials (Peter 2008). The average annual temperature of air is -2.8 °C; in the Austral summer (January and February) the average monthly temperatures rise up to 0.7-0.8 °C (Abakumov & Andreev 2010), but it is necessary to consider that the soil surface temperature is essentially higher when it is free from ice and snow (Markov 1956, 1958). The total annual precipitation reaches 729 mm; the number of days with precipitation is from 22 to 30 days per month. The mean wind velocity is about 9.3 m s^{-1} (Peter et al. 2008) with maxima of about 28 m s^{-1}. The vegetation diversity of the Fildes Peninsula is quite high in comparison with landscapes around other Russian Antarctic stations (Abakumov 2011); mono-species plant communities as well as mixed ones are common for both the coastal part and on the plateau of the peninsula. Accordingly, many authors identified the vegetation as tundra or Antarctic tundra (Casanov-Kathny & Cavieres 2012). Plant communities of King George Island are the most developed and rich of the whole Antarctic. There are many locations on the Fildes Peninsula where the *Deschampsia antarctica* population increases. There are plots of former penguin rookeries, rocks affected by sea petrel (*Laurus dominicanus*) guano and fresh moraines in the periglacial part.

Two other studied plots belong to the coastal part of the continental Antarctic. One of them is Lindsey Island (73°36' S, 103°02' W), interesting because of the very thick and developed layers of guano of the penguins *Pygoscelis antarctica* in the Pacific sector of Antarctica (coastal part of continental sector). This area is almost uninvestigated by biologists. The Leningradskaya Station was selected as an example of primary soil formation in the severe conditions of Antarctic nunataks (rock hills emerging from the surrounding ice sheet). This station is found in the eastern part of the Oates Coast, Victoria Land (69°30' S, 159°23' W, 294 m a.s.l.) on Leningradskiy Nunatak. The parent material is magmatized amphibolic gneiss and schist. The mean annual temperature is -14.2 °C, mean annual precipitation is 59.6 mm, mostly as snow (Abakumov 2011). Average wind velocity is estimated as 8.4 m s^{-1} and with maxima of 37 m s^{-1}. Thus as seen from the climatic parameters, the soils at the Leningradskaya Station and Lindsey Island are developed under more severe conditions than the soils of King George Island. Photos of selected soils are shown in Figure 2.

The soil samples were taken into special containers (volume about 200 cm^3). Samples were stored in a freezer to halt transformation processes. Before analysis, the samples were stored at 0 °C in the laboratory.

Chemical characterization of Antarctic soils

The following characteristics were determined in the soil fine earth and organic materials grounded to the fine earth size (<2 mm):
(i) Carbon and Nitrogen content by C-H-N analyzer LECO CHN-628. Data are related to dry mass (dried at 105 °C). Subsequently, the C/N ratio was calculated.
(ii) C_{HA}/C_{FA} ratios on the basis of separate determination of C_{HA} and C_{FA} in extracts from soil materials (Ponomareva & Plotnikova 1980). According to this procedure, soil fine earth after decalcification by 0.1 M HCl solution and further purification by water was processed by 0.1 M NaOH solution. The extract was separated into humic and fulvic acids fractions by the adding a 0.5 M solution of sulfuric acid. Further, the carbon content in the extract of HAs and FAs was determined by chromic acid oxidation (Walkey & Blake 1934).

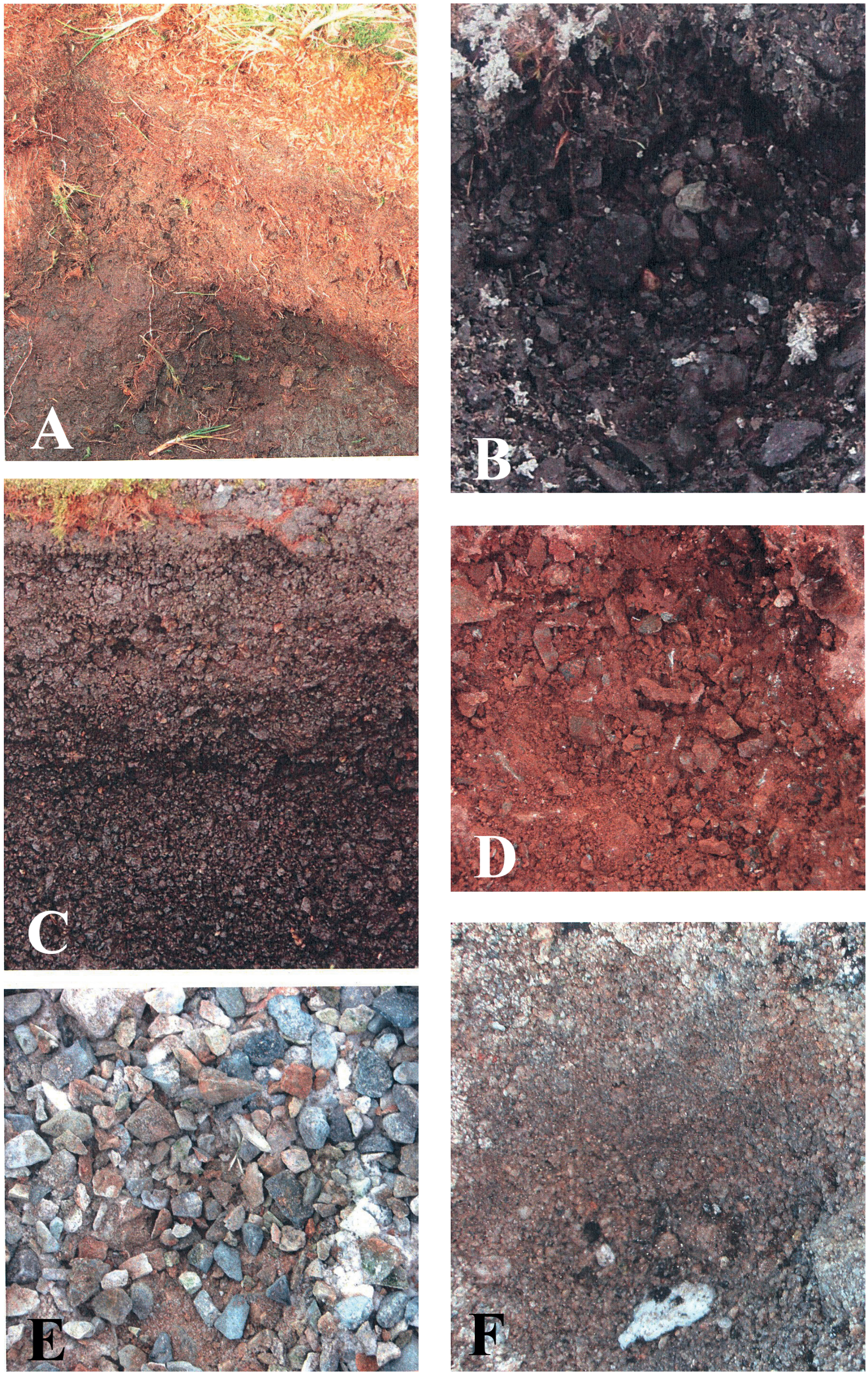

Fig. 2: Soils of selected plots at King George Island (A-D), Lindsey Island (E) and Leningradskiy Nunatak (F). **A** = Profile of about 20 cm depth under vascular plant community (see I-1 – I-5 of Tab. 1 & 2). **B** = Profile of about 20 cm depth under lichen (see II-1 – II-3 of Tab. 1 & 2). **C** = Profile of about 20 cm depth under mosses (see III-1 – III-3 of Tab. 1 & 2). **D** = surface of soil (20 x 20 cm) covered by guano (see IV-1 of Tab. 1 & 2). **E** = surface of soil (20 × 20 cm) at Lindsey Islands (see V of Tab. 1 & 2). **F** = Profile of about 10 cm Leningradskiy Nunatak (see VI of Tab. 1 & 2).

Abb. 2: Abb. 2: Böden der ausgewählten Parzellen auf King George Island (A–D), auf Lindsey Island (E) und auf dem Leningradskiy Nunatak (F). **A** = Bodenprofil unter einer Gefäßpflanzengemeinschaft (Details vgl. Proben I-1 – I-5 in Tab. 1 & 2). **B** = Bodenprofil unter Flechten (Details vgl. Proben II-1 – II-3 in Tab. 1 & 2). **C** = Bodenprofil unter Moosen (Details vgl. Proben III-1 – III-3 in Tab. 1 & 2). **D** = Oberfläche eines Bodens, der mit Guano bedeckt ist (Details vgl. IV-1 in Tabellen 1 & 2). **E** = Oberfläche eines Bodens (20 × 20 cm) auf Lindsey Island. **F** = 10 cm Bodenprofil auf dem Leningradskiy Nunatak.

Humic acids isolation

Humic substances were extracted from each soil sample (5 g) with 0.1 M NaOH solution (soil/solution ratio 1:10) under N_2. After 24 h of shaking, the alkaline supernatant was separated from the soil by centrifugation at 45,000 g for 30 min and acidified to pH 1 with 6 M HCl solution to induce the precipitation of HAs. The supernatant with FAs was then separated from the HAs precipitate by centrifugation at 10,000 *g* for 30 min. The HAs precipitates were demineralized by shaking with a 20 ml of 0.1 M HCl/0.3 M HF solution. The HAs suspension was then dialyzed in distilled water for seven days using 10-12 kDa cellulose dialysis tubing (Sigma-Aldrich) and lyophilized.

Electrophoresis technique

Humic acids, isolated from several Antarctic soils, were characterized by polyacrylamide gel electrophoresis (PAGE) according to Trubetskoj et al. (1997). The apparatus was a vertical electrophoresis device (LKB 2001 Vertical Electrophoresis, Sweden) with gel slab (20 x 20 cm). Acrylamide 9.7 % and bisacrylamide 0.3 % were dissolved in 89 mM Tris-borate buffer with 1 mM EDTA and 7 M urea, pH 8.3. For polymerization, 0.014 mL N,N,N',N'-tetramethylethylendiamine and 0.4 mL of 10 % ammonium persulfate were added to 40 mL of acrylamide/bisacrylamide solution. The electrode buffer had a concentration of 89 mM Tris-borate with 1 mM EDTA, pH 8.3. The sample buffer (0.1 ml) contained 89 mM Tris-borate, 7 M urea, 1 % SDS and 1 mM EDTA, pH 8.3. Electrophoresis was carried out at room temperature for 1 h at a constant current of 25 mA. Immediately after electrophoresis, a polyacrylamide gel slab (PAG) with fractionated HAs was moved to the UV/White light (WL)L-mixed light transilluminator (Vilber Lourmat, Marne-la-Valee, France) in a dark room and photographed under white light (using two tubes T-8.L, 8W) and under UV light (using six tubes T-8.M, 8W, emitting at 312 nm), located under the PAG. After inspection under white and UV light, the PAG with fractionated HAs was stained for 2 h with a solution containing 0.025 % protein-specific dye Coomassie Brilliant Blue R-250, 15 % acetic acid, 15 % ethanol, and 1 % $CuSO_4$. Further, the PAG was washed to a transparent background with a solution containing 10 % acetic acid and 10 % ethanol for 24 h and photographed as well.

UV-Visible absorption

Absorption spectra were recorded on a Cary 3 (Varian) spectrophotometer from 210 to 700 nm in a 1 cm quartz cuvette. The spectra of HAs isolated from different horizons of several Antarctic soils were measured at a HA concentration of 0.1 mg mL^{-1} in a 0.1 M NaOH solution. The E4/E6 ratio (ratio of absorbances at 465 and 665 nm) and specific absorptions at 280 nm and 465 nm at a concentration 0.1 mg mL^{-1} were calculated.

RESULTS AND DISCUSSION

Soil morphology and general properties

Soils of King George Island consist of three main types of horizons: organic, organo-mineral and mineral. Organic soil parts are differentiated in two or three horizons, containing organic remnants with different degrees of transformation / humification. The upper, almost undecomposed horizon of litter is the Oi horizon, consisting of organic matter with only initial morphological features of decomposition. The next lower horizon showing very initial humification is the fermentation layer Oe. The organic sub-horizon with evident darkening of the organic remnant is the Oh horizon. The thickness of these horizons is different in the soils investigated; an Oh layer is common in soils under graminoid plants and mosses. Organo-mineral horizons develop under organic ones and are classified as grey-humus A horizons; they are characteristic for all soils sampled on King George Island. Soils under vegetation were classified as Lithosols by the "Classification and Diagnostics of Soils of Russia" (Shishov 2004) and as Folic (raw humus) Leptosols according to the World Reference Base (WRB) for Soil Resources (Decker et al. 1998). Soils under guano in both studied plots can be identified as Ornithosols (Simas et al. 2007), whereas there is a possibility of calling the soil with well mineralized and transformed guano as post-ornithic soil (Abakumov 2012). These terms (Ornithosol, post-ornithic soils) are not derived from the WRB, as this system has only the qualifier "Ornithic" to indicate a layer 15 cm or more thick with ornithogenic material. Meanwhile, these terms became more frequently used in studies of polar soils and sediments of oceanic islands (Ivanov & Avessalomova 2012, Abakumov 2014). The soil from the Leningradskaya Station should be classified as Leptosol derived from the massive dense bedrock. In this publication, we use the Ornithic classifier only with the aim of specifying the effect of guano on soil formation, but not as a soil taxonomic characteristic.

The basic data on soils (Tab. 1) show that all the samples were acid with the exception of soils under guano and the Leptosol of the Leningradskaya barren. The soils contained a low portion of clay fraction in the fine earth and a high portion of the coarse fraction. No morphological features of podzolisation or cambic process were revealed in our study, whereas this was observed in soils of the Admiralty Bay, Arctowsky Polish station (Bölter et al. 1997).

The studied organic soil profiles were differentiated by their sequence of horizons (Tab. 1). In all of the studied Sub-Antarctic soils, the first horizon of the organic profile was an Oi horizon with initial decomposition and no or slightly transformed organic matter. The main difference between the soils was in the thickness of the Oi horizon, which was highest in case of vascular plant vegetation, lower under lichen vegetation and minimal under moss vegetation. Next after the Oi horizon is the fermentation horizon Oe with partially decomposed organic matter or (and) the humified horizon Oh, which is darker than the two previous ones. The Oh horizon also exists in soils covered by mosses, while it is absent in soils under lichens. It was shown previously (Vlasov et al. 2005, Parnikoza et al. 2011) that transformation of organic matter has being occurred in soils of Sub-Antarctic herb and lichen tundra and results in differentiation of two or three organic sub-horizons with different levels of humification and transformation. The most developed system of organic horizons was typical for soils formed under vegetation dominated by the vascular plant *Deschampsia antarctica*. The reason for this is the relatively high amount of lignin-derived compounds as

Type of soils (WRB/Russian system) source of organic material	Index of sample	Soil horizon index thickness (cm)	Colour	Clay content g kg^{-1} of fine earth	pH
Lithosol / Lithozem under the vascular plant *Deschampsia antarctica,* King George Island	I-1 I-2 I-3 I-4 I-5	Oi (0-6) Oe (7-9) Oh (9-12) A (12-15) AC (15-20)	2.5 YR 10YR 5/2 10YR 5/2 5YR 5/1 5Y 5/1	nd nd nd 80 83	5.3 5.3 5.2 5.2 5.0
Lithosol / Lithozem under the lichen *Stereocaulon glabrum,* King George Island	II-1 II-2 II-3	Oi (0-4) Oe (4-7) A (7-12)	7,5 YR 5/2 5 Y 5/1 5 Y 7/1	nd nd 78	5.7 5.4 5.1
Leptosol Lithozem under the mosses *Sanionia uncinata, Polytricastrum alpimun,* King George Island	III-1 III-2 III-3	Oi (0-3) Oh (3-5) A (5-10)	7 YR 6/4 10YR 6/1 10 YR 7/1	nd nd 81	5.4 5.3 4.9
Ornitosol/ Organogennaya pochva under well decomposed guano, King-George Island	IV-1 IV-2	OC (0-5) C (5-15)	10 YR 4/1 10 YR 5/1	nd 120	6.8 7.1
Orhnitosol/ Organogennaya pochva under slightly decomposed guano, Lindsey Island	V	O (0-10)	7.5YR 4/4	nd	7.0
Leptosol / Petrozem, Leningradskaya Station	VI	O (0-2)	Nd	nd	7.5

Tab. 1: Morphological features and chemical characteristic of Antarctic soils (colour was determined by Munsell Colour Chart, clay content by sedimentation method, pH in water suspension).

Tab. 1: Morphologische Merkmale und chemische Eigenschaften antarktischer Böden.

humus precursors that gives the possibility for humification by polycondensation and polymerization of lignin-derived compounds (ORLOV 1990).

Humus horizons, designated as A, were grey or greyish in colour; they were not deep and were the result of humic substances stabilization and association with the mineral soil matrix. In all soils, the investigated organic horizons were thicker than the organo-mineral ones, which is in good correspondence with previous data (VLASOV et al. 2005, ABAKUMOV et al. 2010). Only soils under graminoid plants were characterised by a transitional AC horizon, while this was absent in the two other soils, which is in good relation with the presence of an Oh (humification) horizon in the soil under *Deschampsia antarctica*. The studied soils under plants were acid, sand-textured and formed on coarse sand-textured debris of massive materials. Ornithosols on guano were completely different to each other. The soil from King George Island was dark grey with well-decomposed guano and shows no evidence of fresh fecal material, whereas the soil from Lindsey Island was brownish, where stones and sands were mixed with sticky guano. Ornithosols were characterised only by an organic horizon O with no differentiation in sub-horizons. This material was at the same time the source of humus precursors enabling in-situ humification.

Only one horizon with organic material was collected from the Leptosol of Leningradskiy Nunatak. This is an example of soil formation and humification in extremely severe conditions of the Antarctic polar desert.

Carbon and nitrogen contents and the CHA/C FA ratios

The three sub-Antarctic soils under different plant communities were clearly differentiated by carbon content in two groups of horizons (Tab. 2): an organic horizon which consists of almost 50 % C of total mass (more than 400 g kg^{-1}; O horizons) and organo-mineral horizons (A horizons) containing 64-72 g kg^{-1} of C. There was evidence of decreasing C/N ratios with soil depth, which was in good correspondence with the degree of soil organic matter transformation. Organic matter of the A horizons was more enriched in nitrogen in comparison with organic ones. Ornithosols contain quite varying C portions, which is in correspondence with the degree of guano transformation caused by climatic conditions and biological activity, as was shown previously (ABAKUMOV 2010, 2012). Carbon contents were 8.72 and 89.22 g kg^{-1} for soil with well-transformed guano and material of the penguin rookery, respectively. This is also the reason for differing C/N ratios, namely about 7:1 in King George Island soil and 2:1 in material from Lindsey Island. We suppose that there were inorganic forms of N in both types of Ornithic soils (SIMAS et al. 2008), and that low C/N ratios were not results of soil nitrogen transformation processes. The Ornithosol from King George Island appears closer to post-ornithic soils, while the Lindsey Island one is an "active" Ornithosol, intensively affected by on-going penguin activity.

The C/N ratio in the Leptosols of the Leningradskiy Nunatak is comparable to those in soils under vegetation on King George Island, while the C content in the O horizon is considerably lower due to lower biological productivity and the more severe climate. We have identified this horizon as O, though not in good correspondence with the WRB classification which requires 20 % of carbon in the fine earth. In soils of the Antarctic, SOM is not really associated with the fine earth; therefore, we have determined it in a bulk sample to provide more precise chemical analyses.

The C_{HA}/C_{FA} ratios and HS contents do not show a distinct pattern of change with profile depth in the studied soils, while the FA fraction shows a decreasing trend with depth. Thus, the

Index of sample	Soil horizon	C g kg^{-1} of fine earth	N g kg^{-1} of fine earth	C/N	C_{HA} g kg^{-1} of fine earth	C_{FA} g kg^{-1} of fine earth	C_{HA}/C_{FA}	A280 C = 0.1mg/ml	A465 C = 0.1mg/ml	E4/E6
I-1	Oi	440.00	20.99	20.96	11.00	33.00	0.33	0.79	0.05	8.4
I-2	Oe	449.00	29.19	15.38	15.10	51.90	0.30	nd	nd	nd
I-3	Oh	445.78	33.98	13.11	24.61	51.40	0.48	1.32	0.13	7.1
I-4	A	64.92	8.51	7.61	8.61	11.40	0.76	1.21	0.14	7.2
I-5	AC	42.70	3.75	11.39	4.41	12.30	0.36	1.12	0.12	7.0
II-1	Oi	440.50	9.70	45.41	11.20	42.80	0.26	0.87	0.07	5.4
II-2	Oe	434.20	15.58	27.87	5.10	39.96	0.13	0.95	0.06	6.0
II-3	A	72.59	6.94	10.45	8.8	10.23	0.86	1.35	0.19	6.1
III-1	Oi	308.62	16.76	25.50	9.85	39.25	0.26	0.70	0.05	10.2
III-2	Oh	421.36	16.99	28.40	11.28	25.31	0.44	0.81	0.07	6.6
III-3	A	72.04	7.16	10.60	4.50	6.45	0.69	0.77	0.08	7.7
IV-1	OC	8.72	1.27	6.86	1.5	2.40	0.63	0.60	0.05	5.5
IV-2	C	0.92	0.14	6.57	0.3	1.20	0.25	1.07	0.09	7.1
V	O	89.22	46.95	1.90	5.00	8.20	0.61	0.71	0.10	7.4
VI	O	102.00	6.00	17.00	7.03	21.01	0.33	0.81	0.17	3.2

Tab. 2: Carbon (C) and nitrogen (N), humic (C_{HA}) and fulvic (C_{FA}) acids content, C/N and C_{HA}/C_{FA} ratios, specific absorption coefficients A280 and A465, E4/E6 ratios of HAs, isolated from Antarctic soils.

Tab. 2: Kohlenstoff- (C) und Stickstoffgehalte (N), Gehalte an Humin- (C_{HA}) und Fulvosäuren (C_{FA}), C/N- und C_{HA}/C_{FA}-Verhältnisse, spezifische Absorptionskoeffizienten A280 und A465, E4/E6-Verhältnisse von Huminsäuren, extrahiert aus antarktischen Böden.

variability of the humic to fulvic acids ratio values are caused by the high variability of the HAs portions between the soil horizons. HAs accumulation was revealed for the Oh horizons; this supports the morphological diagnostics of the soils and suggests that humification is a current process in the Oh layer. The C_{HA}/C_{FA} ratios of soils under guano were not different from the other studied soils. This may be caused by the high water content and greater amount of acid-extractable organic acids which is usually determined as a part of the fulvic acids fraction (ORLOV 1990, ABAKUMOV 2010). The Leptosol from the Leningradskaya plot is characterized by the lowest C_{HA}/C_{FA} ratio among the organo-mineral horizons. We suppose that this is caused by the severe climatic conditions and shorter temperature period above 0 °C than in case of the maritime landscapes of King George Island.

The obtained data show that there is on-going humification and changes of C/N ratios due to organic matter transformation in all organic horizons of the investigated soils. This affirms that soil organic matter transformation and humification is the main and active process in soils of Antarctica. SOM transformation in Ornithic soils occurs in conditions of high levels of inorganic N forms, which provide extremely low C/N ratio values. There is also vertical migration and accumulation of the FAs within the soil profiles.

Optical characteristics of humic acids isolated from Antarctic soils

The coefficients of optical absorbance are indexes, which evaluate the degree of humification in soils (PONOMAREVA & PLOTNIKOVA 1980). The obtained data (Tab. 2) indicate the trend of increasing specific absorption coefficients A280 and A465 with soil profile depth. From fresh organic matter to a more decomposed one, the HAs became more humified. This trend has been observed in all investigated soils. A similar index for the optical characteristics is the E4/E6 ratio. In general, these ratios should be higher with lower degrees of humification and, conversely, lower with a higher degree of humification. Thus, the trend of decreasing E4/E6 ratios from upper to lower soil horizons confirms the data of specific absorption coefficients obtained for the soils investigated.

As result we can conclude that humification increases with the depth of soil horizons and the degree of transformation of the organic remnants. This confirms the existence of active humification as a current process in Antarctic soils.

Electrophoresis of humic acids, isolated from Antarctic soils

For confirmation of the current humification process in Antarctic soils, HAs isolated from different organic and organo-mineral horizons of three soils under different plant communities (soil samples I-III) and one soil under a well-mineralized guano (soil sample IV, Tabs. 1 & 2) were fractionated using polyacrylamide gel electrophoresis (PAGE) in the presence of denaturating agents (Figs. 3 & 4). The inspection of the electrophoresis results in white light demonstrated that all HAs samples were separated into three discrete naturally coloured pale brown fractions: H-MS (starting fraction), M-MS (middle fraction), L-MS (bottom gel fraction). The electrophoretic mobilities (EM) of identically marked fractions were similar in all soil HAs investigated. It has been previously shown (TRUBETSKOJ et al. 1999) that the molecular size (MS) of electrophoretic fractions of different soil HAs decreased with increasing EM. We could assume that in Antarctic soils the fraction H-MS has the highest MS, fraction L-MS has the lowest, and fraction M-MS has medium MS.

Electropherograms inspected under white light (Figs. 3a & 4a) showed the essential changes in colour intensity of naturally coloured pale brown electrophoretic fractions of HAs from organic to organo-mineral horizons. There is a trend of increasing the brown colour intensity of all three fractions in HAs samples of organo-mineral horizons isolated from soils under vascular plants and lichens. The HAs of organo-mineral horizons isolated from soils under mosses and well mineralized guano demonstrated increasing brown colour of fractions M-MS and L-MS. In other words, there is a redistribution of the electrophoretic fractions due to the organic matter transformation and humification. A similar picture has been previously shown for young soils of a podzol soil chronosequence, where more transformed layers of forest floors showed greater percentages of the L-MS fraction (Abakumov et al. 2010). Summarizing, the colour intensity was higher in the organo-mineral layers than in corresponding organic layers. This is in a good correspondence with the increment of the specific absorption coefficients A465 and the general trend of decreasing of E4/E6 ratios with soil profile depth.

The electropherograms were then inspected under UV light (Fig. 3b & 4b). In HAs isolated from organo-mineral horizons of soils under vascular plant and lichen, the fluorescence intensity of L-MS fraction was higher than in organic ones; simultaneously, fluorescence intensity of H-MS fraction was practically absent. In the HAs of organo-mineral horizons isolated from soils under the mosses and well-mineralized guano, an only weak decrease of fluorescence intensity of H-MS and some increase of fluorescence intensity of L-MS was detected.

It is well known that humification involves biotic and abiotic transformations of biological materials into mature HS. Hence, the portions of non-transformed biological material in the initial humus precursors must exceed the ones in the more mature soil HSs. To find experimental evidence immediately after inspection under white and UV lights, electropherograms were stained with the protein-specific dye Coomassie Brilliant Blue (Fig. 3c & 4c). The results showed that protein-like materials concentrated mostly in H-MS and M-MS fractions, whereas L-MS fractions of all HAs investigated were not coloured by the protein-specific dye. The organo-mineral layers of all soils have shown the almost complete absence of the Coomassie Brilliant Blue colour, which might be an additional indicator of current humification process. This becomes clearer after comparison of Coomassie Brilliant Blue colour of HAs isolated from organo-mineral horizons of the studied Antarctic soils with well-humified chernozemas as a reference. Thus, we can suggest that the humification leads to the decrease of protein-like components in HAs composition, i.e. they are only stable in the H-MS fractions of HAs.

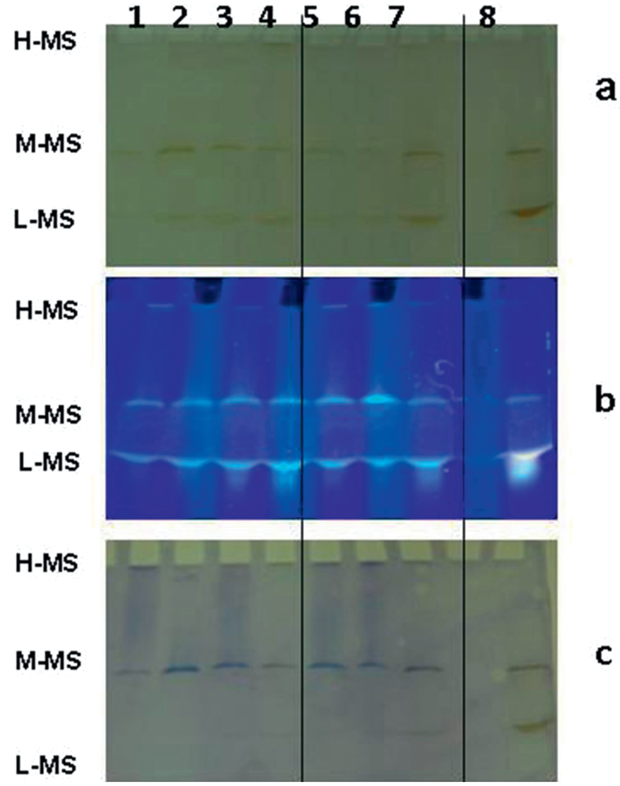

Fig. 3: Electrophoresis in 10 % PAG of 0.1 mg HAs, isolated from horizons Oi (1), Oh (2), A (3), AC (4) of soil under the vascular plant *Deschampsia antarctica* and horizons Oi (5), Oe (3), A (3) of soil under the lichen *Stereocaulon glabrum* (both soils from King George Island). As a control 0.025 mg chernozem HAs (8) was used.
a = PAG with naturally coloured HAs under white light; b = PAG inspected under UV light (312 nm) with detection of fluorescence distribution between fractions; c = PAG inspected after staining with protein-specific dye Coomassie Brilliant Blue R-250 for detection of protein-like matter distribution between fractions.

Abb. 3: Elektrophorese in Polyacrylamid-Gel (10 %) von 0,1 mg Huminsäure, extrahiert aus den Horizonten Oi (1), Oh (1), A (3), AC (4) der Böden unter der Gefäßpflanze *Deschampsia antarctica* und der Horizonte Oi (5), Oe (3), A (3) der Böden unter den Flechten *Stereoaculon glabrum* (beides Böden auf King George Island). Als Kontrolle wurden 0,025 mg Schwarzerde (8) verwendet.
a = Polyacrylamid-Gel mit Huminsäuren natürlicher Färbung unter weißem Licht; b = Polyacrylamid-Gel inspiziert unter UV-Licht (312 nm) mit Detektion der Fluoreszenzverteilung zwischen den Fraktionen; c = Polyacrylamid-Gel geprüft nach der Färbung mit proteinspezifischem Farbstoff Coomassie Brilliant Blue R-250 für den Nachweis der proteinähnlichen Massenverteilung zwischen den Fraktionen.

CONCLUSIONS

Horizons of organic matter ininvestigated Antarctic soils are characterized by different origins. These are organic materials derived from graminoid plants, lichens and mosses. An additional important source of organic matter is guano of penguins. The complex investigation of morphological organization of soil profiles, C and N contents and ratios, HAs and FAs portions with the relation to optical indexes and electrophoretic properties allow us to conclude that there is current transformation of SOM in studied soils with increasing humification of SOM with soil depth. Soils of the Sub-Antarctic islands show humus organo-mineral horizons of varying development stages, with the highest intensity of nitrogen enrichment between the organic and organo-mineral soil materials investigated.

There are several common characteristics of the investigated soil profiles: decreasing of C/N ratios with soil depth, accumulation of FAs versus accumulation of HAs, decline of E465/E656 ratios in correspondence with growth of the A465 absorbance coefficient and strong differentiation of electrophoretic properties of HAs. The most humified organic matter is found in organo-mineral horizons (with lower portion of peptides, and highest portion of L-MS fraction). The above-mentioned

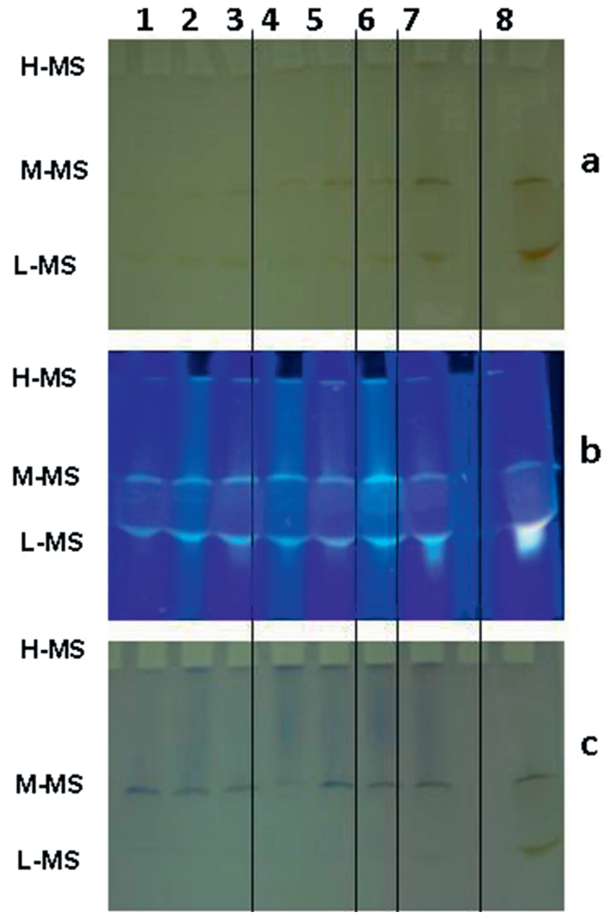

Fig. 4: Electrophoresis in 10 % PAG of 0.1 mg HAs, isolated from horizons Oi (**1**), Oh (**2**), A (**3**) of soil under the mosses *Sanionia uncinata* and *Polytricastrum alpinum*; horizons OC (**4**), C (**5**) of soil under the well mineralized guano (both soils from King George Island); horizon O (**6**) of soil under the weak mineralized guano (Lindsey Island) and horizon O (**7**) Leptosol (Leningradskiy Nunatak). As a control 0.025 mg Chernozem HAs (**8**) was used.
a = PAG with naturally coloured HAs under white light; b = PAG inspected under UV light (312 nm) with detection of fluorescence distribution between fractions; c = PAG inspected after staining with protein-specific dye Coomassie Brilliant Blue R-250 for detection of protein-like matter distribution between fractions.

Abb. 4: Elektrophorese in Polyacrylamid-Gel (10 %) von 0,1 mg Huminsäure, extrahiert aus den Horizonten Oi (**1**), Oh (**2**), A (**3**) der Böden unter den Moosen *Sanionia uncinata* und *Polytricastrum alpimun*; Horizonte OC (**4**), C (**5**) des Bodens unter hochmineralisiertem Guano (beides Böden auf King George Island); Horizont O (**6**) des Bodens unter dem schwach mineralisierten Guano (Lindsey Insel) und Horizont O (**7**), Leptosol (Leningradskiy Nunatak). Als Kontrolle wurde 0,025 mg Schwarzerde HAs (**8**) verwendet.
a = Polyacrylamid-Gel mit Huminsäuren natürlicher Färbung unter weißem Licht; b = Polyacrylamid-Gel inspiziert unter UV-Licht (312 nm) mit Detektion der Fluoreszenzverteilung zwischen den Fraktionen; c = Polyacrylamid-Gel geprüft nach der Färbung mit proteinspezifischem Farbstoff Coomassie Brilliant Blue R-250 für den Nachweis der proteinähnlichen Massenverteilung zwischen den Fraktionen.

indexes confirm the humification phenomena in Antarctic soils. Protein compounds decrease with the humification rate increasing, and a decreased portion of these were revealed for humus in the A horizons of the soils investigated.

Humification appears also in Ornithosols as well as in a Leptosol under very severe climatic conditions, which is confirmed by presence of all electrophoretic fractions in HAs. FAs prevails in the humus composition of the soils investigated.

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