

Research Article

Characterization of Humic Acids Isolated from Selected Soils of Livingston Island by CP/MAS ^{13}C NMR and ESR Spectroscopy

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Received 9 March 2022; Revised 6 July 2022; Accepted 16 July 2022; Published 5 August 2022

Academic Editor: Vasanthavigar M

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The tundra and tundra barrens of the maritime Antarctica represent a unique type of terrestrial ecosystem, geographically confined to the region of the Antarctic Peninsula and a number of surrounding archipelagos. Antarctic soils are underestimated in the quantity of organic matter (OM) pools, organic remnant humification/mineralization rates, and biogenic-abiogenic interactions. The structure of reserves for humic substances within the permafrost zone, as well as the role of the molecular composition of organic substances, are still poorly understood. In this study, we investigate humic acids of selected sub-Antarctic soils in terms of elemental and structural composition to evaluate OM stabilization degree and to assess carbon distributions in the molecules by solid-state CP/MAS ^{13}C NMR and ESR spectroscopy. The results obtained show that the studied humic acids consist mainly of aliphatic structural fragments. According to ESR spectroscopy, it was noted that the most stable molecules by the data of ESR spectroscopy are formed in postornithogenic soils. In contrast, the average portion of the aromatic compounds is about 30% in humic acids, extracted from soils with evident ornithogenic effect.

1. Introduction

Polar soils play a key role in global carbon circulation and stabilization, as they contain the maximum reserves of soil organic matter (SOM) within the pedosphere [1]. The low microbiological activities and degree of transformation of SOM in cold climates lead to the accumulation of a significant amount of SOM in the soils and the biological sediments [2].

Global climate change and permafrost degradation result in huge pools of SOM undergoing microbial degradation [1] and leading to the mineralization of OM [3]. Polar SOM is a vulnerable carbon source prone to remobilization with increasing temperature [1]. A better understanding of the permafrost SOM effects on greenhouse gas emissions requires accurate qualitative and quantitative knowledge of its spatial distribution (e.g., biodegradability, chemical composition, and humification rate) [4–6]. The polar tundra ecosystems are a unique type of terrestrial ecosystem [7–9].

Antarctica is conventionally divided into two parts—eastern and western. The western part of Antarctica is subject to climate change to a greater extent than the eastern one. This is due to the active accumulation of OM in the soils of maritime Antarctica; with climate change, this OM can be mineralized relatively quickly and make an additional contribution to climate change. The main sources of organic carbon in the soils of this region are plant residues, as well as the waste products of birds [10]. Humic acids (HAs) have a significant conservation role, imparting stable features to soils, thus participating in the deposition of SOM. In terms of ecosystem services, humic substances in soils participate in the regulation of the atmosphere. This is associated with the formation of stable compounds to biotic and abiotic influences in the composition of HAs. Therefore, their study is a priority in the changing climate of the planet. The climatic and ecological conditions are different from the continental regions of West Antarctica. At the station “Juan Carlos I” (SAS), it was noted that the permafrost table is

probably located at a depth of 2–3 m, and soil freezing occurs mainly in those areas that are covered by snow [11, 12]. It is expected that the role of Antarctica in a global carbon cycle will be significant since the content and reserves of biogenic carbon compounds are essential to the development of microorganisms and vegetation in this region [13].

Numerous studies of soil organic carbon quality and content in polar soils have been carried out recently [14–16]. The structure and molecular composition of Antarctic SOM and HAs have been investigated by applying CP/MAS ^{13}C NMR methods [17, 18]. HAs are heterogeneous systems of high and low molecular weight compounds formed from the transformation of plant and animal residues in terrestrial and aquatic ecosystems [19, 20]. The stabilization process of OM is the transformation of OM into a state inaccessible to soil microorganisms, thus the resistance of OM to biodegradation [21]. Previous studies report that, in organometal soils of East Antarctica, the aliphatic carbon prevails over aromatic compounds in permafrost-affected soils [22]. It was shown that this feature is typical for soils from different Antarctic regions, including soils formed on penguin rookeries [10]. Nowadays, there are currently no data on the qualitative composition of SOM from Livingston Island. For comparison, the same model in the organization of organic molecules is observed in the Arctic polar environment. Selective stabilization of alkyl compounds in deeper soil layers has been proposed as a reason why aliphatic compounds are more abundant than aromatic compounds. Minor SOM transformation processes can result from low soil temperatures inhibiting microbial degradation [18]. Birds play an important role in the redistribution of plant debris in Antarctica [23, 24], as they transport significant amounts of OM [25]. The influence of birds on the ecosystem of west Antarctica is quite wide: the redistribution of OM and the spread of plants to remote areas. As a result, birds can affect the morphological, chemical, and molecular composition as well as microbiome features [23, 24, 26]. However, published data on the composition of SOM for Antarctica are rare, and further studies are needed to detail its structural compounds and their distribution. Recently, CP/MAS ^{13}C NMR are successfully used to detail soils found in the polar region and show that OM is characterized by a low abundance of aromatic compounds [19, 27]. Another effective way to analyze the structure of HAs is electron spin resonance (ESR) spectroscopy. According to this method, based on the content of free radicals in the HAs molecules, we can claim the degree of aromaticity of HAs. The concentration of free radicals obtained during ESR spectroscopy positively correlates with the degree of humification of SOM. According to Kononova [28], the content of free radicals is determined by the degree of condensation of polyphenols. Thus, according to this method, we can determine the degree of resistance of HAs molecules to biodegradation [29].

This work expands on a previous study about the molecular composition of Antarctic soils [19, 23, 24, 27]. This paper presents for the first time information on the quality of SOM according to CP/MAS ^{13}C NMR and ESR spectroscopy. To achieve the goal of the present investigation, we define the following set of objectives: (1) to describe the

molecular composition of HAs from the soils studied; (2) to investigate the elemental composition of HAs; (3) to investigate ESR spectra of HAs.

2. Materials and Methods

2.1. Study Sites. Sampling sites are located in the vicinity of the Bulgarian Antarctic station (BAS) “St. Kliment Ohridski” (S 62°38′25.7″, W 060°21′54.9″) (Figure 1). The parent rock of the Hurd peninsula is presented by sandstone, Mayers Bluff formation (Late Mesozoic-Cenozoic magmatic arc) [30]. Soil formation is spatially concentrated in the coastal parts on rocks and sea terraces [31–33]. The soil cover represents disjunctive soil areas. Soils are formed in areas with bird nests, as well as in areas near water bodies. The distinctive feature of this island is the wide distribution of two species of vascular plants—*Deschampsia antarctica* and *Colobanthus quitensis*, associated with the ornithogenic factor. In the nesting areas of birds, the chemical parameters of the soil and the composition of microbial communities change significantly [34]. The bird’s waste is the main source of organic carbon in the island and coastal parts of Antarctica [7, 35]. In ornithogenic soils, the content of OM is significantly increased in comparison with native soils, during the mineralization of organic residues in the soil; readily, hydrolyzable compounds are formed, which are rapidly incorporated in the geochemical cycle and are used by plants [10]. The average annual air temperature is -5°C , the average January temperature is 0°C , and the average July temperature is -7°C . Annual precipitation is 140 mm. Most of the land is characterized by the presence of permafrost at a depth of about 2–3 meters.

Moraine ridges and other signs of glacial features of the relief begin at 700 m from the station along the coast. Eluvia and talus rocks occupy the highest areas. Volcanic tephra also accumulates in the island’s landscapes. The long-term presence of colonies of penguins and migratory birds had a significant effect on the biogeochemical transformation of the landscape and the formation of ornithogenic tundra dominated by *D. antarctica*. The decisive factor for the postornithogenic succession and the development of a pronounced soil-vegetation cover is the enrichment of terrestrial ecosystems with OM of marine origin and ornithochoria—the transfer of seeds and vegetative parts of plants, including for the construction of nests. Thus, for several islands in the maritime Antarctica, one can single out an ornithogenic factor of ecogenesis and soil formation [7, 25, 34, 35]. It is considered that postornithogenic soils forms mature OM [10, 19, 23, 24].

2.2. Soil Sampling. Samples were taken and described by the international soil sampling methodology [36] during the 2018/2019 field season. The samples collected are representative of the soil micromorphology of the terrestrial ecosystems at Livingston Island. The sampling was performed by considering the snow-free areas along the coastline of the island in terms of the landscape features and accessibility to the sites. The soils have been selected from

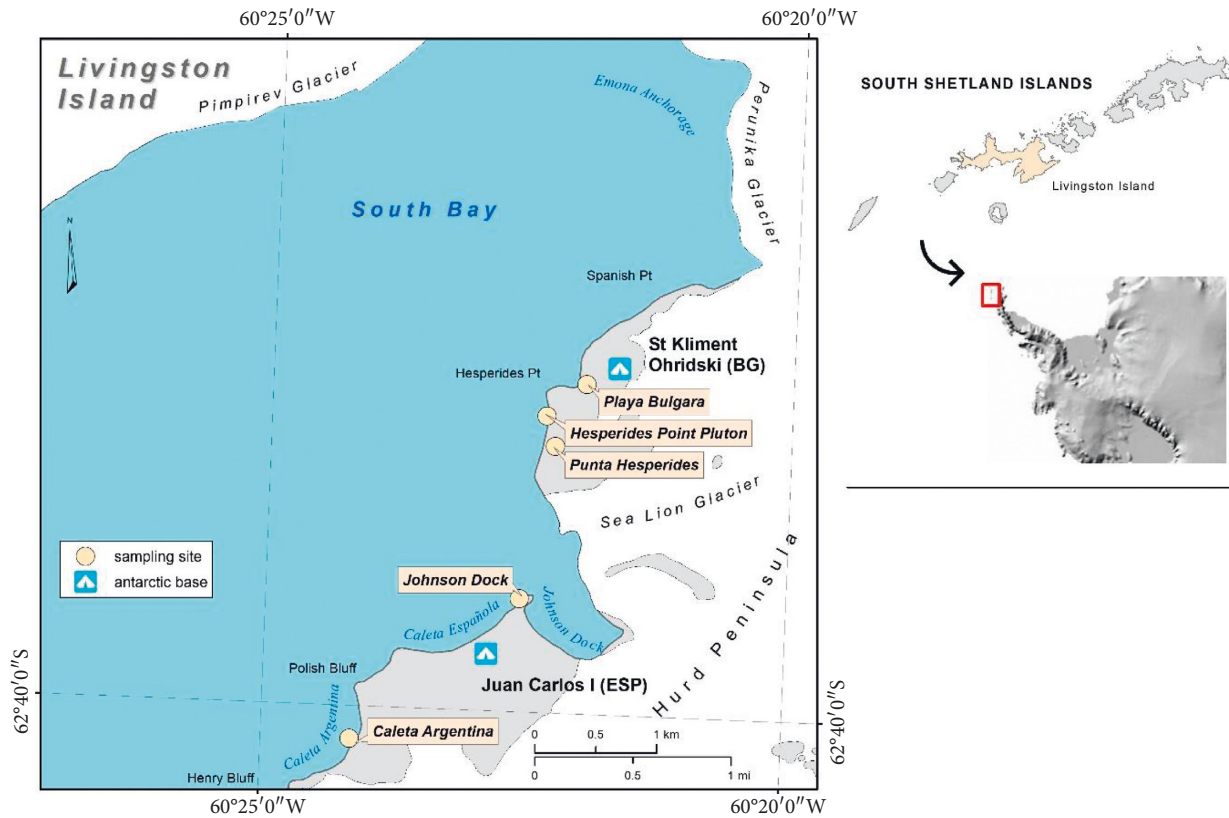


FIGURE 1: Study sites on Livingston Island, Antarctica.

five locations on the island for humic substance isolation. All soils have top humus layers with high carbon content and distinguishable layers of suprapermfrost accumulation of OM. The samples were transported from Antarctica on a research vessel and analyzed in the Department of Applied Ecology at Saint-Petersburg State University. Soil samples were transported by the ship of the Russian Antarctic Expedition "Academic Fedorov" at a temperature of +4°C.

2.3. Sampling Locations. The first point of the study was Hesperides Point Pluton (S 62°38'41.3", W 060°22'22.1") with elevation 7m above sea level (asl) (Figure 1). The sampling location is situated on a small bay in the foothill of Hesperides hill with pebble stones covering the shore. At about 20 m inland, a thick moss layer is formed between rock sediments. The soil underneath is mixed with many remnants of mollusks (*Patella*) and reaches 2-3 cm depth. The next point was Playa Bulgaria (S 62°38'32.5", W 060°21'59.9") with elevation 71 m asl. The site is located in the southern part of the Bulgarian beech on a hill formed by gabbro and diorites of the main boundary fault (MBF). Mosses, lichens, and *D. antarctica* are distributed between rock sediments. Soil has a high content of angular stones, and two layers with varying depths can be distinguished. The next point was Punta Hesperides (S 62°38'49.3", W 060°22'16.7") with elevation 50 m asl. The sampling location is situated on the snow-free top of a plateau like a hill in the west of Sea Lion Lake. A layer of lichen and mosses creates thin organically cryoturbated soil. A couple of abandoned

skua nests and many bird remnants are found. The soil is poorly structured with a sandy texture mixed with stone particles. The next point was Caleta Argentina (S 62°40'08.5", W 060°24'07.7") with elevation 2 m asl (Papua penguin rockery), and it can be described as a postornithogenic locality. Currently, the number of penguins has decreased to 15–20 couples situated in the south part of the beach at about 2 m a.s.l. The coastline is rocky with a solid cover of decomposed Antarctic green alga *Prasiola crispa* and distributed *D. antarctica* tufts. The last point was Johnson Dock (S 62°39'30.0", W 060°22'33.8") with elevation 1 m asl. This is a small rocky cove in the terminal moraine. The coast has a cape-like shape with two marine terraces. The upper terrace is formed by large pieces of slope sediments, mosses, and *D. antarctica* in between, where a solid soil layer is formed underneath. The soil thickness varies from 10 to 40 cm.

2.4. Soil Analysis. Soil samples were air-dried (24 hours, 20°C) in a laboratory, grounded, and passed through a 2 mm sieve. The chemical analyses (TOC, nitrogen, and pH) were performed by the elemental analyzer (Euro EA3028-HT Analyser, Italia) for TOC and nitrogen content dry combustion method and pH by using a pH-meter (pH: 150M, Russia) in water (H₂O). The W (degree of oxidation) index (1) determines the number of electropositive and electro-negative atoms about carbon atoms:

$$W = \frac{(2 * O) - H}{C}, \quad (1)$$

where W is the degree of oxidation; O is the oxygen mole content of HAs; H is the hydrogen mole content of HAs; and C is the carbon mole content of HAs.

With an excess of hydrogen, the difference is negative, which corresponds to the reduction conditions in HAs. With an excess of oxygen, the difference is positive, and the compound has a higher oxidation condition. The content of carbon (C), hydrogen (H), nitrogen (N), and oxygen (O) varies in HAs within a wide range; therefore, the atomic ratios C/N, H/C, and O/C are usually used to present information. H/C_{mod} (2) is the number of substituted hydrogen atoms in HAs (the use of H/C_{mod} allows avoiding errors in the determination of the hypothetical hydrocarbon skeleton of the studied HAs). O/C ratio is an indicator of the degree of oxidation of HAs [37].

$$\frac{H}{C_{mod}} = \left(\frac{H}{C} + 2 * \frac{O}{C} \right) * 0.67, \quad (2)$$

where H/C_{mod} is the number of substituted hydrogen atoms in HAs; H is the hydrogen mole content of HAs; C is the carbon mole content of HAs; O is the oxygen mole content of HAs.

HAs were extracted according to the recommendation of the International Humic Substances Society (IHSS) [38, 39] in modification by Vasilevich [6].

- (i) Soil sieving: sieving the grinded sample through the 2 mm mesh.
- (ii) Double extraction of humic substances with alkaline solution (NaOH 0.1N) for 48 hours each time.
- (iii) Gravity filtration of the extractant: the soils will be sedimented to the bottom of the flask, and the supernatant will contain the NaOH with the extracted humic substances.
- (iv) Precipitation of the humic substances: for every sample, calculate the amount of H_2SO_4 1N solution in the proportion of 50 ml H_2SO_4 1N per 100 ml of supernatant.
- (v) Dialysis of HAs: dialysis was carried out in dialysis cellophane bags with a pore diameter of 12–14 kDa. During the previous 24 hours, the humic substances will be precipitated to the bottom of the flask. After 24 hours, the supernatant liquid (acid-soluble fraction) is siphoned off, and the HAs precipitate is squeezed out in a centrifuge at 3000 rpm for 15 minutes. After centrifugation, the HAs gel is placed in bags made of dialysis cellophane and placed in large containers with distilled water for 7–10 days to completely remove excess sodium sulfate.
- (vi) Drying of HAs preparations: HAs preparations from dialysis bags are transferred into Petri dishes or small crystallizers, after which they are dried in a vacuum oven over containers with dry $CaCl_2$.

HAs extraction yields were calculated as the percentage of carbon recovered from the original soil sample [6].

2.5. Solid State CP/MAS ^{13}C NMR Spectroscopy. Solid-state CP/MAS ^{13}C NMR spectra of HAs separated from soils were measured with a Bruker Avance 500 NMR spectrometer in a 3.2 mm ZrO_2 rotor. The magic angle spinning frequency was 20 kHz in all cases, and the nutation frequency for cross polarization was $u1/2p$ 1/4 62.5 kHz. Repetition delays were 3 seconds. The number of scans was 6500–32000. Contact time is 0.2 μs . Groups of structural compounds were identified by their chemical shifts' values: alkyl C (0 to 45 ppm), O/N-alkyl C (45 to 60 ppm), OCH group (60–110 ppm), aromatic C (110–160 ppm), carboxyl group (160–185 ppm), and quinone group (185–200 ppm).

2.6. ESR Spectroscopy. The ESR spectra were recorded on a JES FA 300 spectrometer (JEOL, Japan) in X-diapason with a free-radical modulation amplitude of 0.06 mT and a microwave power in the cavity of 1 mW. Magnesium powder with fixed radical concentration was used as an external standard. The concentration of the paramagnetic centers in powdered samples was determined by comparison with relative signal intensities of the external standard using the program JES-FA swESR v. 3.0.0.1 (JEOL, Japan) [40].

2.7. Statistical Analysis. The statistical analysis has been performed in PAleontological STatistics (PAST) software. The hierarchical clustering method (Ward's method) was chosen for the combination to group the study samples. This analysis uses the method of variance to estimate the distances between clusters. The method minimizes the sum of squares (SS) for any two (hypothetical) clusters that can be formed at each step [41]. The Pearson correlation has been used to determine the statistical relationship between the studied parameters in the soils [42].

3. Results and Discussion

3.1. Chemical Characteristics of Study Soils. The soils investigated in Livingston Island are identified as Cryosols [36]. The soil group Cryosols are characterized by the presence of permafrost table in the soil profile. In places where birds nest, there is an accumulation of OM, higher nitrogen content, and a slightly acid soil reaction (Table 1). In the Antarctic region, this is the typical way for nutrients to enter the soil cover [19, 23, 24, 27].

The climate in the western part of Antarctica is much milder than in the eastern part, and the presence of liquid moisture causes the growth of vascular plants and is a relatively high indicator of biodiversity in comparison with the eastern part of Antarctica. The studied areas are characterized by well-developed moss biocenoses, as well as by areas occupied by *D. antarctica*. In terms of carbon content, the highest level is observed in postornithogenic soils with *D. antarctica*, while the lowest level is noted in Soil ID 1. Soils developed in the nesting area are inaccessible to plants for some time because the guano excess is toxic to vegetation. The SOM content in the continental part of Antarctica is relatively lower than in the maritime part. The concentration of SOM in the soils of Mount Mosses is 0.67%

TABLE 1: Description and basic characteristics of soils from Livingston Island, Antarctica ($n = 5$).

Location	Description of study soils	TOC		N		C/N	pH	Color ^a	Soil type by WRB classification ^b
		Mean	SD	Mean	SD				
1 Hesperides Point Pluton	Soil forms under bird nest (skua) abandoned, contain <25% of fine earth	0.09	0.01	0.01	0.006	9	5.85	10 YR 4/7	Hyperskeletal cryosol (ornithic)
2 Playa Bulgaria	Soil under mosses contains the layer of dense rock in profile	1.19	0.04	0.08	0.007	15	5.95	10 YR 4/4	Leptic cryosol (ornithic)
3 Punta Hesperides	Soil under mosses contains <25% of fine earth	0.49	0.05	0.06	0.006	8	5.82	10 YR 4/7	Hyperskeletal cryosol
4 Caleta Argentina	Soil under grasses (<i>Deschampsia antarctica</i>) contains a layer of dense rock in profile	3.47	0.06	0.37	0.05	9	5.75	10 YR 5/2	Leptic cryosol
5 Johnson Dock	Soil under grasses (<i>D. antarctica</i>) contains a layer of dense rock in profile	0.52	0.04	0.08	0.007	6	6.60	10 YR 4/4	Leptic cryosol

^aMunsell soil color charts, 2010 [43]. ^bIUSS Working Group WRB World Reference Base for Soil Resources 2014 [36].

TABLE 2: Elemental composition of the studied HAs.

Soil ID	C		H		N		O		C/N	H/C	O/C	H/C mod	W
	Mean	SD	Mean	SD	Mean	SD	Mean	SD					
1	45.70	0.18	5.57	0.13	4.73	0.11	44.00	0.21	11	1.45	0.72	2.42	0
2	38.37	0.15	5.62	0.19	4.40	0.19	51.62	0.18	10	1.74	1.01	3.09	0.28
3	44.58	0.2	5.46	0.18	4.75	0.17	45.21	0.32	11	1.46	0.76	2.48	0.07
4	41.01	0.11	5.89	0.12	6.17	0.12	46.94	0.12	9	1.71	0.86	2.86	0.01
5	37.88	0.13	5.46	0.1	4.99	0.2	51.68	0.15	9	1.71	1.02	3.09	0.33
Std. error	1.58		0.07		0.3		1.6		—	—	—	—	—
Yamal region, Arctic, Russia	36.1		4.6		5.5		53.8		6.56	0.13	1.49	—	—
King-George Island, maritime Antarctica	35.5		4.4		5.7		54.4		6.23	0.12	1.53	—	—
Leningrad region, temperate climate, Russia	48		6		2		39		24	1.52	0.68	—	—

Gravimetric concentrations are given for C, H, O, and N content. C/N, H/C, O/C, H/Cmod, and W were calculated from the mole fraction of C, H, O, and N content. Sample numbers correspond to Table 1 ($n = 5$).

C, Leningradskaya station (Russia) is 0.48% C, and Russkaya station (Russia) is 0.27% C. This is the result of the absence of the introduction of OM by birds and other animals [44]. In Casey Station, Wilkes Land, East Antarctica lichens accumulated up to 417 g/kg of TOC [45, 46]. On King George Island, maritime Antarctica, the SOM content is increased compared to the continental part, and the carbon content ranges from 0.61% to 1.3% C, depending on the position in the landscape. The highest carbon content is obtained in areas confined to bird nesting sites [25, 44]. Compared to soils of a temperate zone, soils from the northwestern part of Russia (Leningrad oblast), here, in the mineral horizons, the SOM content is lower and ranges from 0.32 to 1.97% C [47].

The soils of the islands of West Antarctica contain on average a relatively high organic carbon content compared with continental soils. Birds in Antarctica are the main factor in the transport and accumulation of SOM [13, 17, 35]. The formation of ornithogenic soils induces an increase in nutrients and the formation of moss-lichen communities. During the transformation of OM, specific organic acids (HAs) are formed. The elemental and molecular composition of HAs can provide reliable data on the chemical processes (hydrogenation/dehydrogenation, oxidation/reduction, humification, and hydrophobicity) occurring in soils. The processes of formation and degradation of SOM largely determine the flows of carbon dioxide and methane

between the soil and the atmosphere and affect the global climate.

3.2. Elemental Composition of Study HAs. The elemental composition of HAs is an important indicator of the condensation and oxidation/reduction processes in molecules. Characteristic features of polar soils are the predominance of hydrogen and low oxygen content [5, 27, 48, 49]. The obtained elemental composition of HAs molecules is presented in Table 2.

The carbon content in the studied HAs varied slightly, which is typical for polar soil [5, 48]. Soil sample 4 distinguishes by the highest nitrogen content in the HAs composition. At the nesting sites, the formation of vegetation is impossible for some time, and this is due to the acidic conditions in soils. Over time, the necrophilic plants occupy these areas. During the postornithogenic succession, the content of available nitrogen in the soil increases significantly, and this is an important sign of ornithogenic soil formation [10]. We assume that this is due to the predominance of vascular plants vegetation during the transformation when organic nitrogen enters the soil. The samples show a high H/C ratio, which indicates high levels of hydrogen addition to OM and the formation of the oxygen-containing group [50, 51]. The lowest concentrations are

observed in no. 1 and no. 3 samples, which indicates the process of increasing aromatic fragments in the molecules of HAs and their higher resistance to biodegradation [21]. This may be the result of relatively fast processes of mineralization of easily hydrolyzable compounds.

From the W (degree of oxidation) index, we see that all studied samples are formed under oxidizing conditions. The index indicates the leading oxidation/reduction processes in HAs molecules; in the studied preparations, a low oxidation state is observed, which is caused by the processes of humification of SOM [37]. Graphical representation of the elemental composition of HAs from soils identifies the patterns of their formation [52, 53]. The method is based on constructing H/C_{mod} (the number of substituted hydrogen atoms in HAs) and O/C diagrams and serves as a technique to demonstrate the contribution of oxidation and condensation to changes in the elemental composition of HAs (Figure 2). In sample nos. 1 and 3, the condensation of molecules in the composition of HAs occurs. A rather high level of the H/C ratio is noted among HAs of polar biomes, which indicates the accumulation of aliphatic carbon chains and oxygen-containing fragments in HAs [54, 55].

3.3. CP/MAS ^{13}C -NMR Spectroscopy of Study HAs. CP/MAS ^{13}C -NMR spectroscopy identifies the abundance of specific structural fragments in the HAs molecule. From the obtained spectra (Figure 3), several peaks related to the zones of C-alkyl, N-alkyl, -OCH group, and carboxyl fragments have been noted. The sum of the aliphatic fragments ranges from 0–110 and 185–200 ppm, and of the aromatic fragments ranges from 110 to 185 ppm. In the study spectra, a peak in the carboxyl group is noted, while it is possibly magnetically duplicated from the -OCH group, the magnetic equivalence of nuclei effect; this method of analysis does not allow for reliable counting of these structural fragments [56]. The content of structural fragments of the studied HAs molecules is presented in Table 3.

According to the data obtained, the quantitative composition of studied HAs molecules is presented by aliphatic fragments (65–69%). The predominance of mosses and lichens leads to the formation of aliphatic structural fragments. Precursors of humification which are formed from mosses and lichens have practically no aromatic structures and consist of carbohydrates and proteins. Aromatic compounds, in particular lignin, usually are formed in precursors of humification from vascular plants [21, 58]. In mosses and lichens, up to 10 percent contain tannins and flavonoids, which make a significant contribution to the aromaticity of the studied samples.

The quantitative molecular composition of HAs is similar to data obtained from the Arctic systems and other Antarctic regions [5, 57, 59].

In the samples formed under *D. antarctica* (soil ID 4-5), the quantitative composition of aromatic fragments is low (31–33%). The quality of precursors of humification leads to the formation of aliphatic structural fragments in the HAs molecule. The predominance of aliphatic fragments in HAs can be caused by the low content of lignin in the plant

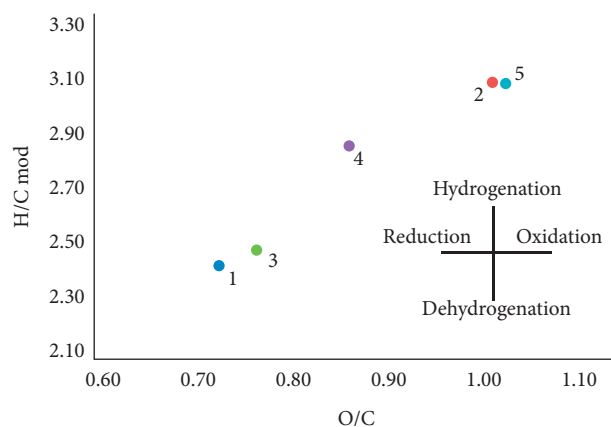


FIGURE 2: Elemental ratios of the studied HAs isolated from the study soil.

residues of *D. antarctica*. According to the data of aromatic fragments composition, the largest number is observed in sample nos. 1–3. The precursors of humification here are formed from mosses and lichens, mosses lack lignin and contain up to 20% tannins and flavonoids, and lichens contain up to 2% tannins and flavonoids and up to 10% of lignin [37].

The following parameters were used to standardize the quantitative characteristics of HAs molecules: the hydrophobicity degree (AL h , $r + AR$ h , r) and degree of decomposition of organic matter (C, H-AL/O and N-AL) (Figure 4).

More active humification processes occur in samples 1, 3, and 4, where the process of hydrogenation (the addition of hydrogen to OM) is to a lesser extent. This leads to the humification of OM and its stabilization to biodegradation [21]. Based on the molecular composition (Table 3), in these samples, the dominance of signals in the C, H-AL region is observed among the studied samples and the least in the quinone group region.

3.4. ESR Spectroscopy of Study HAs. Calculations of the integrated intensity of the absorption line allow us to estimate the concentration of unpaired electrons in the HAs samples. Free (unpaired) radicals are direct participants in the stepwise polymerization process of humus formation in soils. An increase in the mass concentration of paramagnetic centers in HAs molecules indicates a higher ability of HAs to polymerization [29]. Figure 5 shows ESR spectra typical for all studied HAs molecules. The least number of free radicals is observed in postornithogenic soils formed under *D. antarctica*.

The ESR spectra are characterized by the presence of one broad line with a g -factor, from 2.01 to 2.31, which indicates the presence of free radicals in the structure of HAs (Table 4). g -factor is a factor that connects the magnetic moment of an elementary particle with the classical value of the magnetic moment. The obtained values are close to the g -factor of a free electron ($g = 2.0023$). A slight increase in the g -factor may indicate a bond of HAs with oxygen-containing

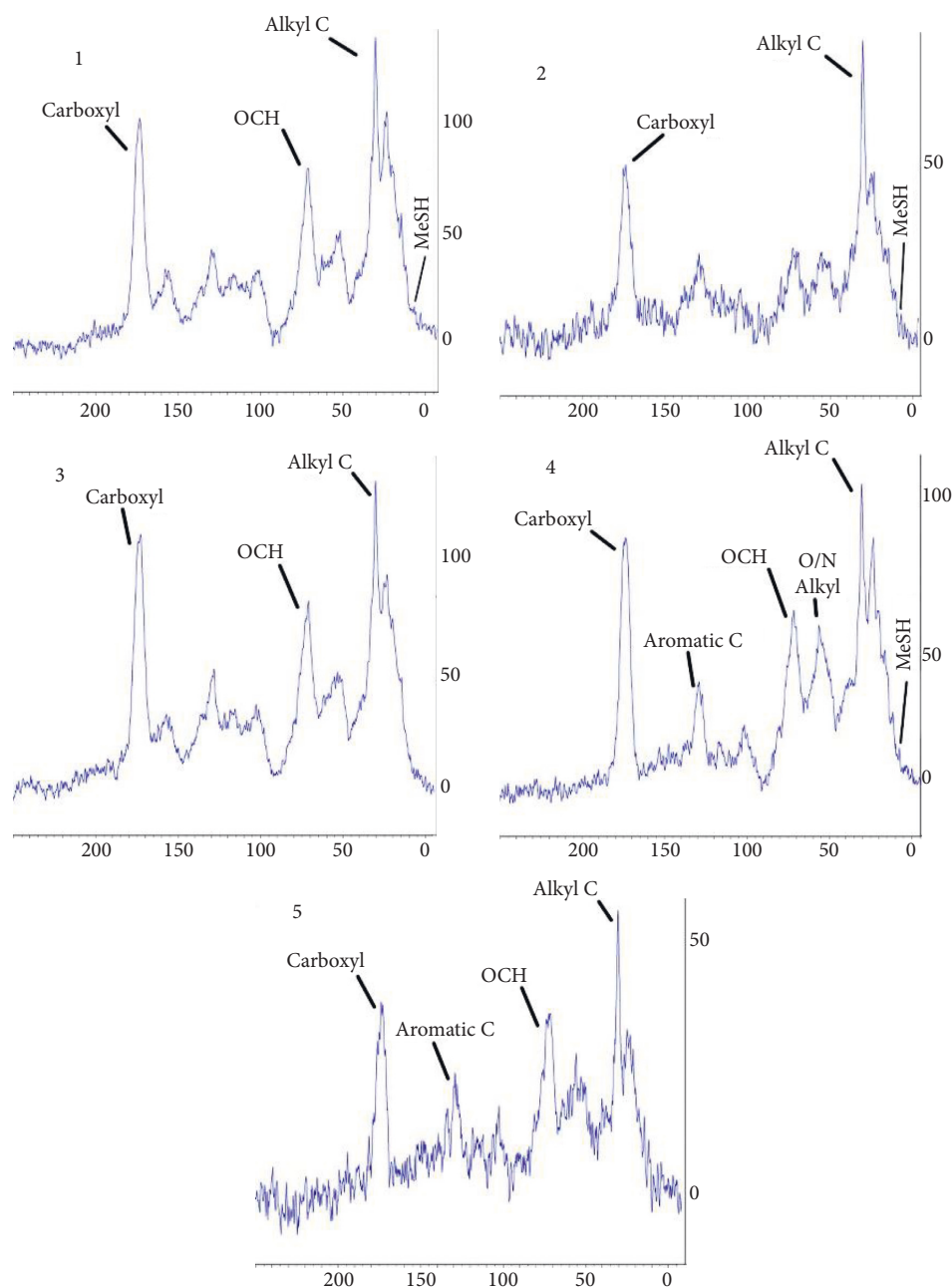


FIGURE 3: CP/MAS ^{13}C NMR spectra of the HAs from Livingston Island, Antarctica.

functional groups. In sample no. 2, the g -factor is higher, which may indicate a larger shift of the electron density of the unpaired electron in the HAs structure to the oxygen atom in the course of its interaction with other oxygen-containing HAs components [29]. In sample no. 2, the highest content of O, N-AL functional fragments is observed according to CP/MAS ^{13}C NMR spectroscopy among the studied samples. This indicates the existence of a highly delocalized molecular orbital in the structure of HAs.

According to cluster analysis (Ward's method) based on ESR spectroscopy (Figure 6), the studied HAs are divided into three groups. The ESR spectroscopy method is a powerful tool for the analysis of humification processes; the

content of free radicals positively correlates with the degree of humification, and the content of paramagnetic centers is determined by the degree of condensation (aromaticity) of HAs molecules [29]. Therefore, based on ESR spectroscopy, we decided to perform a cluster analysis and distinguish the following groups of HAs. The first (ornithogenic) HAs were isolated from soil nos. 1-2, which are underdeveloped soils with the highest number of free radicals. The second group (transitional from ornithogenic to postornithogenic) HAs isolated from soil no. 3 with an average number of free radicals among the studied samples. The third group (post ornithogenic) HAs isolated from soil nos. 4-5, which represent the lowest mass concentration of free radicals.

TABLE 3: Percentage of carbon in the main structural fragments of HAs from the studied surface soil horizons (according to CP/MAS ^{13}C NMR data).

	Chemical shifts in % from ^{13}C						AR ^a	AL ^b	AR/ AL	AL h, r + AR h, r ^f	C, H-Al/O, N-Al ^g
	0–46	46–60	60–110	110–160	160–185	185–200					
1	29	7	22	20	14	8	34	66	0.52	49	1.00
2	24	12	20	20	14	10	34	66	0.52	44	0.75
3	28	7	22	21	14	8	35	65	0.54	49	0.97
4	31	9	22	18	13	7	31	69	0.45	49	1.00
5	25	8	25	21	12	9	33	67	0.49	46	0.76
Soil from King-George Island, Antarctica ^c	44	8	18	21	8	1	29	71	0.41	65	1.69
Soil from Vaigach Island, Arctic ^d	23	8	37	19	11	2	29	71	0.42	42.05	0.52
Soil from King-George Island, Antarctica	22	17	23	15	14	9	29	71	0.41	—	—

^aAromatic. ^bAliphatic. ^cPolyakov and Abakumov, 2020 [56]. ^dPolyakov, 2019 [57]. ^eAbakumov, 2017 [17]. ^fHydrophobicity degree. ^gDegree in the decomposition of organic matter. Sample numbers correspond to Table 1.

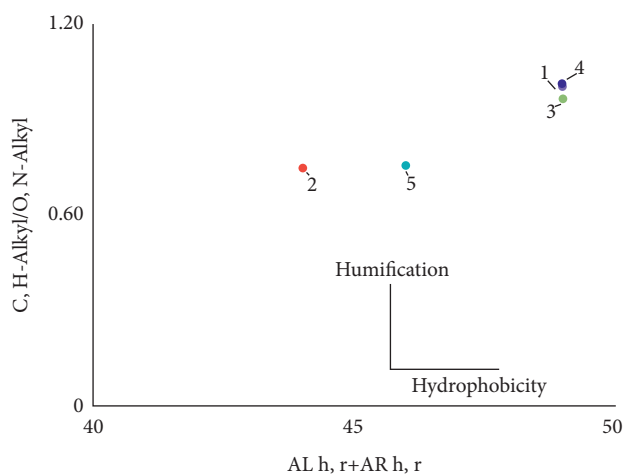


FIGURE 4: The diagram of integrated indicators of the molecular composition of HAs.

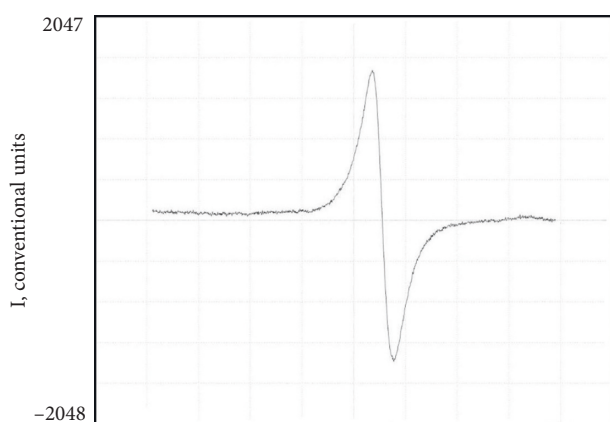


FIGURE 5: The ESR spectra of HAs were isolated from the study soils.

According to linear (Pearson) correlation, the highest correlation between the mass concentration of free radicals, and the molecular composition of HAs by CP/MAS ^{13}C NMR spectroscopy was found in the range of carboxyl groups 160–185 ppm ($r = 0.83$, $p = 0.95$). This may indicate

an increase in the stabilization of unpaired electrons in sample nos. 1–3.

Table 4 shows the content of free radical changes among the studied samples.

The content of free radicals in the HAs molecules in ornithogenic soils (nos. 1–2) is two times higher than in the HAs molecules formed under vascular plants (nos. 4–5). This determines their high ability to participate in polymerization and radical complexation reactions [29]. The content of free radicals in HAs preparations under *D. antarctica* is the lowest. The entrance of vascular plants in the plant cover with a relatively high proportion of cellulose in composition (30–32%) in comparison with mosses and lichens (8–25%) leads to a decrease in the content of free radicals in HAs molecules and indicates the formation of aliphatic structural fragments, which is confirmed by CP/MAS ^{13}C NMR spectroscopy.

3.5. Stabilization of Soil Organic Matter in Antarctica.

The results of the elemental composition and NMR spectroscopy reveal the inhomogeneous composition of the studied HAs molecules in the soils of Livingston Island. The main factor affecting the composition is the quality of humification precursors (plant residues) [60]. The main types of vegetation in this specific Antarctic region (mosses and lichens) contain mainly carbohydrates, lipids, and proteins, which, during transformation, form aliphatic compounds in the composition of HAs molecules [44]. The sources of lignin, precursors for the synthesis of aromatic compounds, are limited in the *D. antarctica*, which contributes to the predominance of aliphatic compounds in the composition of HAs molecules [37]. This is reflected in the molecular composition of HAs molecules, which accumulate more than 60% of aliphatic carbon structures. A long period with low temperatures and high humidity is typical for western Antarctica which leads to restricting the process of humification and mineralization of SOM. The functional composition of HAs in the studied areas corresponded to those previously studied in Antarctica and the Arctic, the general regularity of which is the accumulation of C-Alkyl, O-Alkyl, and–OCH group [5, 17]. According to Mergelov et al. [61] in

TABLE 4: Mass concentration of free radical in HAs.

Soil	Mass concentration of free radical, $N^*10^{15} \text{ spinN}^*g^{-1}$	<i>g</i> -factor
1	4.56	2.03
2	4.89	2.31
3	3.43	2.03
4	2.34	2.01
5	2.21	2.03

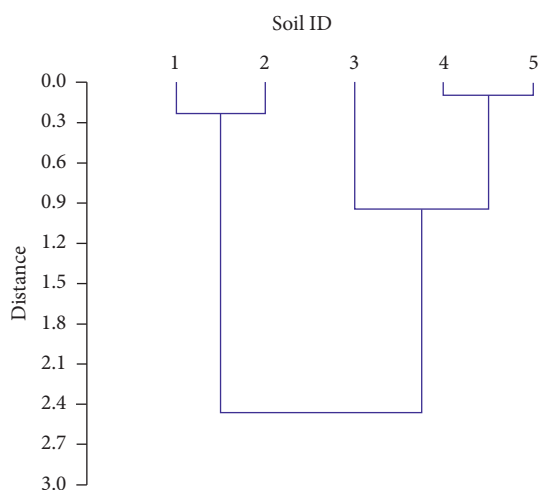


FIGURE 6: Hierarchical clustering based on ESR analysis. Ward's method.

the Antarctica Dry Valley, the formation of organic acids occurs under the action of various bacteria, and these organic acids on the surface of stones serve as primary products for other organisms (fungi and bacteria). According to NMR spectroscopy, the OM obtained from the endoliths of East Antarctica is characterized by the predominance of O/N-alkyl C. The formation of biogenic aggregates in the Antarctic Dry Valley is a characteristic stage in the stabilization of organic matter. Compared to the boreal zone, relatively low aromatic fragments accumulate in the Arctic and Antarctic soils (up to 35% AR in the polar region and 40% AR in the boreal region) [40, 57]. In the boreal zone, in comparison with the polar zone, a more active process of mineralization occurs, which is associated with the high hylomorphism of the territory [47]. In the polar regions, under conditions of low microbiological activity and low temperatures, OM is deposited in the composition of permafrost [1]. Maritime Antarctica differs significantly from the continental part of Antarctica, and this is due to a more favorable climate, a relatively high amount of precipitation (in liquid form), and a variety of flora and fauna. The limiting factor of continental Antarctica is liquid precipitation and vegetation (mosses, lichens, algae) spread along the places of temporary streams, which are the result of melting snowfields and ice, and along wind shelters. In this regard, maritime Antarctica has many more ecological niches, there are vascular plants, a more diverse avifauna

(which is involved in the redistribution of OM), and the presence of liquid moisture causes more active humification processes in the studied area. The relatively high diversity of biotic and abiotic factors in maritime Antarctica compared to the continental part of Antarctica significantly affects the accumulation of OM, their transformation, and stabilization in the soil cover.

4. Conclusion

HAs are heterogeneous systems consisting of high and low molecular weight organic compounds. The absence of a constant chemical structure and high variability of molecules and chemical bonds makes it difficult to interpret the structure and composition of HAs. The study of OM by various methods (ESR and CP/MAS ^{13}C NMR spectroscopy) allows a more detailed assessment of the structure and composition of HAs. The study presents a detailed description of HAs separated from soils from Livingston Island, Western Antarctica. From the data obtained, the aliphatic structural fragments predominate in the studied HAs. The carbon content in the elemental composition of the studied samples is max. in the samples from the post-ornithogenic and transit areas of Livingston Island up to 47.5 and 44.7% as well. According to the graph of elemental ratios, the most dehydrated molecules were found in ornithogenic and transit areas which are indicated in the formation of aromatic structures. As a result of ESR spectroscopy, it was revealed that the largest mass concentration of free radicals ($4.56\text{--}4.89 \times 10^{15} \text{ spin}^*g^{-1}$) is formed on the ornithogenic areas of the island. Ornithogenic and transitional zones are the most resistant areas for the process of degradation of SOM on the island. An increase in the proportion of free radicals and aromatic structures in HAs leads to the stabilization of organic matter in the soils of Livingston Island.

Abbreviation

SOM: Soil organic matter
 OM: Organic matter
 SAS: Spanish Antarctic station
 HAs: Humic acids
 NMR: Nuclear magnetic resonance
 ESR: Electron spin resonance
 BAS: Bulgarian Antarctic station
 TOC: Total organic carbon
 C: Carbon
 H: Hydrogen
 N: Nitrogen
 O: Oxygen
 SS: Sum of squares
 PAST: PAleontological STatistics
 MBF: Main boundary fault.

Data Availability

Data are available within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study was developed under the bilateral project “assessment of the regional contribution of soils of maritime Antarctica islands to global carbon balance with an evaluation of stabilization and humification rate of organic matter” (contract no. KP 06-Russia-29/28.09.2019 RFB bolga-19-54-18003) by the National Science Fund, Ministry of Education and Science, and the Russian Foundation for Basic Research. This work was supported by the grant of Russian Foundation for Basic Research (no. 19-05-50107). Special contribution to the collection of and analysis of the results has been realized within the project “biomonitoring of polar ecosystem component’s state in Livingston Island under global changes” at the Bulgarian Antarctic Institute funded through the National Program for Polar Research 2017–2021 with CM decision no. 718/29.08.2016. The development of the projects would not be possible without the professional collaboration with the National Center for Polar Studies, Sofia University St. Kliment Ohridski (and Bulgarian Antarctic Institute), and the logistical support during the 27th and 28th Bulgarian Antarctic Expeditions.

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