Characterization of organic species and functional groups in pollen, fungi, algae, and bacteria bioaerosols

Palina Bahdanovich[1,2], Kevin Axelrod[1,2], Andrey Y. Khlystov[1,2], Vera Samburova[1,2,*]

[1]Desert Research Institute, 2215 Raggio Parkway, Reno, NV 89512, USA
[2]University of Nevada, Reno, 1664 N Virginia St, Reno, NV 89557, USA
*Correspondence: vera.samburova@dri.edu

Table S1. Standard compounds used for ^{1}H-NMR bioaerosol functional group and chemical shift assignment.

^{1}H-NMR Standards	**Compounds**
1. Saccharides *(DMSO-d_6 and D_2O)*	D-(+)-Glucose, Fructose, Galactose, Mannose, Sucrose, Starch (potato origin)
2. Sugar Alcohols *(DMSO-d_6 and D_2O)*	Glycerol, Erythritol, Inositol, Mannitol, Arabitol
3. Fatty Acids *(DMSO-d_6 and D_2O)*	Palmitic, Stearic, Glutaric, Tridecanoic, Adipic acids
4. Triacylglycerides *(DMSO-d_6 and D_2O)*	Tri-arachidin, Tri-oleate, Glyceryl tri-heptadecanoate, 1-stearoyl-rac-glycerol, Glyceryl tri-palmitate
5. Amino Acids *(DMSO-d_6 and D_2O)*	Proline, Leucine, Glutamic Acid, Arginine, Phenylalanine
6. Carboxylic Acids *(DMSO-d_6 only)*	Succinic, Malonic acids
7. PAHs *(DMSO-d_6 only)*	Phenanthrene, Retene
8. Oxygenated PAHs *(DMSO-d_6 only)*	Anthraquinone, Perinaphthenone
9. Aldehydes *(DMSO-d_6 only)*	Acetaldehyde
10. Aliphatic Alcohols	Isopropyl *(DMSO-d_6 only)*, Methanol (*DMSO-d_6 and D_2O*)
11. Aromatic Alcohols *(DMSO-d_6 only)*	Phenol

Table S2. Calibration standards for bioaerosol GC-MS saccharide analysis and UPLC-MS amino acid analysis. Internal standard (IS) used for saccharide analysis is glucose-d7 and nonadecanoic acid (C19:0) for fatty acid analysis.

Saccharide Standards (GC-MS)	Amino Acid Standards (UPLC-MS)	Fatty Acid Standards (UPLC-MS)
Sucrose	Phenylalanine	Dodecanoic (C12:0)
Trehalose	Tryptophan	Myristic (C14:0)
α-D-arabinose	Leucine	Palmitic (C16:0)
ß-D-arabinose	Isoleucine	Stearic (C18:0)
α-D-fructose	GABA	Oleic (C18:1)
ß-D-fructose	Methionine	Linoleic (C18:2)
D-(+)-galactose	Valine	Linolenic (C18:3)
D-(+)-glucose	Proline	Nonadecanoic (C19:0) (IS)
α-lactose	Tyrosine	Eicosanoic (C20:0)
α-L-mannose	Alanine	
ß-L-mannose	ß-alanine	
α-D-xylose	Hydroxyproline	
ß-D-xylose	Threonine	
glucose-d7 (IS)	Glycine	
	Serine	
	Citrulline	
	Glutamic Acid	
	Histidine	
	Arginine	
	Lysine	

Table S3. Chemical shifts of various solvents, determined from solvent spectra and adopted from the "NMR Solvent Data Chart" by Cambridge Isotope Laboratories, Inc. (Cambridge Isotope Laboratories Inc., n.d.). To be consistent with solvent and water peaks present in each spectrum (standard and bioaerosol spectra), these were cut from the calculations as follows: TMS peak was cut from -0.02 to 0.02 ppm, DMSO-d6 peak was cut from 2.45 to 2.55 ppm, H_2O peak (absorption of water due to DMSO-d6 solvent use) was cut from 3.23 to 3.43 ppm, and the acetone peak (located at 2.07 ppm and used while preparing samples) was cut from 2.06 to 2.08 ppm.

Solvent	**Solvent chemical shift (ppm)**
TMS	0.00
DMSO-d_6	2.50
H_2O	3.33
Acetone	2.05 (2.07 in our spectra)
D_2O	4.79

Table S4. Concentrations and standard deviations (in μg/mg) of individual organic compounds found in bioaerosols. "-" stands for bioaerosols that were not tested for specific compounds. All values presented are above the method detection limit (MDL), which varies for each compound.

		Bioaerosols							
	Compounds	**Bacillus**	**Pedobacter**	**Western Gall Rust**	**Spirulina**	**Lodgepole Pine 2020**	**Lodgepole Pine 2021**	**Lodgepole Pine 2022**	**Rabbitbrush**
	Starch	0.45 ± 0.05	-	4.30 ± 0.06	0.64 ± 0.05	0.94 ± 0.06	-	-	-
	α-D-arabinose	0	-	0.24 ± 0.04	0	0	0	0	0.61 ± 0.01
	β-D-arabinose	0	-	0.17 ± 0.04	0	0.04 ± 0.11	0.19 ± 0.04	0.08 ± 0.02	0.42 ± 0.02
	α-D-xylose	0	-	0	0	0	0	0	0
	β-D-xylose	0	-	0.29 ± 0.05	0	0	0	0	0
Saccharides	**α-L-mannose & α-D-fructose**	0	-	1.69 ± 0.28	0	0	0	0	0
	β-D-fructose	0	-	5.20 ± 1.03	2.43 ± 0.48	4.84 ± 2.34	11.43 ± 2.27	7.40 ± 1.47	42.25 ± 0.27
	D-(+)-galactose	0	-	1.05 ± 0.15	0	1.31 ± 1.42	5.32 ± 0.77	3.41 ± 0.49	0.51 ± 0.004
	D-(+)-glucose	0.12 ± 0.02	-	3.07 ± 0.55	1.62 ± 0.29	12.17 ± 5.65	15.79 ± 2.84	12.29 ± 2.21	43.44 ± 1.22
	β-L-mannose	0	-	1.93 ± 0.31	0	0.33 ± 0.62	0.31 ± 0.05	0.36 ± 0.06	0
	Sucrose	1.45 ± 0.11	-	1.41 ± 0.11	0.17 ± 0.01	75.75 ± 12.92	129.19 ± 10.05	160 ± 12.44	0
	α-lactose	0	-	0	0	0	0	0	0
	Trehalose	0	-	0	0	0	0	0	0
	Phenylalanine	0.20 ± 0.01	0.49 ± 0.03	0.04 ± 0.004	0.09 ± 0.004	0.22 ± 0.06	-	-	0.15 ± 0.008
	Tryptophan	0.06 ± 0.007	0.08 ± 0.004	0.49 ± 0.05	0	0.07 ± 0.01	-	-	0.07 ± 0.003
	Leucine	0.12 ± 0.006	0.60 ± 0.03	0.10 ± 0.001	0.16 ± 0.02	0.32 ± 0.09	-	-	0.21 ± 0.02
	Isoleucine	0.04 ± 0.001	0.42 ± 0.03	0.04 ± 0.004	0.07 ± 0.001	0.17 ± 0.06	-	-	0.07 ± 0.02
	GABA	3.71 ± 0.74	0.73 ± 0.02	1.18 ± 0.11	2.90 ± 0.06	1.07 ± 0.36	-	-	0
Amino Acids	**Methionine**	0.16 ± 0.009	0.24 ± 0.004	0	0	0	-	-	0

	Valine	0	0.63 ± 0.02	0.10 ± 0.008	0	0.34 ± 0.12	-	-	0
	Proline	0.23 ± 0.005	0.29 ± 0.004	0.06 ± 0.004	0.05 ± 0.001	3.65 ± 1.77	-	-	15.05 ± 0.44
	Tyrosine	0	0.42 ± 0.03	0	0.17 ± 0.04	0.24 ± 0.07	-	-	0
	Alanine	0.97 ± 0.07	1.02 ± 0.04	0.28 ± 0.03	0.32 ± 0.009	0.38 ± 0.13	-	-	0.28 ± 0.01
	β-alanine	0.10 ± 0.0007	0	0	0	0	-	-	0
	Hydroxyproline	0	0.09 ± 0.01	0	0	0	-	-	0.78 ± 0.006
	Threonine	0.45 ± 0.02	0.62 ± 0.01	0.15 ± 0.008	0	0	-	-	0
	Glycine	0	0	0	0	0	-	-	0
	Serine	0	0	0	0	0	-	-	0
	Citrulline	0.15 ± 0.01	0	0	0	0	-	-	0
	Glutamic acid	13.80 ± 0.15	1.09 ± 0.05	0.12 ± 0.04	3.60 ± 0.04	0.45 ± 0.22	-	-	0
	Histidine	0.16 ± 0.01	0	0	0	0	-	-	1.49 ± 0.13
	Arginine	0	0	0	0	2.55 ± 1.20	-	-	0
	Lysine	1.24 ± 0.05	0.70 ± 0.03	0	0	0	-	-	0
	Dodecanoic acid C12:0	0	-	0	0	-	0	-	0.004 ± 0.006
	Myristic acid C14:0	0.05 ± 0.006	-	0.18 ± 0.02	0	-	0.07 ± 0.009	-	0.03 ± 0.004
Fatty Acids	**Linolenic acid C18:3**	0	-	2.83 ± 0.58	0.85 ± 0.34	-	1.19 ± 0.24	-	1.84 ± 0.38
	Linoleic acid C18:2	0	-	1.67 ± 0.18	0.69 ± 0.32	-	7.35 ± 0.78	-	2.57 ± 0.27
	Palmitic acid C16:0	0	-	3.61 ± 0.53	1.30 ± 0.63	-	6.94 ± 1.03	-	2.69 ± 0.40
	Oleic acid C18:1	0	-	1.20 ± 0.17	0.09 ± 0.07	-	6.70 ± 0.95	-	0.17 ± 0.02
	Stearic acid C18:0	0	-	1.01 ± 0.20	0.13 ± 0.18	-	2.98 ± 0.58	-	0.38 ± 0.07
	Eicosanoic acid C20:0	0	-	0.12 ± 0.02	0	-	0.60 ± 0.11	-	0.04 ± 0.008

27

Table S5. Correlation coefficients of aliphatic, saccharide, and amide ^{1}H-NMR segments and concentrations of individual organic species (saccharides, amino acids, and fatty acids).

	Aliphatic (Segment 1)	Saccharides (Segment 3)	Amides (Segment 7)
Saccharides	-0.610	0.608	0.441
Amino Acids	-0.130	0.129	-0.002
Fatty Acids	-0.442	0.448	0.090

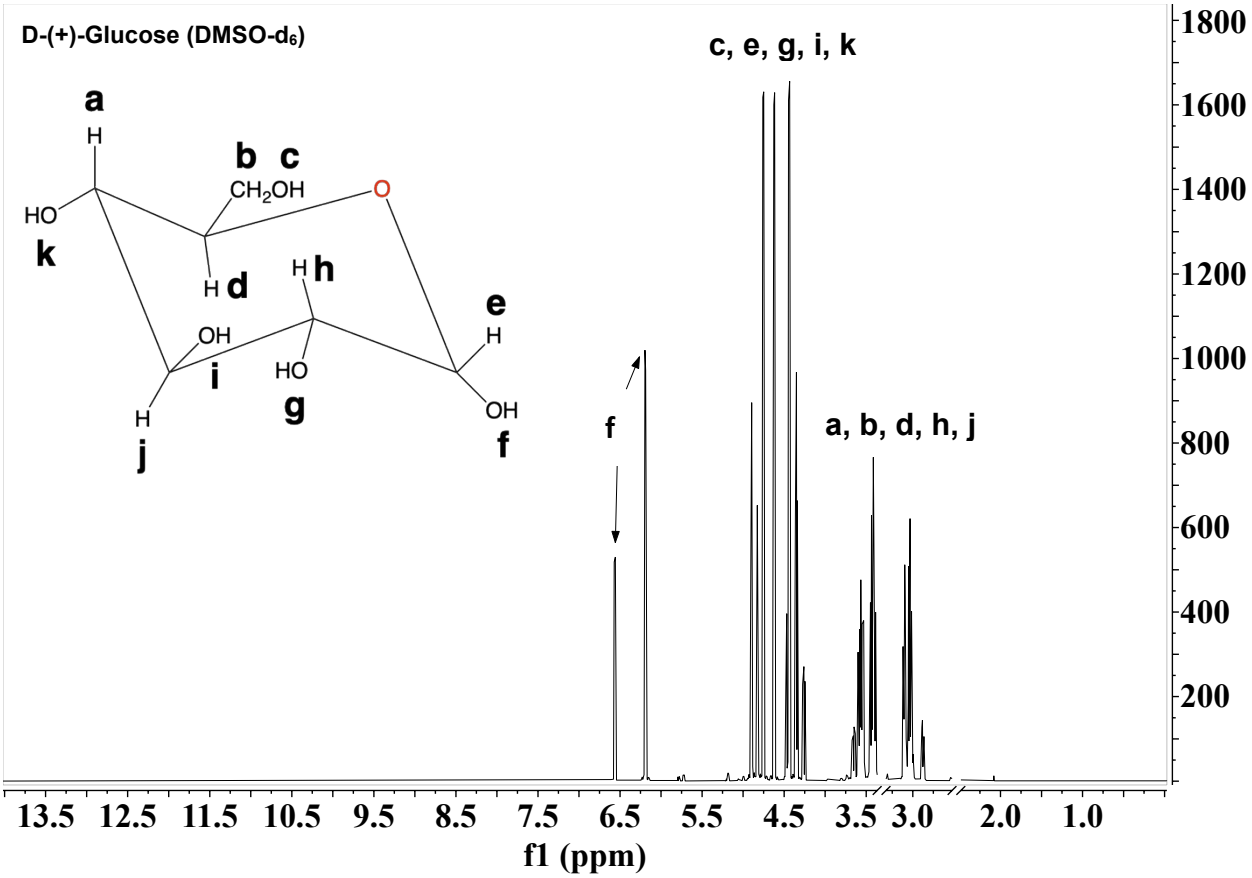

Figure S1. ^{1}H-NMR spectrum (0 – 14 ppm) of D-(+)-Glucose used for assignment of polar (H) and non-polar (OH) regions within the spectrum for bioaerosol analysis.