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A characteristic study of humic acids isolated from Arctic fjord sediments

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Abstract Humic substances are ubiquitous natural materials found in sediments as a product of biochemical transformation reactions representing a significant proportion of organic carbon cycle on earth. This study involves the analysis of humic substances with special emphasis on humic acids (HAs) in sediments collected from the Kongsfjorden System of Arctic region in June, 2017. The characterization of the isolated HAs were done using various spectroscopic techniques viz. UV-visible, Fluorescence, FTIR and NMR. Isolated HAs were also undergone for elemental analysis along with other characterization. The UV spectral analysis results with a lower E_4/E_6 ratio suggesting the presence of HAs with high degree of aromaticity and condensation. Indications for the presence of hydroxyl, methyl, methylene, carbonyl, carboxyl, phenol, alcohol and amide groups were obtained from the FTIR spectrums of HAs. NMR spectral characteristics also confirm the presence of OH group as well as the presence of CH protons adjacent to C=*X*, were *X* can be any electronegative element. This also confirms the presence of carbonyl group which is also evident in the FTIR spectral studies. Presence of aliphatic regions slightly more dominated with long chain and/or alicyclic moieties rather than methyl groups was also inferred from the results of NMR.

Keywords **Kongsfjorden, sediments, humic acids**

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1 Introduction

The understanding of the role of aquatic systems in global biogeochemical cycles needs a critical knowledge on the source, fate and reactivity of organic matters. In determining the relative contribution of autochthonous and allochthonous sources of organic matter due to the overlapping of elemental and isotopic compositions of various end member sources, the bulk organic matter techniques are the effective ones. The limitations in using elemental composition and biochemical compositions for source characterization of sedimentary organic matter makes molecular biomarker approach as an invaluable tool for explaining environmental processes in a quantitative way.

The primarily deposited organic debris embedded in sediments can be classified broadly as humic substances and non humic substances. The humic group includes all those organic molecules which are chemically similar to living matter, whereas the non humic group comprises of compounds that arise during diagenesis from organic debris that is not a part of plants and animals.

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Humic substances are organic materials that occur widely in aquatic, and terrestrial environments with a heterogeneous polyfunctional yellowish black organic macromolecules. These macromolecules comprises a major part of the aquatic organic matter, hence considered to have an important role in geochemical processes like carbon cycle on earth, transportation of metals and other inorganic and organic compounds.

The solubility factor classifies the humic substances into humic acid, fulvic acid and humins. Among these, the humic acids are water insoluble compounds which enhance the solubility property in high pH. The fulvic acid is soluble in acidic conditions, but the humins are insoluble in all pH. The quantity and composition of humic acids differs accordingly with the variability in the parent material (Gracia et al., 1985), vegetation (Singbal and Sharma, 1983) and climate (Dziadowiec et al., 1994). The characteristic properties of humic acids can show similarity to the fulvic acids according to the climatic condition and specific biochemical composition (Dziadowiec et al., 1994). The reduction of humic substances is considered to be the major metabolic pathways in some of the major ecosystems present in the Arctic region (Lipson et al., 2010), also it contributes broadly to the ecosystem respiration (Lipson et al., 2013). The polar soils contains higher amount of free radical than other zonal soils due to the lower degree of organic matter humification and the enhanced hydromorphism under continuous permafrost condition (Chukov et al., 2017). The structure determination of these compounds is a great challenge due to its high complexity and heterogeneity. This study is an attempt to understand the structural characteristics of humic acids isolated from one of the typical Arctic fjords, Kongsfjorden System.

2 Material and methods

The northern location of Arctic fjords makes them sensitive to climatic changes that can provide scientists with important information on the effects of contemporary environmental changes. The sensitivity of fjord environments stems from both the interference and buffer between glaciated land and the ocean (Syvitski and Shaw, 1995). The organic matter plays a major role in the biogeochemistry of fjords not only because the sediment provides a significant reservoir in the global carbon cycle, but also they drive early diagenesis.

2.1 Study area

The study area is an Arctic fjord named Kongsfjorden between 79°N, 12°E which is one of the double-fjord systems on the northwest coast of Spitsbergen, within the Svalbard Archipelago (Figure 1). It is an open fjord with a length of 20 km and width ranging in 4–10 km, without sill, and therefore largely influenced by the processes on the adjacent shelf. This fjord is particularly suitable for studies of effects of climate change on ecosystems, because it is influenced by both Arctic and Atlantic water masses and hence an established reference site for Arctic marine research.

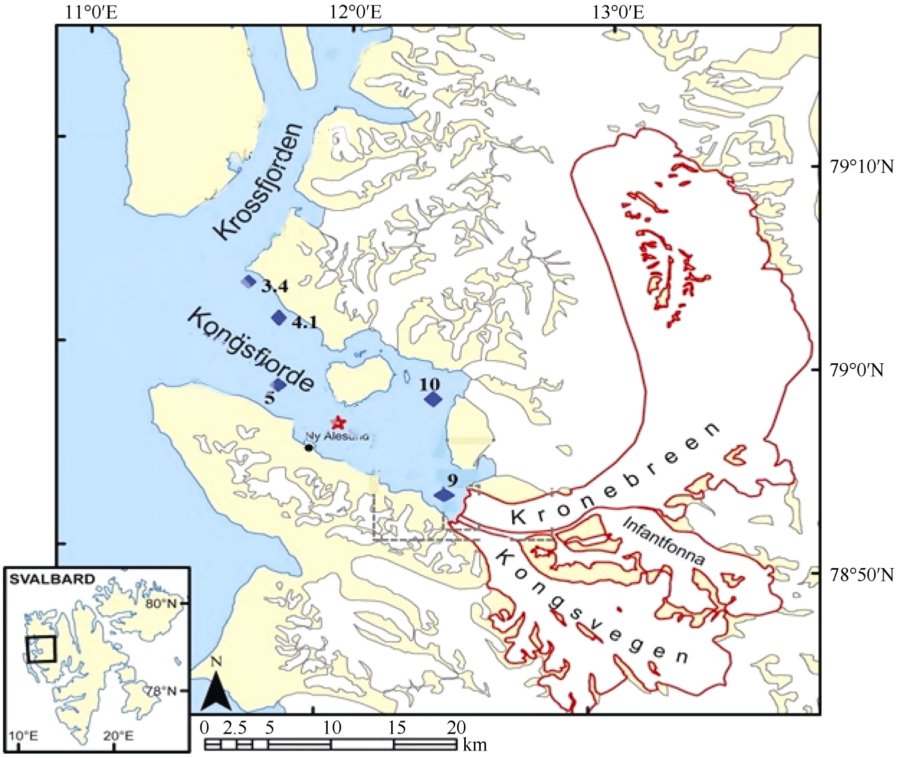


Figure 1 Location map of sampling stations in Kongsfjorden.

The fjord has an inner section which is relatively shallow with maximum depth of 350 m, and communicates with the Greenland Sea through the Kongsfjorden. The seasonal changes of the hydrological conditions were observed in this study area. During winter, water masses exhibits a homogeneous nature while in summer, the freshwater outflow from the glaciers and warmer surface water masses from the West Spitsbergen Current (WSC) causes thermal-salinity stratification.

Sampling of sediments from the Kongsfjorden was done during the summer season in June 2017 (Table 1). The samples were collected with the help of a Van Veen grab, packed in high quality polythene zip locks and preserved in very low temperature of about −20℃ for transportation to our laboratory in Kochi, India.

Table 1 Location of sampling stations in Kongsfjorden

Stations	Latitude	Longitude
3.4	79°02′41.25″N	11°41′51.12″E
4.1	78°58′54.31″N	11°41′44.52″E
5	78°57′49.25″N	11°49′34.51″E
9	78°53′43.54″N	12°13′56.27″E
10	78°58′21″N	12°20′19″E

2.2 Isolation and characterization

The isolation of HAs was performed according to the IHSS method (IHSS, 2010a, 2010b) with slight modification. It involves the treatment of sediment samples with alkaline NaOH. About 1 kg of samples was taken from each station for the isolation process of HAs. These samples initially undergo the process for the elimination of weakly bound carbonates, sulfates and hydroxides with 1 N HCl. The removal of HCl solution after a few minutes is then followed by the extraction process with 1 N NaOH and shaken for 24 h in a rotary shaker and allowed to rest overnight. The supernatant solution was decanted and the pH was adjusted within 1–2 with 6 M HCl. The supernatant was again allowed to rest for a whole day. The precipitation of insoluble HAs in the fulvic acid solution was observed in this stage of the isolation process. The separation of precipitate was done with filtration and then freeze dried for further characterization by UV-visible, FTIR, NMR spectroscopic techniques along with Carbon Hydrogen Nitrogen Sulphur (CHNS) elemental analysis.

The total organic carbon content in Arctic kongsfjorden sediment samples were analysed using PRIMACS$^{\mathrm{MCS}}$ TOC analyzer of SKALAR. The UV-visible spectroscopic analysis was done with the help of UV-visible spectrophotometer of Thermofischer. The elemental compositions of HAs were investigated using CHNS elemental analyzer of Elementar Vario EL III. Relative quantities of C, H, N and S were measured directly from the analyzer on an ash-free basis. For FTIR analysis Avatar 370 FTIR spectrometer was used. NMR data were acquired using the instrument Bruker Advance 400 MHz NMR spectrometer fitted with QNP ^{1}H probe.

3 Results and discussion

3.1 Quantification

Considering the geographical and climatic specialties of Artic fjord-Kongsfjorden, it is understood that the presence of refractory organic compounds are comparatively less in those sediments. The HAs content in 1 kg Arctic sediment obtained through IHSS 2010 method is much less than that obtained through the modified procedure of IHSS (IHSS, 2010a, 2010b). This variation in the amount of HAs obtained clearly indicate the effect of modification done on the IHSS method which has to be revised for enhancing the extraction of HAs for polar samples. Similar studies done on HAs reveal the low decomposition rate of organic matter in Arctic regions.

3.2 Total organic carbon (TOC)

The TOC analysis of the HAs samples indicates that the total carbon (TC) content of the Arctic fjord-Kongsfjorden is less (Table 2). The inorganic carbon (IC) and TOC levels are comparable except in stations 9 and 10 where the TOC is much lesser. This low carbon content reveals the lower rate of carbon degradation which in turn hints to low pollution levels in this area.

Table 2 TOC content of various stations in Kongsfjorden

Stations	TC/%	IC/%	TOC/%
3.4	4.754	2.206	2.548
4.1	4.801	2.358	2.443
5	5.032	2.863	2.168
9	4.145	3.073	1.072
10	4.022	2.855	1.666

3.3 Carbon hydrogen nitrogen oxygen (CHNO) analysis

The elemental composition and atomic ratios of elements in HAs isolated are reported in Table 3. The null hypothesis method indicates that polluted HAs have higher Nitrogen content than unpolluted HAs (Bird and May, 1981).

Table 3 Elemental composition and atomic ratios of HAs isolated from each stations of Kongsfjorden

Stations	N/%	C/%	H/%	O/%	C/H	C/N	C/O	O/H
3.4	0.49	2.75	0.15	96.61	18.33	5.61	0.02	6.44
4.1	2.25	16.20	2.70	78.85	6	7.20	0.20	0.29
5	0.11	1.57	0.12	98.20	78.50	14.27	0.02	8.18
9	0.57	3.83	0.12	95.46	31.91	6.71	0.04	7.95
10	0.06	3.20	0.16	96.58	20	53.33	0.03	6.03

By comparing the results above, it is observed that the Nitrogen content of HAs at all stations is less except for station 4.1 which has a considerable level of Nitrogen. Similarly, the Carbon content in all stations are approximately similar but for station 4.1, a high level of Carbon content is observed which is an indication of high level of degradation in this region. The predomination of Oxygen is also observed in these samples. Hence, the study of these values suggests the presence of terrestrial derived organic matter and oxic sediments with oxygenated bottom water to the fjord (Raiswell et al., 1987).

The atomic ratios, C/N and C/H provides the information regarding the lower rate of humification processes in station 4.1 whereas, station 5 reveals a higher level of humification. The reduction in humification accelerates the lignin mineralization (Anuradha et al., 2011). The inferences of O/H also showed accelerated humification (Ramesh and Ronald, 2008). This indicates an ordinary level of humification occurring in the study area with an exception of station 4.1.

3.4 Texture analysis

The affluence of humic acids in sediments depends on the productivity of the environment (Table 4). The textural analysis of sediments is used to assess the rate of accumulation on surface sediments (Table 5) (Aiken et al., 1985). The results of the texture study revealed that station 9 was characterized by larger clay content and have the highest humic acid content in it and the other stations were impoverished in humic acid level. The concentrations of organic compounds in sediments are highly influenced by surface area of the sediments (Brian et al., 1997) and its well established fine textured clay particles absorb higher amounts of organic matter than coarse textured soil when supplied with similar input of organic material (Harsink,1994).

Table 4 Humic acid content of various stations in Kongsfjorden

Sl. No	Stations	Weight of humic acid/g
1	3.4	0.60
2	4.1	0.70
3	5.0	0.62
4	9.0	0.73
5	10.0	0.57

Table 5 Texture of HAs obtained from Kongsfjorden

Stations	Clay/%	Silt/%	Very fine sand
3.4	25	73	2
4.1	19	70	11
5	28	72	0
9	47	53	0
10	27	58	15

3.5 UV-visible spectroscopic characterisation

The ratio of absorbance at 465 nm and 665 nm in UV spectrophotometer is termed as E_4/E_6 ratio (Table 6). The relationship of E_4/E_6 is related to the aromaticity and to the degree of condensation of aromatic carbon chain of the humic acids and could be used as a humification index (Table 7) (Stevenson and Schnitzer, 1982; Konnonova, 1996). The condensation of aromatic components increases with molecular weight and thus decreases E_4/E_6 (Stevenson, 1994; Chen et al., 1977). E_{280}/E_{664} ratio also supports the same (Albrecht, 2004). In the present study, E_4/E_6 ratio is below 1 so as the E_{280}/E_{664} values except the three stations viz. 5, 9 and 10 which exhibits values around 1 and above. Thus, it results in higher condensation degree of aromatic carbon chain and low humification index.

Table 6 Characteristic ratios interpreted from the UV-visible spectroscopy

Stations	E_4/E_6	E_{280}/E_{664}	E_{270}/E_{400}
3.4	0.1851	0.972	8.923
4.1	0.1584	0.825	5.261
5	0.1660	1.096	8.543
9	0.1666	1.055	7.210
10	0.1904	1.098	8.653

Coefficient E_{270}/E_{400} characterizes the degradation of phenolic/quinoid core of HA to simpler carboxylic aromatic compounds (Uyguner and Bekbolet, 2005). High levels of phenolic OH points to the presence of poorly decomposed humic acids (Lavti and Paliwal, 1981). The study results indicate an average value of 7.7 for E_{270}/E_{400} ratio showing the degradation of phenolic/quinoid core as well as the mineralization of lignin and other materials during the initial stages of humification.

3.6 FTIR characterization

FTIR spectroscopy is mostly used for characterization of hydroxyls, aliphatic C–H, carboxyls, carbonyls and amides that are found specifically in HAs (Stevenson, 1994; Amir et al., 2004, Sierra et al., 2005a). This provides information regarding structural and functional properties of HAs. At stations 3.4, 4.1, 5 and 9, a peak was observed at 1654, 1656, 1638 and 1635 cm^{-1} respectively (Figure 2). The characteristic bands at these wavelengths suggest the presence of highly conjugated delocalized π electrons with reduced double bond character (–C=O) of quinones (Silverstein et al., 1991). The presence of a peak at 1400–1500 cm^{-1} indicates the presences of –OH deformation and CO stretching of phenolic OH, CH deformation of CH_3 and CH_2 groups and COO– (Sierra et al., 2005a; Adani et al., 2006). IR spectra of stations 5, 9 and 10 showed peaks at 1459, 1461 and 1466 cm^{-1} respectively. Presence of peaks in the region 1000–1250 cm^{-1}

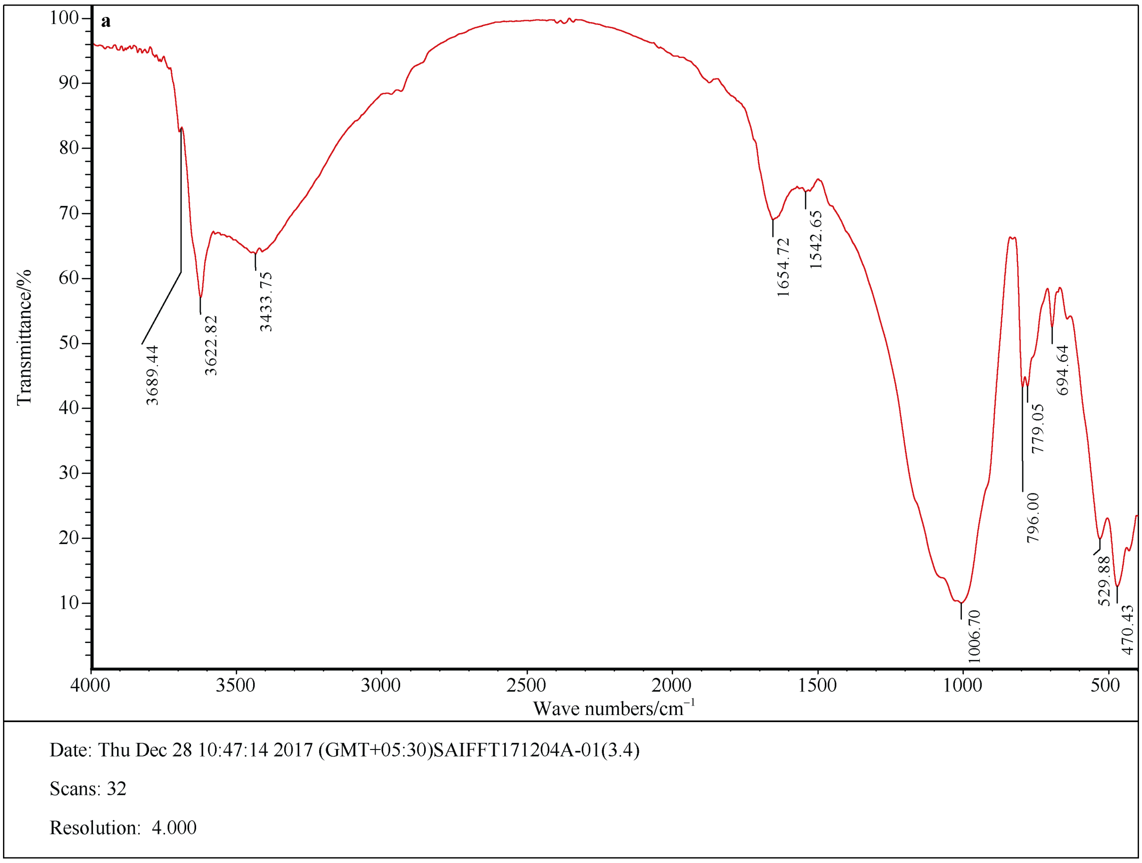
Table 7 Compilation of the characteristics of humic acid obtained from the Arctic fjord sediments

Location			Wt. of HAs /g	Total organic carbon			Textural properties of HAs			Ratios interpreted from UV-visible spectroscopy			Elemental composition and atomic ratios of HAs							
Stations	Latitude	Longitude		TC/%	IC/%	TOC/%	Clay/%	Silt/%	Very fine sand	E_4/E_6	E_{280}/E_{664}	E_{270}/E_{400}	N/%	C/%	H/%	O/%	C/H	C/N	C/O	O/H
3.4	79°02′ 41.25″N	11°41′ 51.12″E	0.60	4.754	2.206	2.548	25	73	2	0.1851	0.972	8.923	0.49	2.75	0.15	96.61	18.33	5.61	0.02	6.44
4.1	78°58′ 54.31″N	11°41′ 44.52″E	0.70	4.801	2.358	2.443	19	70	11	0.1584	0.825	5.261	2.25	16.20	2.70	78.85	6	7.20	0.20	0.29
5	78°57′ 49.25″N	11°49′ 34.51″E	0.62	5.032	2.863	2.168	28	72	0	0.1660	1.096	8.543	0.11	1.57	0.12	98.20	78.50	14.27	0.02	8.18
9	78°53′ 43.54″N	12°13′ 56.27″E	0.57	4.145	3.073	1.072	47	53	0	0.1666	1.055	7.210	0.57	3.83	0.12	95.46	31.91	6.71	0.04	7.95
10	78°58′ 21″N	12°20′19″E	0.73	4.022	2.855	1.666	27	58	15	0.1904	1.098	8.653	0.06	3.20	0.16	96.58	20	53.33	0.03	6.03

shows the presence of –CO stretch or –OH deformation of phenol or alcohol groups (Sierra et al., 2005a). All stations in this study showed a peak around 1010 cm^{-1}. The 775–850 cm^{-1} region shows the NH_2 wagging and twisting and third overtone N–H stretching (Stevenson, 1994; Amir et al., 2004; Sierra et al., 2005a). IR spectra of HAs in all the stations of the present study showed a peak at 778 cm^{-1}. Thus, the FTIR study indicated the presence of hydroxyl, methyl, methylene, carbonyl, carboxyl, phenol, and alcohol and amide groups in the isolated humic substances from Kongsfjord sediments.

3.7 NMR characterization

NMR liquid spectra of HAs contain numerous narrow resonances. In the NMR spectrum, two peaks are obtained at δ values 2.5 and 3.4 for stations 3.4 and 4.1 (Figure 3). In stations 5, 9 and 10, a small peak is obtained only at δ value 3.4 which indicates the presence of OH groups which are more H-bonded (Pavia et al., 2008).



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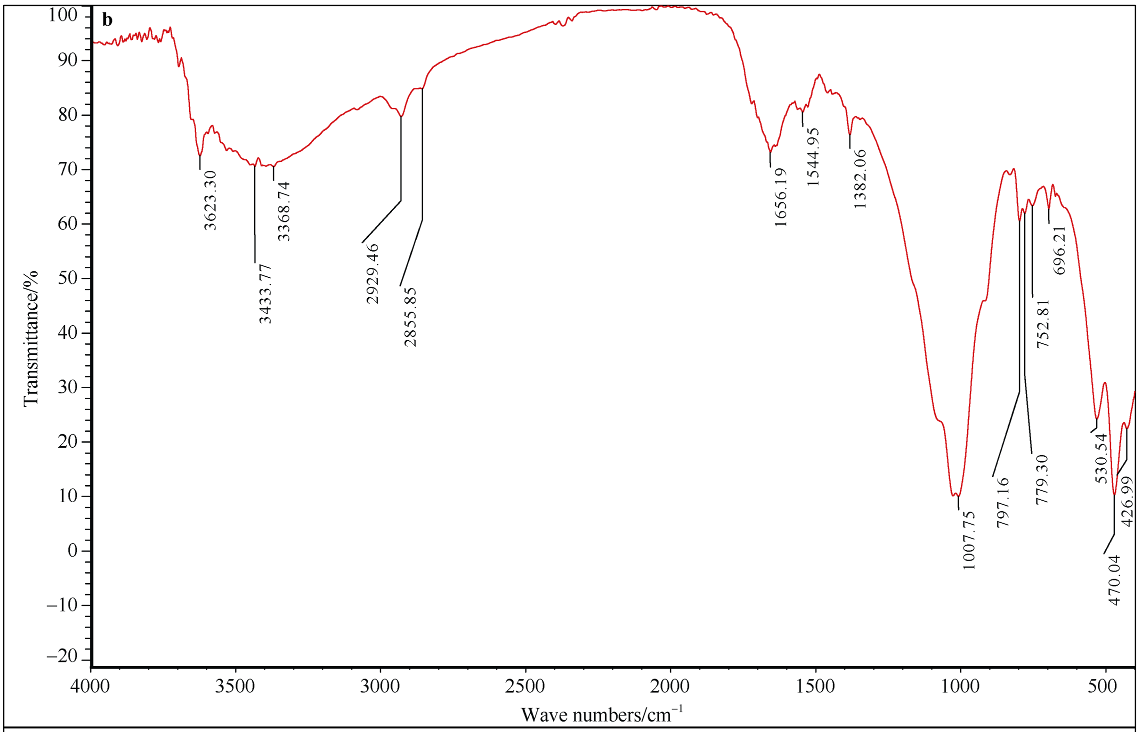

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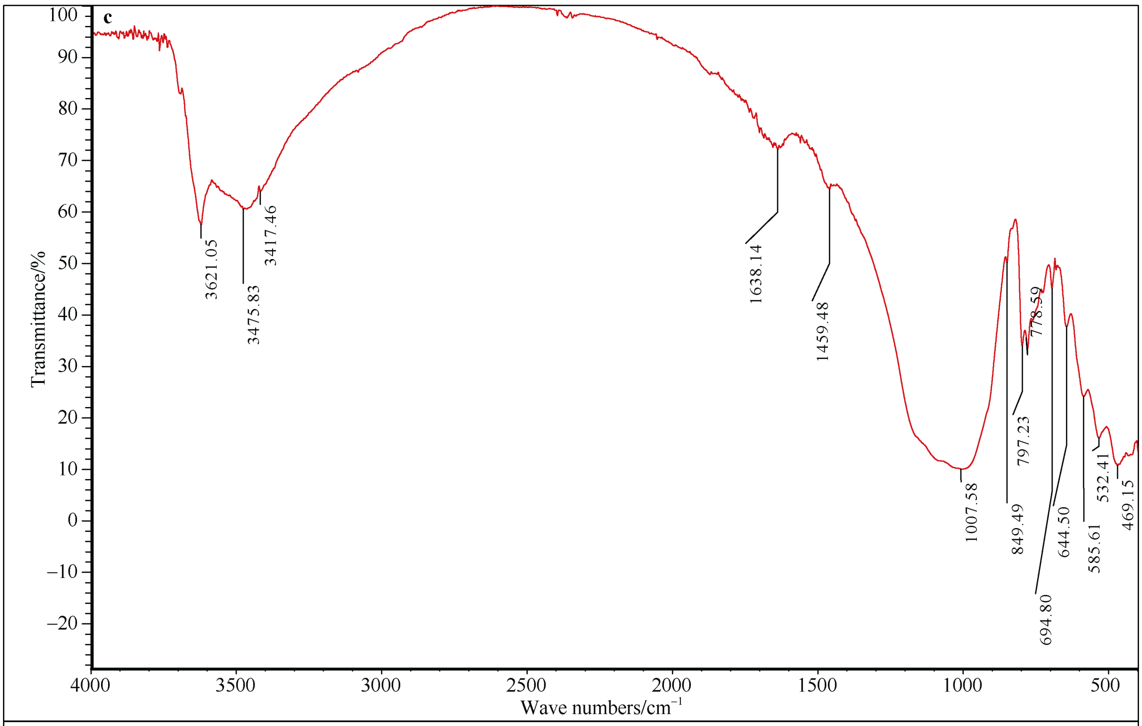

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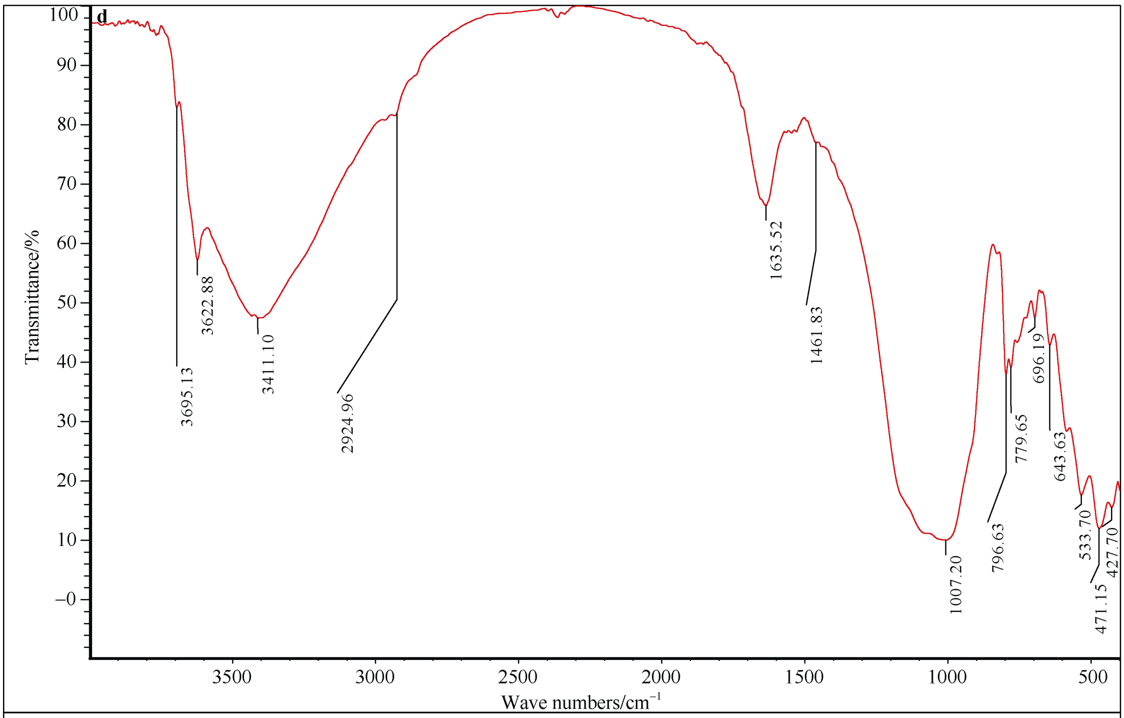

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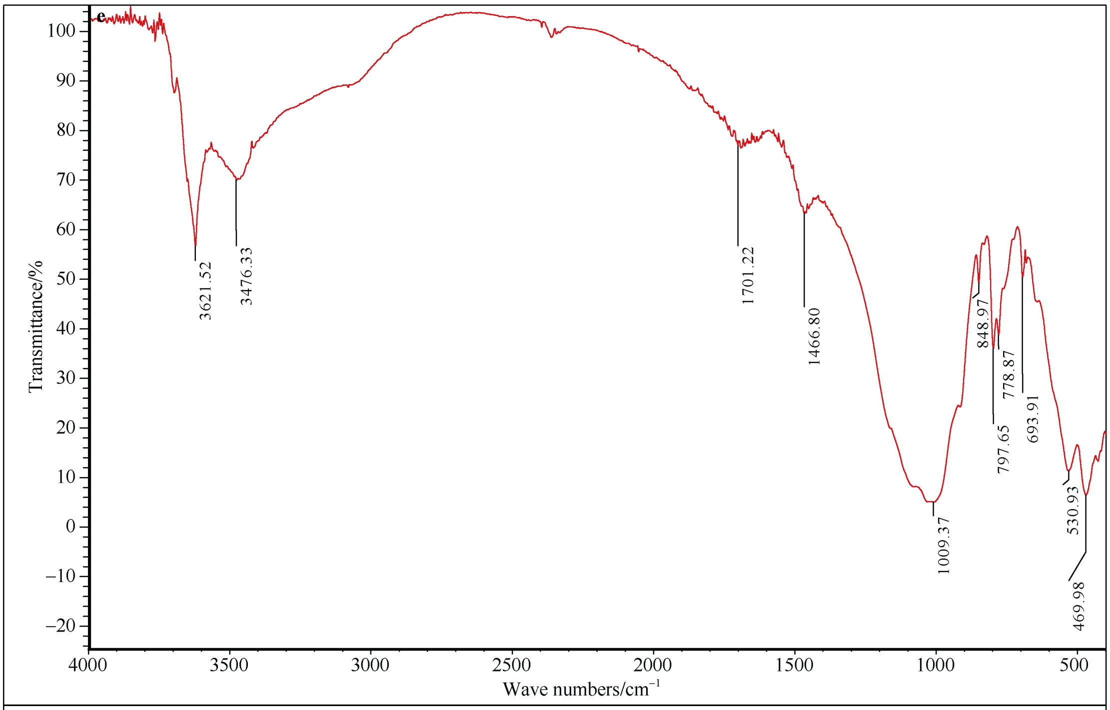

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Scans: 32

Resolution: 4.000

Figures 2 FTIR spectrums of HAs isolated from sediments of each stations of Kongsfjorden. **a**, station 3.4; **b**, station 4.1; **c**, station 5; **d**, station 9; **e**, station 10.

The presence of OH groups in the isolated HAs were also confirmed by the FTIR spectral characteristics. The presence of small peak at δ value 2.5 indicates the presence of CH protons adjacent to C=X were, X can be any electronegative element. In the present study, FTIR spectral characteristics have revealed the presence of carbonyl groups in the isolated HAs (Pavia et al., 2008). In this study, station 3.4 shows two resonance peaks at 1.282 and 1.290 ppm and station 4.1 at 0.972 and 1.266 ppm. Presence of aliphatic regions slightly more dominated with long chains and/or acyclic moieties rather than methyl groups is inferred from these results.

4 Conclusion

Artic fjord-Kongsfjorden samples collected from distinct geographical and climatic conditions contains lesser levels of Nitrogen, Carbon and Hydrogen which indicate degradation and pollution in lower rates. The predominating Oxygen content suggests the presence of terrestrial derived organic matter and oxic sediments with oxygenated bottom water to the Arctic fjord. The presence of HAs with higher degree of condensation and low humification index was suggested from the low E_4/E_6 values obtained from the UV visible charctaeristics of HAs from the study area. Also, the low E270/400 ratios depict the lower levels of phenolic/quinoid compounds. The presence of hydroxyl, methyl, methylene, carbonyl, carboxyl, phenol, alcohol and amide in the humic substances were inferred with FTIR spectroscopy. NMR spectral characteristics also confirm the presence of OH group as well as the presence of CH protons adjacent to C=X, were X can be any electronegative element. This also confirms the presence of carbonyl group which is also evident in the FTIR spectral studies. Indications of the presence of aliphatic regions slightly more dominating with long chains and/or alicyclic moieties rather than methyl groups were also understood with NMR spectral datas. The difficulty in the structural characterization of HAs due to its complexity was overcome in this study to an extent. It was possible to determine the characteristic features of HAs from the Kongsfjorden samples that contain high levels of phenolic compounds and low level of carboxylic groups in its highly humified state with high molecular weight and long chains.

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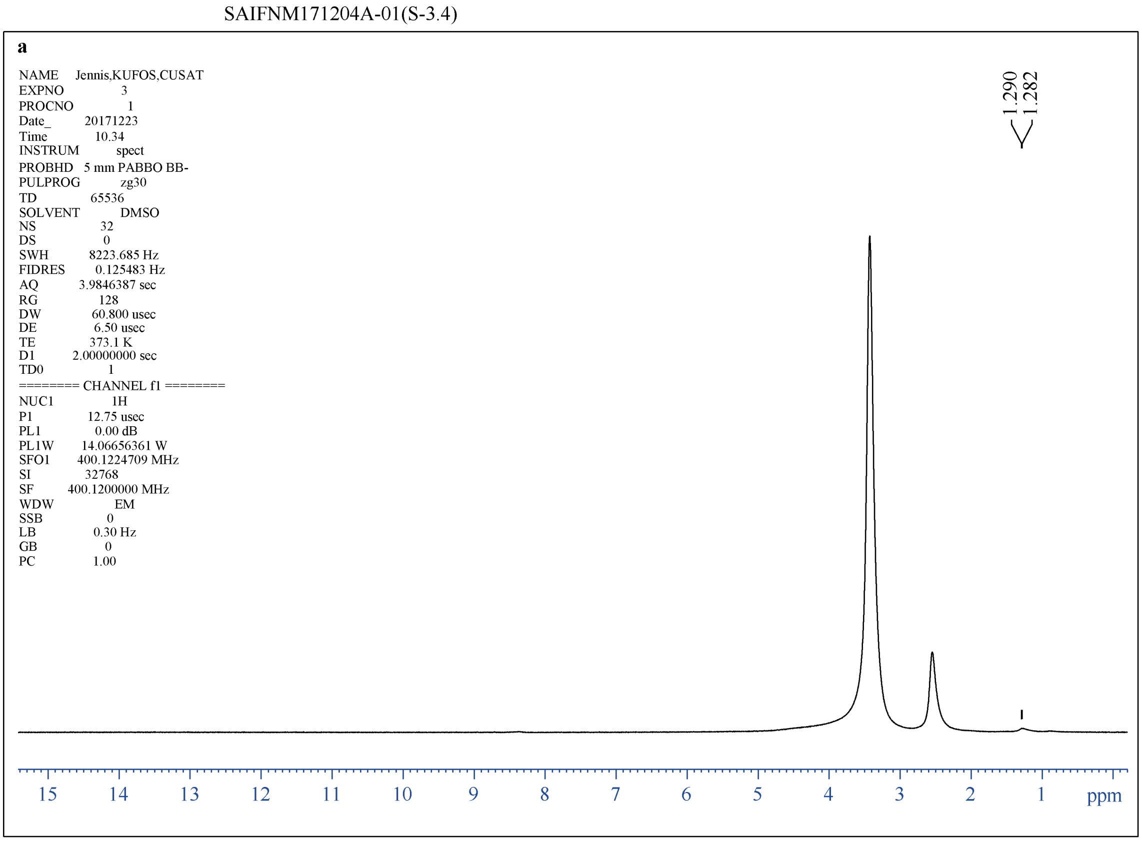

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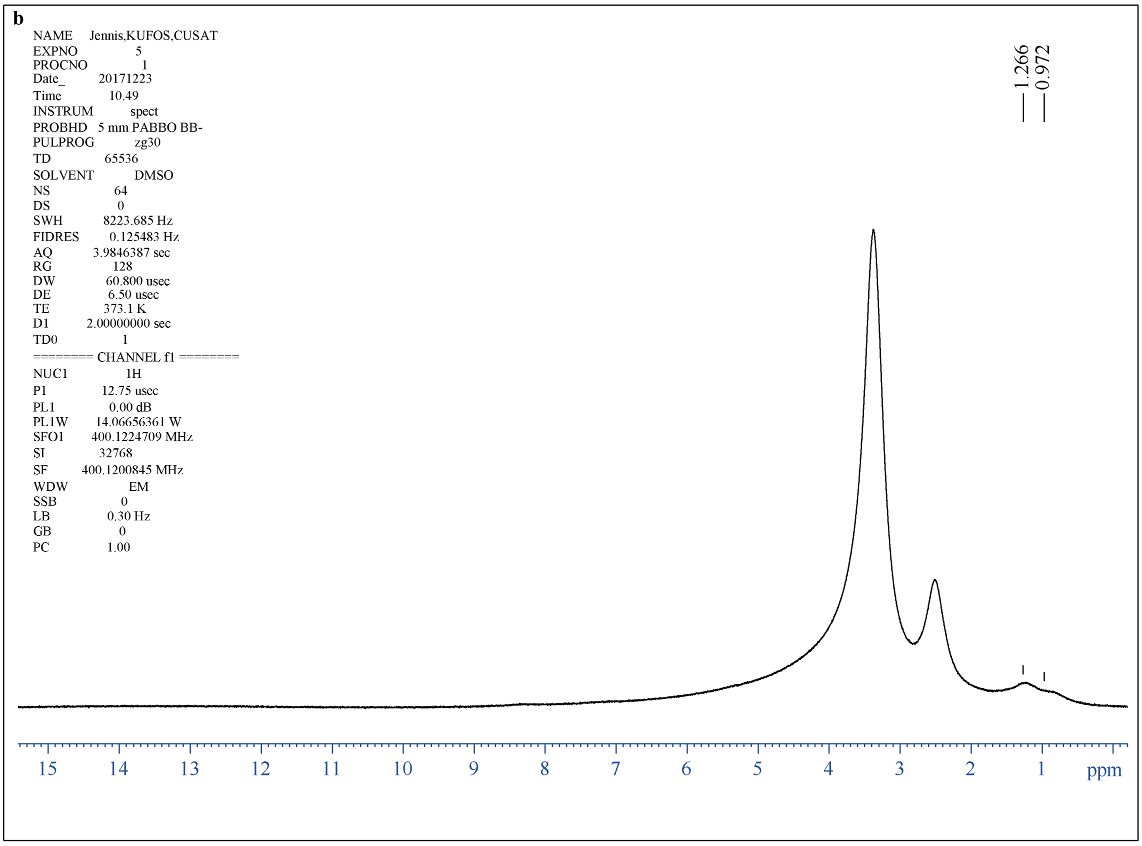

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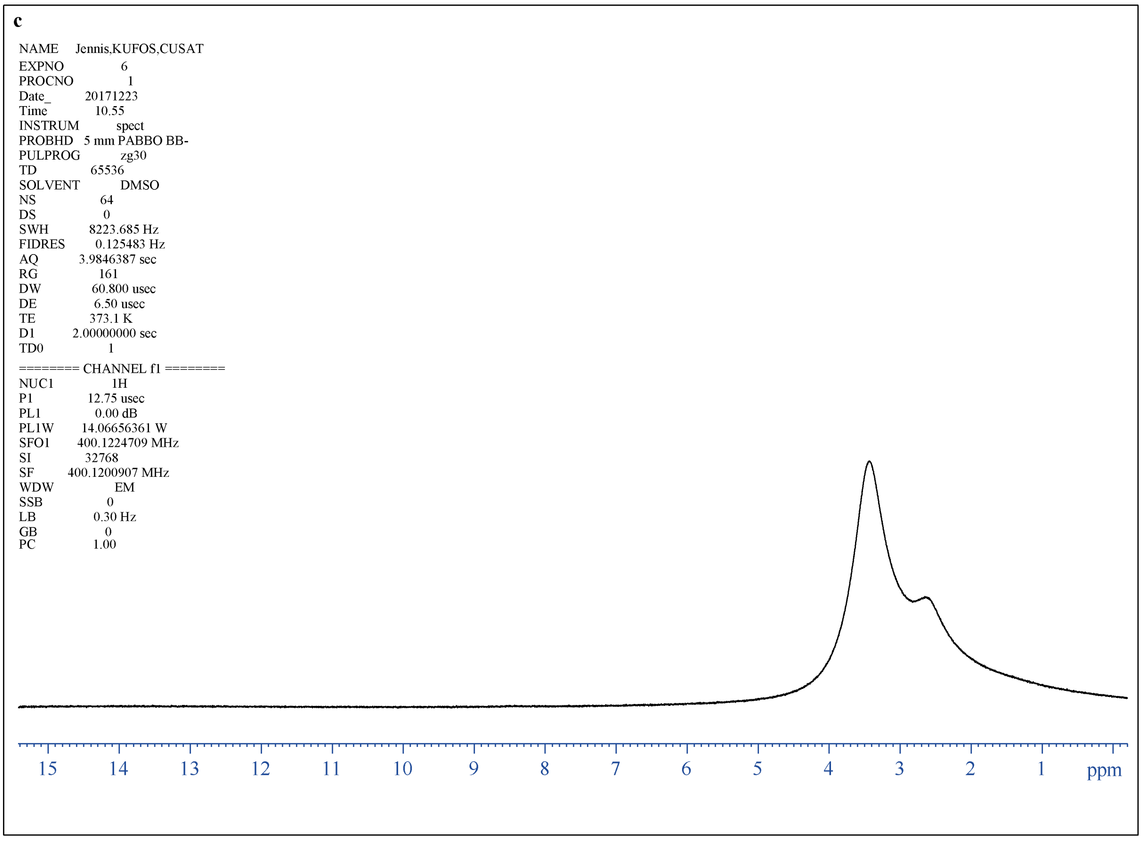

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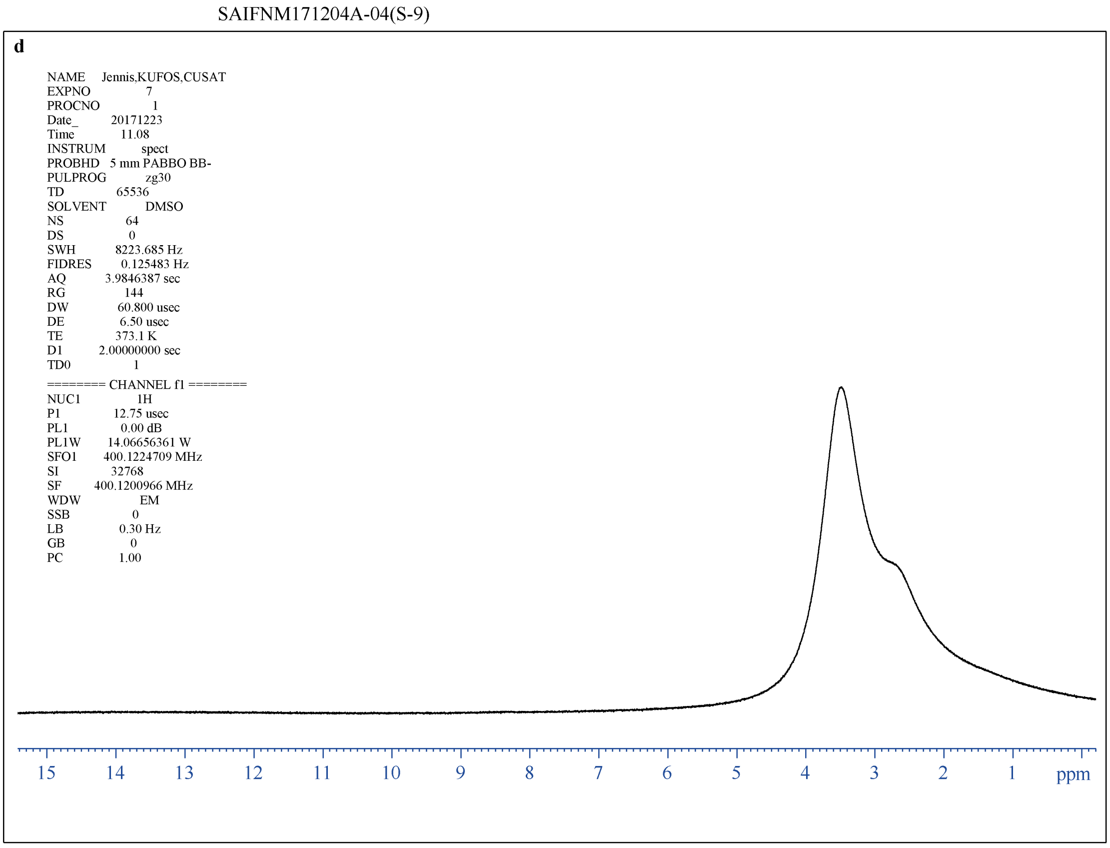

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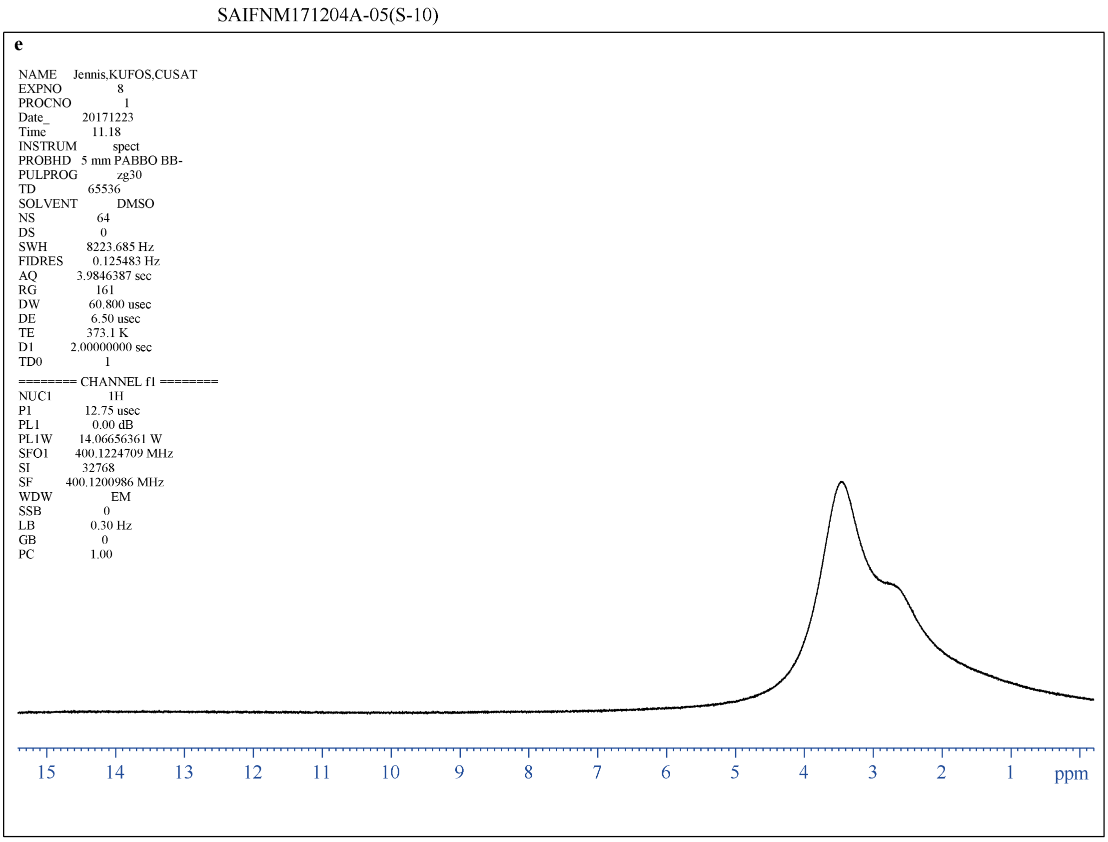

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NUC1              1H
P1            12.75 usec
PL1            0.00 dB
PL1W       14.06656361 W
SFO1      400.1224709 MHz
SI            32768
SF        400.1200986 MHz
WDW               EM
SSB               0
LB             0.30 Hz
GB                0
PC             1.00
```

Figures 3 NMR spectrums of HAs isolated from sediments of each stations of Kongsfjorden. **a**, station 3.4; **b**, station 4.1; **c**, station 5; **d**, station 9; **e**, station 10.

References

Aiken G R, McKnight D M, Wershaw R L, et al. 1985. Humic substances in soil sediment and water. New York: Wiley.

Amir S, Hafidi M, Merlina G, et al. 2004. Elemental analysis, FTIR and ^{13}C-NMR of humic acids from sewage sludge composting. Agronomie, 24(1): 13-18.

Anuradha V, Nair S M, Kumar N C. 2011. Humic acids from the sediments of three ecologically different estuarine systems-a comparison. Int J Environ Sci, 2(1): 174.

Bergamaschi B A, Tsamakis E, Keil R G, et al. 1997. The effect of grain size and surface area on organic matter, lignin and carbohydrate concentration, and molecular compositions in Peru Margin sediments. Geochim Cosmochim Ac, 61(6): 1247-1260.

Bird J O, May A J C. 1981. Statistics for technicians, gen editor, math. Science, 95-96.

Chen Y, Senesi N, Schnitzer M. 1977. Information provided on humic substances by E_4/E_6 ratios 1. Soil Sci Soc Am J, 41(2): 352-358.

Chukov S N, Ejarque E, Abakumov E V. 2017. Characterization of humic acids from tundra soils of northern Western Siberia by electron paramagnetic resonance spectroscopy. Eurasian Soil Sci, 50(1): 30-33.

Dubois M, Gills K A, Hamilton J K, et al. 1956. Colorimetric study by phenol-sulphuric acid method. Anal Chem, 26: 350.

Dziadowiec H, Gonet S, Plichta W. 1994. Properties of humic acids of Arctic tundra soils in Spitsbergen. Pol Polar Res, 15(1-2): 71-81.

Francioso O, Sanchez-Cortes S, Tugnoli V, et al. 2001. Spectroscopic study (DRIFT, SERS and 1H NMR) of peat, leonardite and lignite humic substances. J Mol Struct, 565: 481-485.

Garcia I, Polo A. 1985. Influence of vegetation on the characteristies of the organic matter of the soils of Alfagnara (Sierra de Alfacar-Granada, Spain). Anales Edafol Agrobiol, 44b: 81-82.

Graham B, Mayol-Bracero O L, Guyon P, et al. 2002. Water-soluble organic compounds in biomass burning aerosols over Amazonia 1. Characterization by NMR and GC-MS. J Geophys Res-Atmos, 107(D20): 8047-8062.

Hassink J. 1994. Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. Soil Biol Biochem, 26(9): 1221-1231.

International Humic Substances Society (IHSS). 2010. Natural organic matter research, Isolation of IHSS samples. http://www.humicsubstances.org/ isolation.html.

IHSS. 2010. Natural organic matter research, Elemental composition and stable isotopic ratios of IHSS samples. http://www.humicsubstances.org/elements.htm.

Jayasree G, Chandramohanakumar N. 1993. Influence of humic acid on metal sorption by estuarine sediments. Environmental Chemistry, Chemical Oceanography Division.

Kononova A M M. 1966. Soil organic matter. Oxford: Pergamon Press, 40-41.

Lipson D A, Jha M, Raab T K, et al. 2010. Reduction of iron (III) and humic substances plays a major role in anaerobic respiration in an Arctic peat soil. J Geophys Res-Biogeo, 115(G4): G00I06.

Lipson D A, Raab T K, Goria D, et al. 2013. The contribution of Fe (III) and humic acid reduction to ecosystem respiration in drained thaw lake basins of the Arctic Coastal Plain. Global Biogeochem Cy, 27(2): 399-409.

Pavia D L, Lampman G M, Kriz G S, et al. 2008. Introduction to spectroscopy. Cengage Learning.

Schnitzer M, Preston C M. 1983. Effects of acid hydrolysis on the ^{13}C NMR spectra of humic substances. Plant Soil, 75(2): 201-211.

Sierra M M D, Giovanela M, Parlanti E, et al. 2005a. Structural description of humic substances from subtropical coastal environments using elemental analysis, FT-IR and ^{13}C-solid state NMR data. J Coastal Res, 42: 370-382.

Sierra M M D, Giovanela M, Parlanti E, et al. 2005b. Fluorescence fingerprint of fulvic and humic acids from varied origins as viewed by single-scan and excitation/emission matrix techniques. Chemosphere, 58(6): 715-733.

Silverstein R M, Bassler G C, Morrill T C. 1991. Spectrometric identification of organic compound. New York: John Wiley & Sons.

Singhal R M, Sharma S D. 1983. Comparative studies of infra-red spectra of soil humic acids of Doon Valley Forests. J Indian Society Soil Sci, 31(2): 182-186.

Stevenson F J. 1994. Humus chemistry: genesis, composition, reactions. New York: John Wiley & Sons.

Uyguner C S, Bekbolet M. 2004. Photocatalytic degradation of natural organic matter: Kinetic considerations and light intensity dependence. Int J Photoenergy, 6(2): 73-80.