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The detection of organic matter in terrestrial snow and ice: implications for astrobiology

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Abstract: The discovery of icy bodies in our Solar System has opened up the possibility that life may exist on and below their surfaces. Snow and ice are good sites for the preservation of biomarkers as they trap and preserve organic matter that is deposited onto their surfaces. Terrestrial samples of snow and ice collected from Ben Macdui in north east Scotland have been analysed for organic compounds contained within them. A range of *n*-alkanes and *n*-alkanoic acids were found in both samples. The particulate matter contained proportionally more higher weight *n*-alkanols than the melted water. This is because higher molecular weight molecules are less soluble in water. Therefore, the volume of snow and ice to be sampled on other icy bodies is an important factor, as many important biomarkers have high molecular weights and may not be detected in small quantities of melted water.

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Introduction

Snow and ice are potential sources of biomarkers for astrobiological investigation, as they occur on bodies of astrobiological significance, for example Europa and Mars (Bada & McDonald 1995). However, analysing melted water alone may not yield all the available organic content, as many hydrocarbon biomarkers are poorly soluble in water.

On the present Martian surface we cannot sample liquid water, but we could sample ice at relatively shallow depths or at the surface in polar regions. In fact, the polar ice caps of Mars are suggested to be strong candidates for the preservation of a biomolecular record (Bada & McDonald 1995), and biotechnology is being developed for the detection of biomolecules in Martian ice (Hansen *et al*. 2002; Tsapin *et al*. 2003).

There is widespread evidence from terrestrial samples of ice and snow, including ice cap cores that contain organic compounds. Specific studies include the measurement of polycyclic aromatic hydrocarbons (Kawamura *et al*. 1994; Peters *et al*. 1995; Vehvilainen *et al*. 2002), carboxylic acids (Legrand & De Angelis 1996), polychlorinated biphenyl (PCBs) (Gregor *et al*. 1995) and DNA (Willerslev *et al*. 1999). Some input to this can be attributed to anthropogenic emissions (fossil fuels and other industry sources), but contributions are also related to natural wildfires (Legrand & De Angelis 1996), and bacteria incorporated within wind-blown dust particles (Christner *et al*. 2003). In some cases, organisms actually inhabit ice and snow, and hence can contribute to their organic content (Willerslev *et al*. 1999). They include snow algae that confer distinct colouration to their host environment (Thomas & Duval 1995; Williams *et al*. 2003). These records show that ice can be a valuable record of surface chemistry. Where winds are strong, as is very evident on Mars, particulate matter is blown across icy surfaces to which it adheres. This is likely to be the mechanism for incorporating a biosignature within Martian ice (Jones 1991).

The ability to analyse organic compounds in ice will also be of major importance in the investigation of the Jovian moon Europa. Europa has an outer shell of ice at least 10 km thick, and represents bodies in the outer parts of planetary systems where temperatures are extremely low.

Organic compounds recorded in snow and ice

A range of organic compounds have been recorded in terrestrial samples of snow and ice. Particulate organic compounds are scarce in the atmosphere but act as good geochemical tracers. Alkanes, esters, organic acids and polycyclic aromatic hydrocarbons (PAHs), the most widely documented, have been systematically detected in aerosols (Masclet *et al*. 2000). The deposition and concentration of PAHs have been investigated on the Greenland ice sheet (Jaffrezo *et al*. 1994). Other studies have been conducted on the Agassiz Ice Cap where PAHs have provided long-term records of atmospheric deposition (Peters *et al*. 1995). The deposition of semi-volatile organic compounds (SVOC), such as persistent organic pollutants (POPs), have been studied owing to their environmental concern (Herbert *et al*. 2006). POPs can be transported over long distances by bioaccumulation resulting in harmful environmental effects. Investigations have focused on the diffusion of SVOCs into fresh and aged snow (Herbert *et al.* 2006). Ion chromatographic measurements of organic ions, including formate, acetate and methane sulphonate, in Antarctic ice have also been performed (Saigne *et al.* 1987).

Acid precipitation over the Antarctic ice results in the deposition of carboxylic acids, and these compounds have also been studied in remote mountain glaciers (Lee *et al.* 2002). These organic acids are relatively weak but contribute greatly to the organic content of ice in remote areas where small levels of pollution occur. Other substances investigated in terrestrial snow and ice include nutrients and organic nitrogenous compounds. The distributions of such substances have been studied in the marginal ice zone of the Fram Strait (Kattner & Becker 1991), where concentrations of dissolved organic nitrogen (DON) were found to be enriched in the surface ice layer.

Methanogens in glacier ice

Methane is a greenhouse gas that occurs in our atmosphere and that of Mars. Methane trapped in the Greenland ice sheet has been measured from ice cores. The ice cores provide a window into past climatic events and a recent ice core taken from the Greenland ice sheet reveals a high concentration of methane at the base of the core (Skidmore *et al.* 2000). This anomalously high abundance of methane can only be explained by methane-producing bacteria, frozen in the base of the ice sheet (Tung *et al.* 2005). *In situ* metabolism of methanogenic archaea explains the increase in methane at these depths. An investigation into the basal ice beneath a high Arctic glacier revealed microbial life living within the debris-rich layer at the base of the glacier. Aerobic chemoheterotrophs, anaerobic nitrate reducers, sulphate reducers and methanogens were discovered (Skidmore *et al.* 2000). *In situ* microbial production of carbon dioxide and methane plays an important role in carbon cycling during glacial periods and provides a model for a habitat for life on Mars in the basal sediments beneath the north Martian polar ice cap. The possibility of chemosynthetic ecosystems in the Mars subsurface has been examined by Boston *et al.* (1991). A methanogen and sulphur-based microbial ecology is possibly the basis for primary microbial production. Tests on the subsurface would require locating geothermal regions associated with ground ice and sampling the ice for geochemical analysis.

Study site

The Cairngorm Mountains in north east Scotland (see Fig. 1) is the only sub-arctic site in the UK, providing a unique climate, ecology and terrain, with a mixture of continental and oceanic climates. The area receives over 100 days of snowfall per year and has the only perennial snow cover in the UK (Phillips & Parnell 2006). The region covers over 3800 km² and, with a large proportion of highland in excess of 1000 m, the landlocked nature of the area results in maritime conditions at lower altitudes and continental regimes at higher altitudes. A temperature gradient of 2.2 °C/300 m from the summit of Ben Macdui to the base was recorded in April 1957 with the dominant prevailing wind from the south west ranging between 177 and 265 km h^{-1} on the summit plateau (Phillips & Parnell 2006). Our snow and ice sample site is located on the north east shoulder of Ben Macdui, at 1250 m above sea level, in Coire Sputan Dearg.

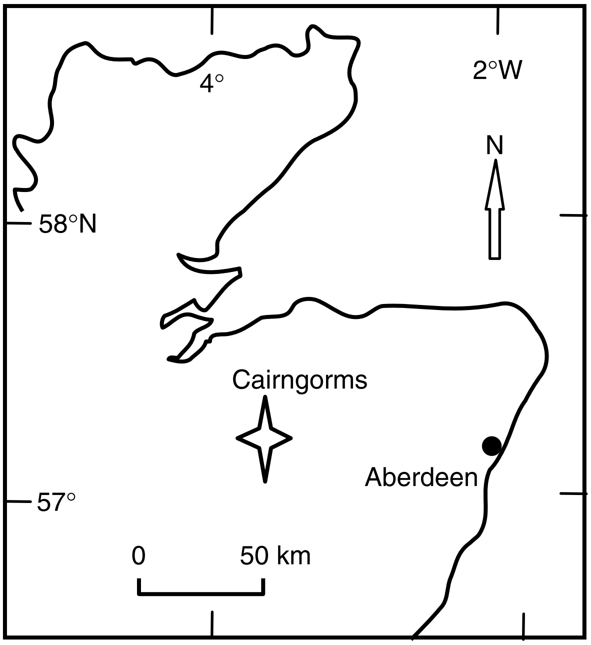

Fig. 1. Location map of the Cairngorm Mountains, north east Scotland.

Methodology

The rationale for the study is to determine whether the organic compounds detected in the snow and ice, and adhered particulate matter within the snow and ice, reflect the initial biological input.

Field sampling

A snowfield surviving from the previous winter was located within a north-east-facing corrie on Ben Macdui. The age of the snowfield is important as it allows for the entrapment of organic compounds from the atmosphere and also for biological input, including insects and vegetation. A snow pit approximately 1 m deep was excavated down to the soil/vegetation base and ice samples were collected at intervals throughout the profile. Distinct ridges of wind-blown particulate matter on the surface of the snowfield were clearly evident (see Fig. 2), forming dense accumulations of material. Multiple layers of wind-blown particulate matter (see Fig. 3) were visible down the profile, indicative of periods of limited snowfall, allowing for the accumulation of particulate matter with cleaner layers representing prolonged periods of snowfall. Four 25 cm × 25 cm × 25 cm cubes were cut from the

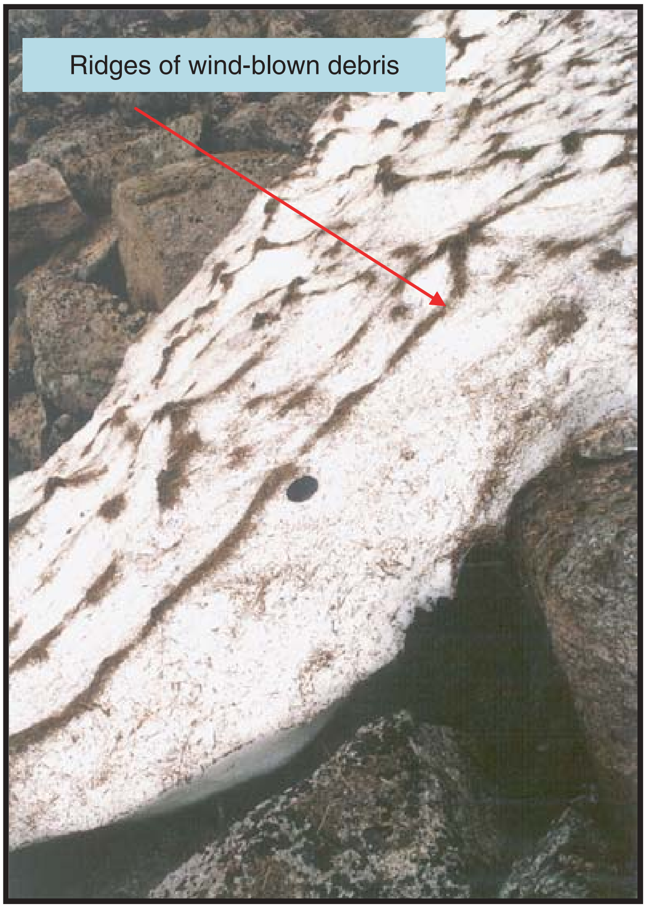

Fig. 2. Snow and ice sample site, Ben Macdui, Cairngorm Mountains. The image illustrates wind-blown ridges of debris accumulated on the surface of the snowfield.

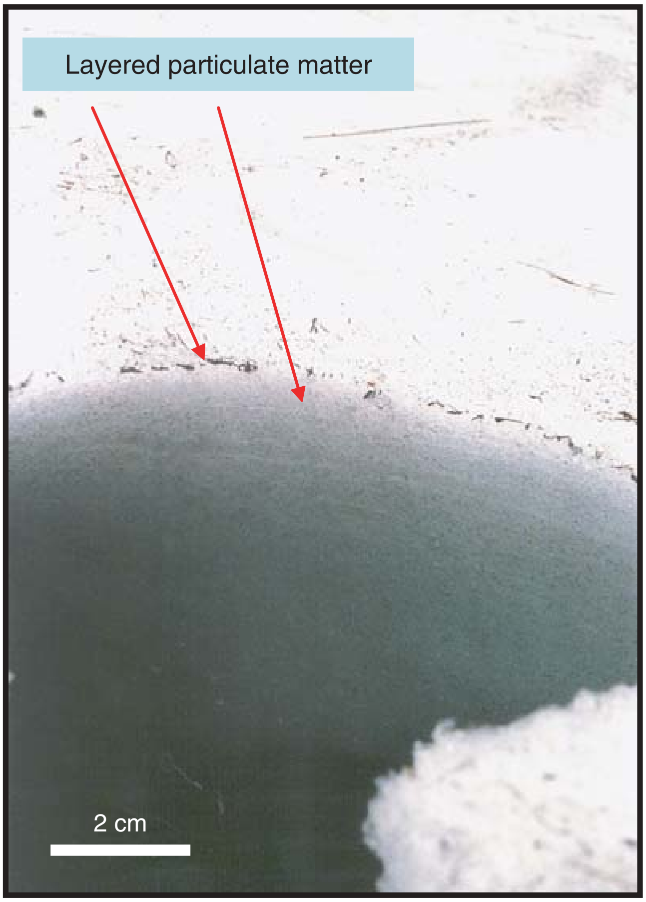

Fig. 3. Layers of particulate matter observed down profile through an excavated snow pit from the snowfield shown in Fig. 2. The layers of particulate matter represent periods of little or no snowfall, allowing for the accumulation of debris. Cleaner snow and ice layers represent periods of prolonged snowfall where debris accumulations have not had time to form.

profile using a sterile blade and wrapped in aluminium foil to avoid contamination. Samples were then insulated and returned to laboratory freezers within 8 h of collection to preserve the initial integrity of the snow.

Laboratory analysis

On return to the laboratory, samples were prepared for analysis in a gas chromatograph-mass spectrometer (GC-MS) by dividing a snow and ice sample cube, from the upper section of the profile, into two glass beakers cleaned with dichloromethane (DCM)/methanol. Small quantities of the samples were removed from the freezer and placed in clean glass beakers, melted at room temperature and reduced in volume by placing them within a fume hood for 48 h. Two different 200 ml volumes of melted water were then taken from each of the two beakers and extracted by five-stage liquid/liquid organic solvent extraction. The first extraction was performed with a 1:1 (v/v) mix of DCM and methanol and latter stages used gradually less methanol. Particulate matter contained within the snow and ice samples was filtered from the two melted water samples on pre-extracted filter paper and then soxhlet-extracted with 97:3 DCM/methanol for 48 h. Using flash chromatography, extracts were separated into apolar and polar fractions by elution with 1:3 (v/v) DCM/hexane (for the apolar fraction) and 1:2 (v/v) DCM/methanol (for the polar fraction). Polar and apolar fractions were then analysed by a GC-MS. GC-MS analysis was performed on an Agilent Technologies 6890 GC fitted with an Agilent HP-5MS (5% phenyl methyl siloxane; 0.25 μm film thickness; 25 mm id; 30 m length) and connected to an Agilent Technologies 5975 MS operating in Scan mode. The oven heating program involved heating from 80 to 290 °C at 4 °C min^{-1} and then holding for 30 min. Sample injection was through a split/splitless injector held at 300 °C and operating in splitless mode.

Results and discussion

Table 1 shows the mass of extracted samples from the particulate matter and melted snow and ice, and the proportion of extract separated into polar and hydrocarbon fractions. The percentage of particulate matter by mass filtered from the two samples is different. The larger percentage of particulate

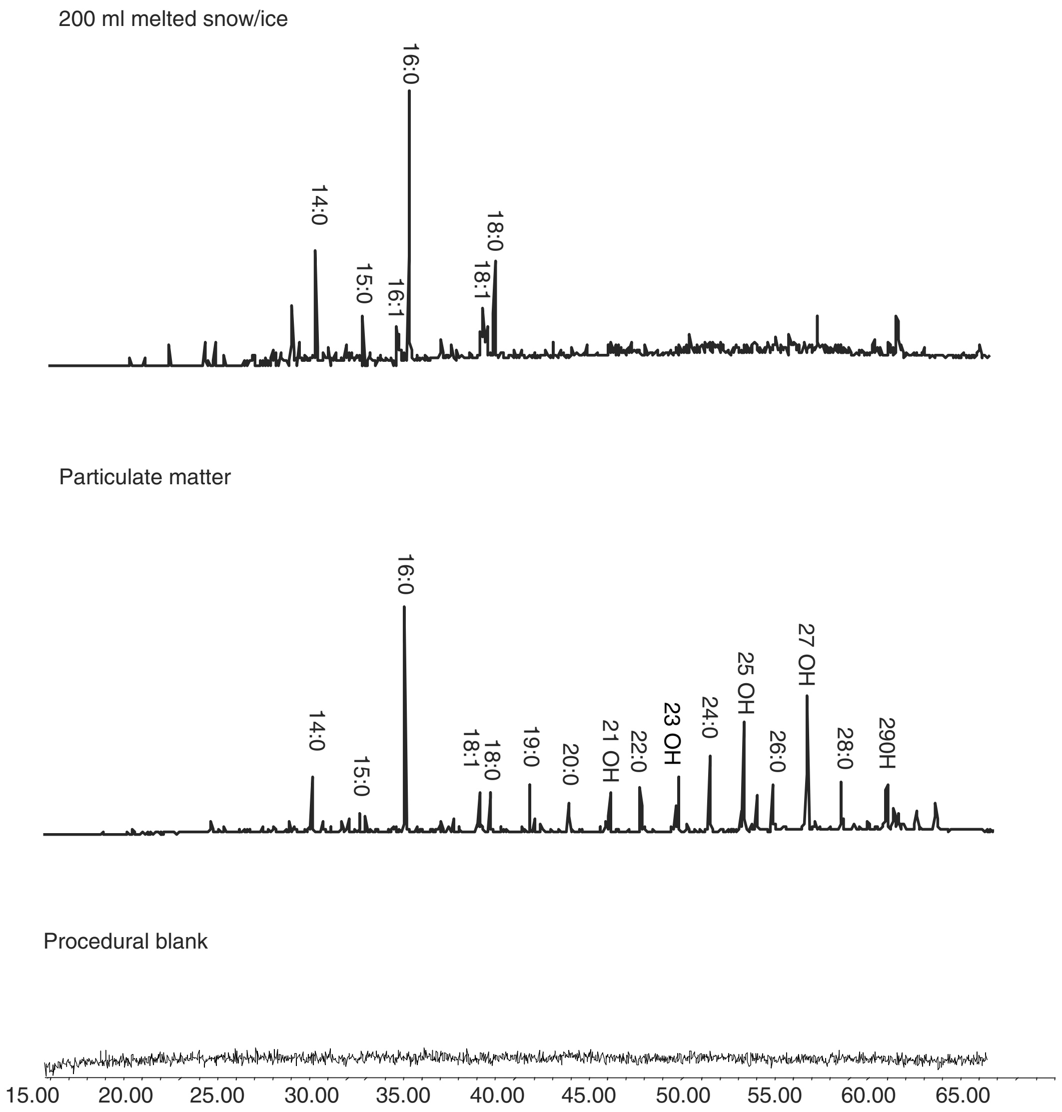

Fig. 4. GC-MS traces of the summed m/z 17+117 ion chromatograms analysed within the melted snow and ice and particulate matter samples. 16:0 = C_{16} saturated alkanoic acid, 16:1 = C_{16} monounsaturated n-alkenoic acid, 21OH = C_{21} unsaturated alkanol.

Table 1. *Mass (mg g^{-1}) of initial sample extracts and polar and hydrocarbon separated fractions for two melted water and two particulate matter samples*

Sample	Sample extract (mg g^{-1})	Hydro-carbon (mg g^{-1})	Polar (mg g^{-1})	Percentage of particulate matter by mass
Melted water 1	5.5	1	3	–
Melted water 2	4.0	0.5	3	–
Particulate matter 1	31	3	22	15.7
Particulate matter 2	19	2	15	12.3

matter results in a larger mass of sample extract. A larger extract mass was recovered from the two particulate matter samples compared with that obtained from the two melted snow and ice water samples. The extracts are polar rich with proportionally more polar than hydrocarbon material fractionated from both melted snow and ice water and particulate matter sample extracts.

The GC-MS traces of the summed m/z 17+117 ion chromatograms for the melted snow and ice and particulate matter (see Fig. 4) are clearly different. A series of saturated straight chain fatty acids are present, containing a range of saturated alkanoic acids, monounsaturated *n*-alkenoic acids and unsaturated alkanols. The melted water gives a biological/microbial signature (Harwood & Russell 1984). There is no evidence of wind-blown contamination onto the snow surface from anthropogenic emissions. This is contrary to previous studies in the area where heavily contaminated black snowfalls associated with back-trajectories originating in eastern Europe have been recorded (Davies *et al.* 1992).

The GC-MS trace of the summed m/z 17+117 ion chromatograms analysed within 200 ml of melted snow and ice contains low carbon numbers ranging up to C_{18}. The particulate matter mass chromatogram trace contains higher

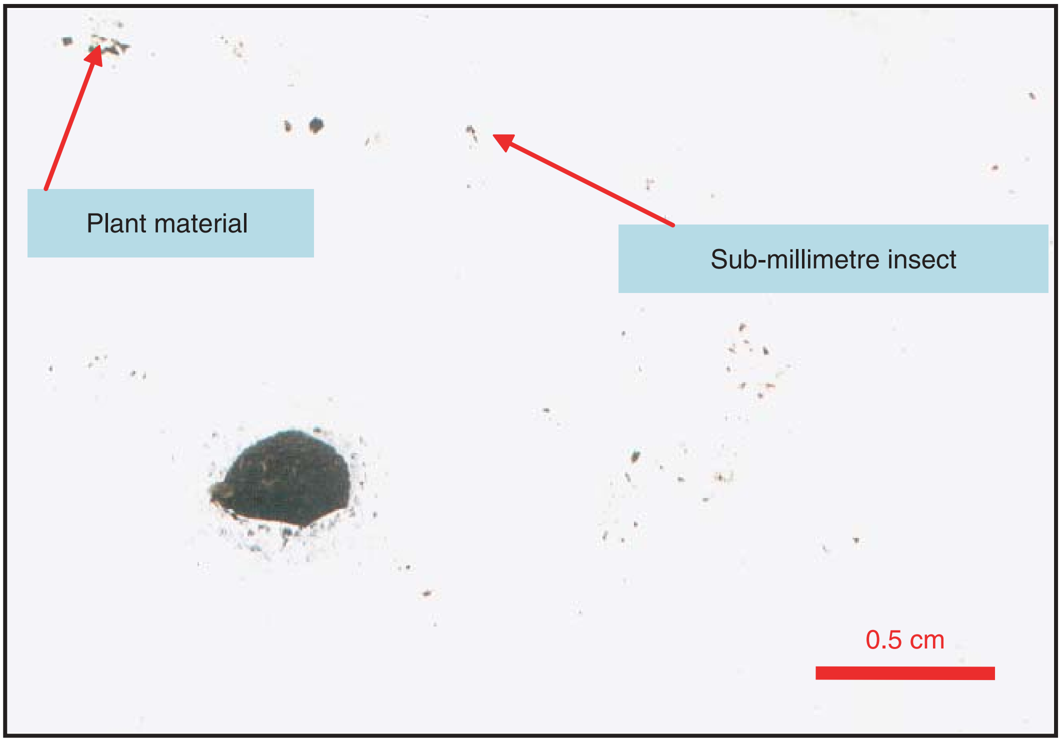

Fig. 5. Detailed observation of the snow and ice surface and near surface. Plant-derived material and a small sub-millimetre-sized insect are present.

carbon numbers up to C_{29}. The particulate matter contains proportionally more higher molecular weight *n*-alkanols than the melted snow and ice water. There are two explanations for the marked differences between the low carbon numbers in the melted snow and ice and the higher carbon numbers in the particulate matter. First, the melted snow/ice sample has a biological/microbial signature (Harwood & Russell 1984). The signature may be a consequence of the higher molecular weight *n*-alkanols occurring in the particulate matter being poorly soluble in water and subsequently not being detected in snow and ice melt water. The solubility of C_{20} *n*-alkanol is 10% that of the solubility of the C_{16} *n*-alkanol. This would account for the absence of higher carbon number compounds in the melted water.

This study focused on a water ice source. Mars has a northern permanent water ice cap and southern carbon dioxide ice cap (Hansen *et al.* 2005), with both exhibiting a seasonal carbon dioxide ice cap. Carbon dioxide ice requires much colder temperatures (–125 °C) and the solubility factor would vary considerably. This study is only relevant to water ice deposits and therefore solubility is only applicable to icy bodies with a similar snow and ice chemistry to that of the Earth.

Close observation of the snow and ice surface and near surface revealed plant-derived material and a small sub-millimetre-sized insect (see Fig. 5). The plant-derived material was identified as a combination of heather and grasses originating adjacent to the snowfield. The sub-millimetre insect was located 0.2 cm below the snow and ice surface and was identified as a small snow fly. The fly may have fallen during migration above the snowfield and become trapped on the surface. Alternatively the fly may have implemented a strategy to survive until the snowfield melted in late summer. The fly may have used freeze tolerance, whereby a significant volume of water is removed from cells in the insect, ice then forms around the outside of the cell and remains until the late summer thaw when the fly is then revived. This variety of inputs into the snow and ice, post-deposition, contributes to its organic content.

Implications for astrobiology

The saturated straight chain fatty acids in the particulate matter, between C_{20} and C_{29}, are indicative of higher plant detritus, possibly originating from highland grasses surrounding the snowfield. Although higher plants are not relevant to astrobiology, identification of the particulate matter under a low-magnification microscope revealed that a small proportion of the material was wind-derived heather from a lower altitude. This indicates that some of the biological input originated from a distant source approximately 0.5 km away. This is relevant for sampling snow and ice on planetary surfaces as any biological input may have originated away from the local sample area. It is a good analogue for Mars as wind is thought to be the driving mechanism behind the putative incorporation of organic compounds into Martian ice (Jones 1991).

The snowfield investigated provides an extreme niche for bacteria to survive. Large populations of bacteria are present in surface snow and firn at the South Pole (Carpenter *et al.*

2000), with evidence indicating that the bacteria can metabolize at temperatures as low as −17 °C. Snow and ice would therefore make a good target for sampling on other icy bodies.

Future mission sampling campaigns on other snow and ice-bearing bodies in the Solar System should consider sample volumes as an important factor. Small volumes of snow and ice will not yield the full range of organic compounds contained within the snow and ice at detectable levels, as higher molecular weight compounds only occur in small quantities. Therefore, larger volume samples would need to be acquired in order to concentrate the organic compounds sufficiently to enable detection.

Conclusions

Snow and ice fields are good places to search for biological signatures on other bodies in our Solar System owing to their ability to trap, store and preserve organic matter deposited into them. Snow and ice samples collected from Ben Macdui in the Cairngorm Mountains have been analysed for organic compounds extracted from snow, ice and trapped particulate matter. Extracts of the melted snow were found to be different to the extracts of particulate organic matter trapped within the snow. Extracts of the melted snow contained a range of *n*-alkanols up to C_{18} and extracts of the particulate organic matter trapped in the snow contained *n*-alkanols up to C_{29}. A limited range of soluble compounds is present in the melted water, in comparison with the particulate matter which contains larger amounts of less soluble organic compounds. These considerations should influence sample analysis on other planetary bodies. Many hydrocarbon biomarkers have high molecular weights and because they are very poorly soluble in water they will not be detected in melted snow and ice alone.

Future research

Future research will include the development of long-term snow sampling assays. This would provide a measurement of accumulated organic matter over a set period of time and would allow for the quantification of the extent of organic deposition during a single winter season. This data could be used to assess the importance of wind-blown deposition of organic compounds and provide estimations of the potential extent and concentration of wind-blown organic matter stored within the Martian ice caps.

The NASA Phoenix Lander will be launched in August 2007 and land in May 2008 near the Martian northern polar ice region between 65 and 75°N. This region is a mixture of rock and ice and the timing of the landing coincides with the northern winter (DiGregorio 2005). Airborne organics are expected to be incorporated into the snow and ice layers, with deep accumulations over an extensive period of time. The lander will provide an insight into the near-surface chemistry and could potentially make a record of organic compounds that were once abundant on the planet.

There is currently no evidence available for microbes living in glacial ice without initially melting it before analysis. Methods are being developed to study microbes *in situ* in solid ice. Two methods are currently being refined. First, a biospectrologging tool is being developed that can be lowered into a fluid-filled borehole in glacial ice (Price 2001). This can take measurements using fluorescence to establish the three microbe states: viable, dead or dormant. Second, epifluorescence microscopy is being developed for use in liquid veins in solid ice to establish the three microbe states (Price 2001). Proving that microbes can live under extremes of temperatures as low as −50 °C in glacial ice could provide an analogue for microbial life in diapirs in Europan ice.

Other techniques being developed include sampling systems for the long-term monitoring of PAH wet deposition (Cereceda-Balic *et al.* 2002). The assay is being developed to sample wet deposition, including snow. The system will include a filtration and concentration stage of the trace organic compounds. PAHs can be charged into, and transported through, the atmosphere as both gases and aerosols. This system will provide an *in situ* assessment of the influence of atmospheric organic pollutants in remote regions and assess the effectiveness of wet deposition of such organic compounds.

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