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Determination of amino acids in ice samples

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Abstract

This study investigates amino acid concentrations and compositions in ice as a preliminary approach for applying amino acid analysis to ice core study. For the amino acid analysis, 0.425 to 0.750 kg ice samples are used. The ices from Antarctica have 16.8 and 18.3 nmol kg^{-1} of amino acids and these concentrations are close to laboratory blank levels, while the ice from the Chongce Ice Cap in China has 160 nmol kg^{-1} of amino acids. Therefore, the analysis for Antarctic ice is estimated to need more than several kg of ice. In contrast, the analysis from the Chongce Ice Cap should be reducible to only several hundred grams of ice because the concentrations in this cap are higher than those in Antarctica. Further, amino acid sources are present in glacier surroundings of temperate regions, whereas polar regions are far from sources. Serine, aspartic acid and alanine are the major components in the samples. However, the amino acid compositions show variation with each sample.

1. Introduction

Ionic species, such as inorganic ions and light carboxylic acids, in ice cores have provided important information on past climatic and environmental conditions. The chemical species yield data on historical events including biogenic emission, biomass burning, anthropogenic emission and volcanic eruption (*e.g.*, Hammer, 1977; Hammer *et al.*, 1980; Legrand and Angelis, 1996; Olivier *et al.*, 2003). However, there are some other candidates for the source tracers in cases when interpretation is difficult. For example, natural events, such as volcanic eruptions and large forest fires, increase concentrations of chloride, nitrate and sulfate ions in the atmosphere. In addition, anthropogenic effects such as biomass burning and fossil fuel combustion can also increase the atmospheric concentrations of these constituents.

In contrast, amino acids that build proteins apparently originate from and are abundant in living organisms, for example, approximately 50% of the human body's dry weight is protein (McMurry and Castellion, 1999). Moreover, amino acids contained in glacier ices seem to originate mainly from microorganisms, such as snow algae and bacteria, that breed on glacier surfaces in summer season (Kohshima, 1987, 1989; Ling and Seppelt, 1990, 1993; Yoshimura *et al.*, 1997; Takeuchi *et al.*, 2001), plant products, such as pollen, in glacier surroundings (Koerner *et al.*, 1988; Bourgeois, 1990, 2000; Liu *et al.*, 1998; Reese *et al.*, 2003; Nakazawa *et al.*, 2004) and the bacteria in precipitation (Herlihy *et al.*, 1987; Sattler *et al.*, 2001; Mace *et al.*, 2003). Thus, the amino acids may have good environmental information in ice core study. Since these biological activities increase in summer season and decrease in winter, amino acid-rich layers in an ice core may indicate summer layers. In addition, amino acid concentrations in each summer layer may be useful as a good marker of meteorological conditions such as temperature and solar radiation that affect these activities. However, no previous study has reported the amino acids in glacier ice. Therefore, as a preliminary approach to ice core research, this study aims to survey the level of amino acid concentrations in ice from the Antarctic ice sheet and Chongce Ice Cap in central Asia, and to compare their compositions.

Table 1. Detection system for high-performance liquid chromatography (HPLC).

HPLC	Shimadzu LC-10AD
Detector	Fluorescence detector RF-10AXL (350-460 nm)
Integrator	Shimadzu chromatopac GR7Ae plus
Column	GL Science Intersil ODS-3 (150 mm L, 4.6 mm ID, 5 μm particle size)
Guard column	GL Science Intersil ODS-3 (50 mm L, 4.6 mm ID, 5 μm particle size)
Column temperature	40℃
Eluent	E1: 5 mM $Na_2HPO_4 \cdot 12H_2O$/5 mM $NaH_2PO_4 \cdot 2H_2O$ (pH 7.05)
	E2: E1/CH_3CN (2: 1, v/v)
	E3: CH_3CN/Distilled water (HPLC grade) (4: 1, v/v)
Flow-rate	1.0 mL min^{-1}

2. Sampling sites and sample preparation

Ice core sampling was conducted at two distinct sites; Asuka camp in East Antarctica (71°31′S, 24°8′E; 930 m a.s.l.) and Chongce Ice Cap in the West Kunlun Mountains, China (35°14′N, 81°07′E, 6327 m a.s.l.). Ice blocks were taken from the surface of an iceberg off Syowa Station (69°00′S, 39°35′E) in the Antarctic Ocean. One section was collected from each ice core; from the Asuka core, the increment between 100.8 and 101.3 m depth, while from the Chongce core, the increment between 2.8 and 3.0 m depth were used. For the amino acid analysis, 0.750, 0.500 and 0.425 kg ice blocks were used for the Asuka ice, the iceberg ice and the Chongce ice, respectively.

To prevent contamination by laboratory controls, precleaned glasswares, which include filters, were used in all laboratory work. The glasswares were prepared by washing in alkaline detergent, placing in 1N HCl overnight followed by rinsing with organic-free water obtained with a Milli-Q system and then heating in a furnace at 450℃ for 3 to 4 h. Additionally, to eliminate contamination of each sample, about 1 cm of the surface was scraped off with a clean knife in a cold room, and then the sample was melted in a beaker. The first melted ice used to wash the surface of the ice samples was discarded. This procedure was repeated 3 to 5 times, depending on the sample. The melted ice discarded in this way came to 0.145 to 0.110 kg. After the ice was completely melted, the melt water was immediately filtrated through 47 mm GF/F glass fiber filters with a pore size of 0.7 μm.

Subsequently, the samples were separated into two portions, a filter sample and filtrate sample. These 6 sub-samples (for amino acid analysis) were hydrolyzed with 6N HCl at 105 ℃ for 22 h. The HCl supernatant was then transferred to a flask and dried under vacuum. After drying, the dried residue was desalted using cation exchange resin (Dowex 50W-X8), derivatized with *o*-phthalaldehyde and then analyzed by gradient reversed-phase high-performance liquid chromatography (HPLC) with UV fluorescence detection in order to determine the total hydrolyzable amino acid content of the ices.

Table 2. Working gradient conditions used for amino acid determination.

Time (mim)		E1	E2	E3
Program 1	Program 2		(%)	
0.01	0.01	95	5	0
4.0	4.0	85	15	0
8.0	8.0	80	20	0
16.0	16.0	73	27	0
18.0	18.0	70	30	0
25.0	27.0	55	45	0
30.0	32.0	50	50	0
42.0	44.0	35	65	0
42.01	44.01	30	70	0
45.0	47.0	25	75	0
51.0	53.0	20	80	0
51.01	53.01	0	100	0
52.0	54.0	0	0	100
84.0	90.0	0	100	0
84.01	90.01	100	0	0
84.02 (stop)	90.02 (stop)	100	0	0

The program 1 was used for the particulate and dissolved fractions of the blank sample, the particulate fraction of the Asuka sample, and the particulate fraction of the Chongce sample. The program 2 was used for the dissolved fraction of the Asuka sample, the particulate and dissolved fractions of the iceberg sample, and the dissolved fraction of the Chongce sample.

A laboratory procedural blank were also analyzed. For particulate amino acids, a new GF/F glass fiber filter (diameter: 47 mm; pore size: 0.7 μm) was hydrolyzed and the following procedure was the same as that for samples. In contrast, for dissolved amino acids, 4-ml of the organic-free water was concentrated in a flask under vacuum, and passed through the cation exchange resin. The following procedure was the same as mentioned above.

A detection system for HPLC and working gradient conditions used for determination of amino acid are summarized in Table 1 and 2 respectively. Twelve amino acids were measured in this study (Table 3). The yield for the purification of amino acids was 95 to

Table 3. Concentration of amino acids in the ice (nmol kg^{-1}). Abbreviation: Glu (glutamic acid), Ala (alanine), Val (valine), Gly (glycine), Ile (isoleucine), Ser (serine), Thr (threonine), Arg (arginine), Asp (aspartic acid), Met (methionine), Phe (phenylalanine), Tyr (tyrosine).

Sample	Type sample	Glu	Ala	Val	Gly	Ile	Ser	Thr	Arg	Asp	Met	Phe	Tyr	Total
Blank	Particulate metter	1.6	1.3	1.1	0.6	3.9	2.1	1.5	0.5	1.5	1.4	n.i.	0.9	16
	Dissolved matter	0.4	0.5	n.i.	0.2	n.i.	0.4	2.0	n.i.	1.4	0.3	n.i.	n.i.	5.2
Asuka	Particulate metter	1.6	1.6	1.8	0.1	n.i.	3.0	1.2	1.1	0.9	0.5	n.i.	n.i.	12.0
	Dissolved matter	0.1	0.6	0.3	0.2	0.6	1.0	0.8	0.2	0.9	n.i.	n.i.	0.1	4.8
Iceberg	Particulate metter	0.4	1.2	0.3	0.4	1.2	0.8	1.0	n.i.	0.9	n.i.	n.i.	0.1	6.4
	Dissolved matter	1.8	1.1	0.7	0.5	1.4	1.9	1.5	0.2	1.4	1.2	n.i.	0.2	11.9
Chongce	Particulate metter	30	24	15	2.7	7.9	20	17	5.5	20	3.3	2.9	3.0	152
	Dissolved matter	0.5	0.9	1.7	0.4	n.i.	0.9	0.9	0.6	0.9	0.5	n.i.	0.2	7.5

The n.i. is "not identified", meaning that it was impossible to identify the peak of amino acid because amounts were below the detection limit or inseparable from adjacent peaks.

100% (Harada, 1991). The precisions (RSD, n=2) were 13 to 1% except for valine (Val), isoleucine (Ile), methionine (Met), phenylalanine (Phe) and tyrosine (Tyr). The precisions of the five amino acids were not determined.

3. Results and discussion

Total concentrations of particulate and dissolved amino acids in the ice samples from the three sites range between 16.8 to 160 nmol kg^{-1}. Table 3 lists the concentrations of twelve amino acids in the blank and 3 ice samples. The blank values are not subtracted from the sample values because we did not measure amino acid concentrations in the organic-free water used for the blank sample and the blank values were close to the detection limits of 2 to 3 pmol. Therefore, it would be safe here to use the blank values as references.

The total concentrations in the Asuka core and iceberg samples are at similar levels. Moreover, the concentration of each amino acid is at the blank level in both the particulate and dissolved fractions. In the Asuka sample, serine (Ser), Val, glutamic acid (Glu) and alanine (Ala) are the major components in the particulate fraction, and constitute 67% of the total amino acids. Ser, aspartic acid (Asp), Tyr and Ala are the major components in the dissolved fraction, and constitute 69% of the total. In the iceberg sample, Ala, Ile, threonine (Thr) and Asp are the major components in the particulate fraction, and constitute 67% of the total. Ser, Glu, Thr, Ile and Asp are the major components in the dissolved fraction, and are 67% of the total. The Asuka ice section is estimated to have been hundreds of years old based on an average accumulation rate of 0.19 m a^{-1} of ice between 1988 and 1991 (Azuma *et al.*, 1994). In addition, the ice from the iceberg was much older than the Asuka ice because it had once been a part of the Antarctic ice sheet. Amino acids seem to be well preserved in glacier ice because of its low temperature. Therefore the level of amino acid concentrations in Antarctic ice should be generally low.

The Chongce sample shows that Glu, Ala, Ser and Asp are the major components of the particulate fraction and comprise 62% of the total. In contrast, Val, Ala, Ser, Thr and Asp are the major components of the dissolved fraction and are 71% of the total. The total concentration in the particulate in the Chongce ice is much higher than those of the Asuka and the iceberg samples, specifically, 13 times that of the Asuka and 24 times that of the iceberg, even though the dissolved matter in the ices from the three sites have similar and blank levels.

The difference in particulate amino acid concentrations between the Antarctica and Chongce ices seems to be attributable to the difference in the source strength in two regions. The ice samples in lower latitudinal ice caps and glaciers (between about 60°N and 60°S) typically contain higher concentrations of nutrients, such as water-soluble chemical species, than that from the polar ice sheets (*e.g.*, Wagenbach, 1989; Clausen and Langway, 1989; Lyons *et al.*, 1991). The higher concentrations play the more important role in the breeding of snow algae. Moreover, on glacier surfaces in lower latitudes, some surface melting occurs in the summer season, and snow algae grow in the meltwater (Pollock, 1970; Yoshimura *et al.*, 2000). However, the surface of polar ice sheets is virtually lifeless because of the lack of meltwater, even though viable microbial cells and spores are present (Catranis and Starmar, 1991). Thus, the local growth of snow algae may cause the higher concentration of particulate amino acids in Chongce ice compared to that in Antarctic ice.

In addition, the wind-blown-particulate amino acids such as in pollen, may also contribute to the Chongce Ice Cap. Ice cores from lower latitude ice caps and glaciers that are typically within a few tens of kilometers from vegetation sources contain more than 1000 grains L^{-1} of pollen (Ambach *et al.*, 1966; Haeberli *et al.*, 1983; Liu *et al.*, 1998, Reese *et al.*, 2003; Nakazawa

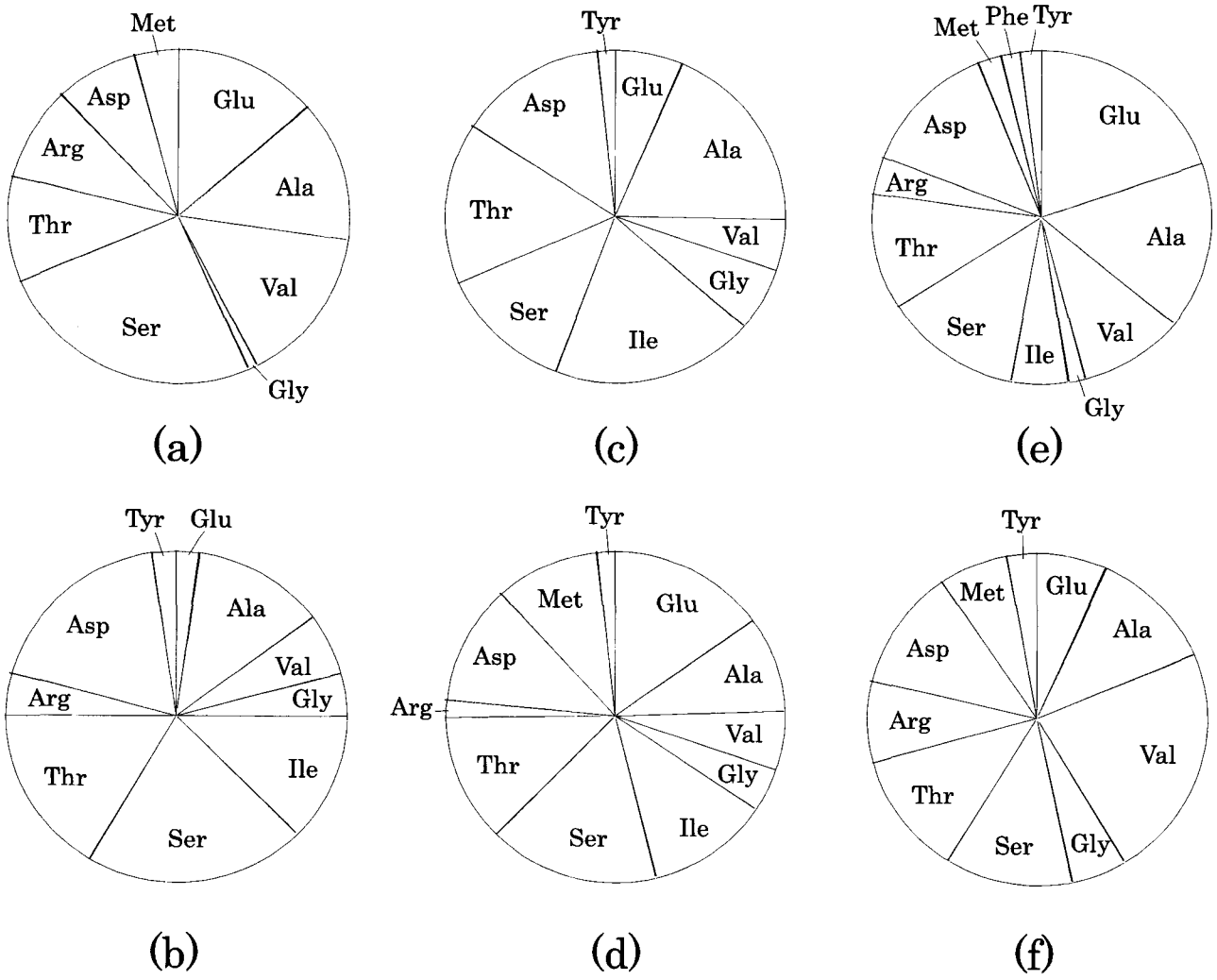

Fig. 1. Amino acid composition in ice samples. (a) Particulate amino acids in the Antarctic Asuka core at a depth of 100.8 to 101.3 m; (b) Dissolved amino acids in the Antarctic Asuka core; (c) Particulate amino acids in the Antarctic iceberg; (d) Dissolved amino acids in the Antarctic iceberg; (e) Particulate amino acids in the Chinese Chongce ice at a depth of 2.8 to 3.0 m; (f) Dissolved amino acids in the Chinese Chongce ice. Abbreviation: Glu (glutamic acid), Ala (alanine), Val (valine), Gly (glycine), Ile (isoleucine), Ser (serine), Thr (threonine), Arg (arginine), Asp (aspartic acid), Met (methionine), Phe (phenylalanine), Tyr (tyrosine).

et al., 2004), whereas the pollen of polar ice sheets mostly originates from sources hundreds to thousands of kilometers away. Thus, the pollen concentration in ice is typically only 10 to 100 grains L^{-1} (McAndrews, 1984; Short and Holdsworth, 1985; Koerner and Bourgeois, 1988; Bourgeois, 1990, 2000). Judging from the above, the ice from lower latitudinal ice caps and glaciers can be expected to contain enough particulate amino acids for analysis. Thus, the sample volume may be reduciable to about several hundred grams considering the laboratory procedural blank if the ice from lower latitude ice caps and glaciers has a concentration similar to that of the Chongce ice sample.

In contrast, the various ice from the polar ice sheets may be difficult to analyze using only several hundred grams of ice samples. This study used the 0.750 and 0.500 kg ice samples for the analysis of Antarctic ices, thereby showing that concentrations of both particulate and dissolved amino acids are at the blank level. This fact seems to be due to few sources of amino acids *in situ* and in the surroundings. It is therefore concluded that more than several kg of ice samples would be needed for analysis in order to be well above the blank level. However, ice core samples are typically limited, and it is therefore essential to secure enough ice volume for ice core study in the polar ice sheets of such as Antarctica.

Ser, Asp and Ala are the major components in 5 out of the 6 sub-samples (Fig. 1). However the amino acid compositions show variation with each sample. The three amino acids were also found to be major compounds in soil organic matter, pollen, marine algae and marine bacteria (*e.g.*, Auclair and Jamieson, 1948; Bieberdorf *et al.*, 1961; Gupta and Reuszer, 1967; Simon and Azam, 1989; Cowie and Hedges, 1992; Cao and Xiang, 1995; Senwo and Tabatabai, 1998), although the amino acid composition differs by type. The exact cause of the differences in amino acid com-

position in the ice is not yet known. In particular, the amino acid composition in the particulate fraction of the Chongce sample may relate to some origin of amino acids. Thus, further research is required to ascertain whether these differences are great enough to have practical significance.

4. Conclusion

This study investigates the concentrations and compositions of amino acids in ice samples from three sites. We conclude that a large ice sample (more than several kg) would be needed for analysis of Antarctic ice, such as the Asuka core and iceberg. Consequently, it is essential to secure enough sample volume for continuous analysis in ice core studies. In contrast, we conclude that the amount of several hundred grams of ice is needed for amino acid analysis of the lower latitudinal ice caps and glaciers, like the Chongce Ice Cap of the West Kunlun Mountains, due to the short distance from amino acid sources, such as snow algae, bacteria and pollen. Therefore, amino acid analysis in ice cores from lower latitude regions seems to be easier than that in those from the polar regions.

On the other hand, the cause of the different amino acid compositions among the samples is an unsettled question. The amino acid composition, especially that in the particulate fraction of the Chongce sample, may relate to the sources of amino acids, and allow us to more easily provide further information to interpret the past climate and environmental conditions. Thus, there is room for further investigation on this point.

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References

Ambach, W., Bortenschlager, S. and Eisner, H. (1966): Pollen-analysis investigation of a 20 m firn pit on the Kesselwandferner (Ötzal Alps). Journal of Glaciology, **6**, 233–236.

Auclair, J.L. and Jamieson, C.A. (1948): A qualitative analysis of amino acids in pollen collected by bees. Science, **108**, 357–358.

Azuma, N., Goto-Azuma, K. and Nakawo, M. (1994): Photogrametric analysis of a 100 m ice core from Asuka Camp, East Antarctica: Preliminary results. Bulletin of Glacier Research, **12**, 69–75.

Bieberdorf, F.W., Gross, A.L. and Weichlein, R. (1961): Free amino acids content of pollen. Annals of Allergy, **19**, 869–876.

Bourgeois, J.C. (1990): Seasonal and annual variation of pollen content in the snow of a Canadian high Arctic ice cap. Boreas, **19**, 313–322.

Bourgeois, J.C. (2000): Seasonal and interannual pollen variability in snow layers of arctic ice caps. Review of Palaeobotany and Palynology, **108**, 17–36.

Cao, S. and Xiang, B. (1995): A comparative study on protein content and amino acid composition of eight species of Unicellular marine feed algae. Acta Oceanologica Sinica, **14**, 141–146.

Catranis, C. and Starmer, W.T. (1991): Microorganisms entrapped in glacial ice. Antarctic Journal of the United States, **26**, 234–236.

Clausen, H.B. and Langway, Jr., C.C.J. (1989): The ionic deposits in polar ice cores. In *The Environmental Record in Glaciers and Ice Sheets*. (H. Oeschger and C.C.J. Langway, Jr. Ed.), pp. 225–247, John Wiley & Sons, New York.

Cowie, G.L. and Hedges, J.I. (1992): Sources and reactivities of amino acids in a coastal marine environment. Limnology and Oceanography, **37**, 703–724.

Gupta, U.C. and Reuszer, H.W. (1967): Effect of plant species on the amino acid content and nitrification of soil organic matter. Soil Science, **106** (6), 395–400.

Haeberli, W., Schotterer, U., Wagenbach, D., Haeberli-Schwitter, H. and Bortenschlager, S. (1983): Accumulation characteristics on a cold, high-alpine firn saddle from a snow-pit study on Colle Gnifetti Monte Rosa, Swiss Alps. Journal of Glaciology, **29**, 260–271.

Hammer, C.U. (1977): Past volcanism revealed by Greenland ice sheet impurities. Nature, **270**, 482–486.

Hammer, C.U., Clausion, H.B. and Dansgaard, W. (1980): Greenland ice sheet evidence of post-glacial volcanism and its climate impact. Nature, **288**, 230–235.

Harada, N. (1991): Study on chronology of marine sediments by the racemization reaction of amino acids in planktonic foraminifera (in Japanese). M.S. thesis, 98 pp., Nagoya University.

Herlihy, J.L., Galloway, J.N. and Mills, A.L. (1987): Bacterial utilization of formic and acetic acid in rain water. Atmospheric Environment, **21** (11), 2397–2402.

Koerner, R.M., Bourgeois, J.C. and Fisher, D.A. (1988): Pollen analysis and discussion of time-scales in Canadian ice cores. Annals of Glaciology, **10**, 85–91.

Kohshima, S. (1987): Formation of dirt layers and surface dust by micro-plant growth in Yala (Dakpatsen) Glacier, Nepal Himalayas. Bulletin of Glacier Research, **5**, 63–68.

Kohshima, S. (1989): Glaciological importance of microorganisms in the surface mud-like materials and dirt layer particles of the Chongce Ice Cap and Gozha Glacier, West Kunlun Mountain, China. Bulletin of Glacier Research, **7**, 59–66.

Legrand, M. and Angelis, M.D. (1996): Light carboxylic acids in Greenland ice: A record of past forest fires and vegetation emissions from the boreal zone. Journal of Geophysical Research, **101** (D2), 4129–4145.

Ling, H.U. and Seppelt, R.D. (1990): Snow algae of the Windmill Islands, continental Antarctica. *Mesotaenium berggreni* (Zygnematales, Chlorophyta) the alga of grey snow. Antarctic Science, **2**, 143–148.

Ling, H.U. and Seppelt, R.D. (1993): Snow algae of the Windmill Islands, continental Antarctica. 2. *Chloromonas rubroleosa* sp. nov. (Volvocales, Chlorophyta), an alga of red snow. European Journal Phycology, **28**, 77–84.

Liu, K.B., Yao, Z. and Thompson, L.G. (1998): A pollen record of Holocene climatic changes from the Dunde ice cap,

Qinghai-Tibetan Plateau. Geology, **26**, 135-138.
Lyons, W.B., Wake, C., Mayeeski, P.A. (1991): Chemistry of snow from high altitude, mid/low latitude glaciers. In *Seasonal Snowpacks, NATO ASI Series, Vol. G28.* (T.D. Davies, M. Tranter and H.G. Jones, Ed.), pp. 359-383, Springer-Verlag, Berlin Heidelberg.
Mace, K.A., Kubilay, N. and Duce, R.A. (2003): Organic nitrogen in rain and aerosol in the eastern Mediterranean atmosphere: An association with atmospheric dust. Journal of Geophysical Research, **108** (D10), 4320, doi: 10.1029/2002JD002997.
McAndrews, J.H. (1984): Pollen analysis of the 1973 ice core from Devon Island Glacier, Canada. Quaternary Research, **22**, 68-76.
McMurry, J. and Castellion, M.E. (1999): Fundamentals of Organic and Biological Chemistry. 2nd Edition. Prentice Hall, Inc., New Jersey, 544 pp.
Nakazawa, F., Fujita, K., Uetake, J., Kohno, M., Fujiki, T., Arkhipov, S. M., Kameda, T., Suzuki, K. and Fujii, Y. (2004): Application of pollen analysis to dating of ice cores from lower latitude glaciers. Journal of Geophysical Research, **109** (F4), F04001, doi: 10.1029/2004JF000125.
Olivier, S., Schwikowski, M., Brütsch, S., Eyrikh, S., Gäggeler, H.W., Lüthi, M., Papina, T., Saurer, M., Schotterer, U., Tobler, L. and Vogel, E. (2003): Glaciochemical investigation of an ice core from Belukha glacier, Siberian Altai. Geophysical Research Letters, **30** (19), 2019, doi: 10.1029/2003GL018290.
Pollock, R. (1970): What colors the mountain snow? Sierra Club Bulletin, **55**, 18-20.
Reese, C.A., Liu, K.B. and Mountain, K.R. (2003): Pollen dispersal and deposition on the Ice Cap of Mt. Parinacota, southwestern Bolivia. Arctic, Antarctic, and Alpine Research, **35** (4), 469-474.
Sattler, B., Puxbaum, H. and Psenner, R. (2001): Bacterial growth in supercooled cloud droplets. Geophysical Research Letters, **28** (2), 239-242.
Senwo, Z.N. and Tabatabai, M.A. (1998): Amino acid composition of soil organic matter. Biology and Fertility of Soils, **26**, 235-242.
Short, S.K. and Holdsworth, G. (1985): Pollen, oxygen isotope content and seasonality in an ice core from the Penny Ice Cap, Baffin Island. Arctic, **38**, 214-218.
Simon, M. and Azam, F. (1989): Protein content and protein synthesis rates of planktonic marine bacteria. Marine Ecology Progress Series, **51**, 201-213.
Takeuchi, N., Kohshima, S. and Seko, K. (2001): Structure, formation, and darkening process of albedo-reducing material (cryoconite) on a Himalayan glacier: a granular algal mat growing on the glacier. Arctic, Antarctic, and Alpine Research, **33** (2), 115-122.
Wagenbach, D. (1989): Environmental records in alpine glaciers. In *The Environmental Record in Glaciers and Ice Sheets.* (H. Oeschger and C.C. Langway, Jr. Ed.), pp. 69-83, John Wiley and Sons, New York.
Yoshimura, Y., Kohshima, S. and Ohtani, S. (1997): Community of snow algae on a Himalayan glacier. Arctic, Antarctic, and Alpine Research, **29**, 128-139.
Yoshimura, Y., Kohshima, S., Takeuchi, N., Seko, K. and Fujita, K. (2000): Himalayan ice core dating with snow algae. Journal of Glaciology, **46** (153), 335-340.