Greenpeace Research Laboratories Analytical Results Report 2025-02

Microplastic fibres and fragments in nearshore surface waters of glacial lakes in Austria in August 2024

David Santillo, Stefan Stadler & Clare Henry, February 2025, 16 pp.

Introduction

A total of 11 (approximately 2.9 litre) composite samples of surface water were initially collected from the shores of a number of glacial lakes in Austria (in the states of Tirol, Kärnten, Salzburg and Oberösterreich) during August 2024. One sample was subsequently lost in the field due to bottle breakage when returning from the sample location. The remaining 10 samples were returned to the Greenpeace Research Laboratories at the University of Exeter (UK) for filtration and analysis using Fourier-Transform - Infrared (FT-IR) microscopy to determine the presence and abundance of microplastics (fibres and fragments) in the samples at the time of collection. Unfortunately a further 3 samples were lost due to bottle damage in transit, resulting in the end in the analysis of just 7 of the original 11 samples.

Details of the samples analysed, including the location, date and time (where recorded) of collection and a description of the surroundings, are provided in Table 1 below. A map showing the approximate locations of the lakes sampled is provided in Figure 1.

Materials and methods

Each sample was collected as a composite of 5 separate fills of a 500ml (nominal volume) bottle, held at the end of a stainless steel sampling pole. The bottle was rinsed three times in the lake water before collecting the first portion. The 5 portions were combined into a single composite sample per site in an amber glass Winchester bottle of approximately 2.9 litre volume (which had been precleaned in our laboratory by rinsing 3 times with 200ml deionised water prefiltered through a 5µm silver filter). In each case, the 5 separate portions were collected within a radius of about 10m from each other and at a depth of between 10-20 cm. The outer surfaces of the Winchester bottles were rinsed with lake water on arrival at each site before removing the lid to collect or transfer the sample, to prevent ingress of dust from the outer surfaces of the bottles. The sampling equipment used in this investigation is illustrated in use (during a previous study) in Figure 2, while Figure 3 shows one of the samples being collected during the current study.

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Sample code	Date/Time	Location	Surroundings	Visible plastic-waste
1	13.8.2024 8:30	Schlatenkees Glaciertongue Lake Osttirol *47.11474, 12.40487*	Directly beneath the glacier tongue is a basin full of glacier water. There starts the river and all the water that comes down from the glacier collects there. Ice and much fine sandy/silty sediment on the shore. People visit it (approximately 10 people within the 2 hours of sampling). It's not an official hiking path, but it's possible to go as an experienced hiker.	Nothing observed but it's a really fast changing environment and debris would be "washed" away. The hiking paths were also quite clean.
2	13.8.2024 11:30	"Auge Gottes" Schlatenkees Moräne Osttirol 47.11488, 12.42514	Directly next to a highly frequented walking path lies the little glacier lake "Auge Gottes", in the moraine of Schlatenkees glacier. Benches are located around the lake. There are fish, many insects (incl. dragonflies) around the place	On the shore or in the water no visible waste was spotted. But on the benches next to the lake (approximate 10 metres away) there was a plastic clip of a plastic bread bag.
3	14.8.2024 13:00	Großglockner "Pasterze" close to Glacier tongue (next to incoming river) 47.08369, 12.72977	Next to the unofficial path where the glacier ice meets the water. It was the closest point we could get. Many tourists around. We sampled approximately 50 metres away from a group of around 50 people	No visible plastic was detected
4	15.8.2024 14:30	Großglockner "Pasterze" close to the middle of the glacier lake 47.07471, 12.74465	Sampling spot was a little half island. The hiking path was approximately 25 metres away. Stony ground. Not many plants. Some insects were around.	Found a worker's glove (with a rubber surface) and a cigarette butt
8	16.8.2024	Kitzsteinhorn Salzburg, Glacier lake Schmiedinger See I	Near a "hiking" path, not much frequented. Stony desert. Next to a ski-slope and a chairlift station (runs only in winter). Some insects (flies and bees) were around. Not many plants	On the shore: some fleece and a small piece of coloured wood
10	22.8.2024 13:55	"Kleiner Eissee" (North shore) beneath Dachstein and Hallstätter Glacier 47.49813, 13.63876	Only reached by with climbing down, no official path down there. Hiking path is around 100 metres away and 30m higher up. No direct influence of people. Some insects, including water beetles diving and swimming there	Old metal cans: one in the water and one on the shore Full of rust (seemed to be really old)
11	22.8.2024 14:15	"Kleiner Eissee" (South shore) beneath Dachstein and Hallstätter Glacier *47.49731, 13.63799*	As above	No visible plastic was detected

Table 1: details of samples received and analysed at the Greenpeace Research Laboratories, along with descriptions of the locations and surrounding

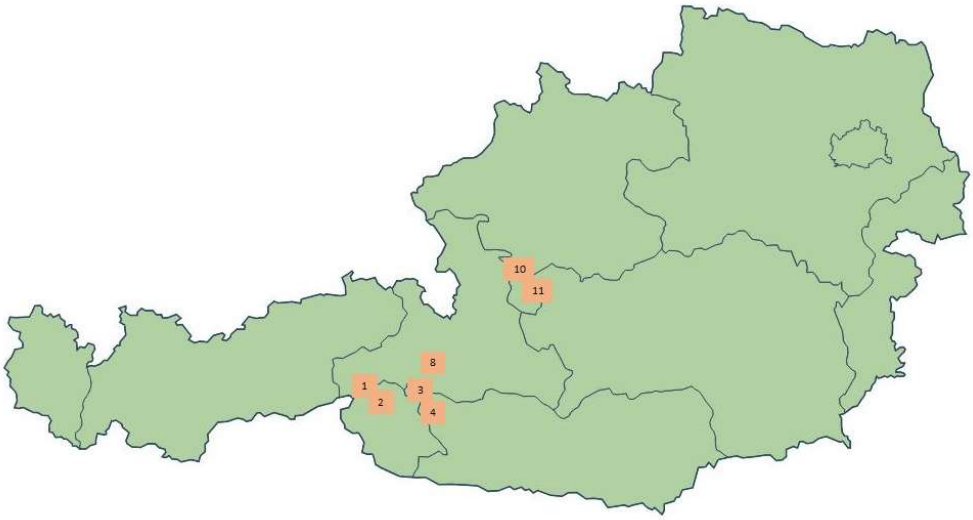

Figure 1: map showing the approximate locations of the 7 samples analysed in this study

On return to our laboratory, samples were filtered through fresh 5µm silver filters (pre-verified as being free from fibres or fragments by inspection under a dissecting binocular light microscope immediately before use), using glassware that had been rinsed three times with 5µm filtered deionised water immediately before each use and which was used wet to avoid adherence of dust particles that can occur during glassware drying.

Filtration took place in the confines of a fume cabinet, pre-cleaned with filtered deionised water and ethanol and with the air flow turned off throughout, located in an analytical laboratory that receives filtered outside air. For additional protection of the samples during filtration, the filter funnel was kept covered with fresh aluminium foil as soon as the sample was introduced. Nitrile gloves were not worn during the filtration of samples (or the subsequent inspection of filters to mark the locations of all fibres and fragments for potential analysis) as previous investigations in our laboratory have shown that these can attract microfibers through build-up of static charge during their use and transfer those microfibres to the glassware more readily than when working with clean, ungloved hands. Hands were washed thoroughly between samples to ensure safety.

Because of the natural turbidity of a number of the samples, it was necessary to use more than one silver filter in order to complete the filtration of the entire volume of sample; 2 filters were needed in the case of sample 3, 3 filters each for samples 10 & 11 and 4 filters each for samples 1, 2 and 4. Only for sample 8 was it possible to filter the entire volume onto a single silver filter.

Figure 2: sampling equipment identical to that used in the current investigation (photographed during an earlier study in Austria in 2023), showing sample pole, 500ml sampling bottle and 2.9 litre composite sample container (Winchester)

Figure 3: collecting a composite surface water sample beneath the Schlatenkees glacier 'tongue' (Lake Osttirol) during the current investigation, illustrating the measures taken to avoid contamination of the sample during collection (incl. use of long steel sampling pole to hold bottle well away from sample taker, minimal personnel accessing the lake shore itself, washing the outer surfaces of the sample bottles with lake water before opening them and collecting the composite sample).

Cotton lab-coats were worn throughout glassware preparation and sample handling in order to minimse the deposition of fibres from clothing, and work areas on benches and microscopes were cleaned with ethanol and lint-free tissues immediately before each procedure.

Two laboratory blanks were prepared (each a 2.9 litre sample of deionised water pre-filtered through a 5µm silver filter and collected in a pre-cleaned Winchester) and subsequently re-filtered using identical protocols to those used for the samples in order to control for potential contamination through fibre or fragment deposition during the sample handling and filtration process.

Immediately after filtering each sample, the silver filters were transferred to clean glass petri dishes (verified under the light microscope as free from visible fibres and particles, on both inside and outside surfaces). Filters were then inspected themselves under the light microscope, at both a low and high magnification, and the positions of all fibres and fragments that could not immediately be recognised and discounted as being natural materials (e.g. phytoplankton, zooplankton, inorganic mineral particles) were marked by scratching a line into the surface of the filter with a sharp needle. This enabled consistent counting of fibres and fragments as candidates to be identified subsequently by FT-IR microscopy, as well as making it easier to locate those materials using the FT-IR microscope camera in order to record the infrared reflectance spectra. Just as importantly, marking the filters in

this way immediately after filtration acts as an additional control against later surface contamination of the filters by materials deposited from laboratory air during filter analysis, since any fibre or fragment that is not associated with a scratch mark can immediately be discounted from further analysis. In practice, this was limited to two fibres that were subsequently deposited on to different filters during the period of FT-IR analysis of all 7 samples.

Individual candidate materials (fibres and fragments) retained on each of the silver filters were photographed under a light microscope and subsequently examined using a PerkinElmer Spotlight 200 FT-IR Microscope System (MCT detector, KBr window) operating in transmittance mode across a wavenumber range from 4000 to 750 cm^{-1} and with a resolution of 4 cm^{-1}. Initially the intention was to analyse the fragments and fibres in reflectance mode without the need to remove them from the surface of the silver filters. In this case, however, instrumental difficulties made this impossible, such that it was necessary instead to conduct the analyses in transmittance mode following manual transfer of each fibre or fragment in turn from the filter surface to a diamond compression cell using fine forceps. The diamond compression cells were cleaned thoroughly with ethanol to remove any residues between samples, and the surfaces of both upper and lower diamond window were verified as being clean through inspection under a light microscope at high magnification before loading the next sample.

Although this method has the disadvantage of being more time consuming and has a higher risk of loss of individual fibres or fragments during manual transfer, the spectra obtained through transmittance FT-IR using a diamond compression cell are generally of a higher energy and quality than those obtained through the use of surface reflectance. This is because (1) the entire section of the fibre or fragment analysed is constrained within a single focal plane within the compression cell, (2) surface scattering of the signal is minimized, (3) spectral interference from surface contamination of the fibre or fragment with other materials, such as biofouling, is also minimized and (4) it is easier to separate the fibres and fragments more completely from other material in the sample, such as sediment or fragments of naturally-occurring organic material, and therefore to obtain a cleaner background spectrum against which the sample spectrum can be compared. In practice, only a small fraction of fibres and fragments could not be transferred effectively from the surface of the filters into the diamond compression cells for analysis, but this was because they broke up in the surface of the filter when handled (and were therefore unlikely to have been synthetic polymers) and not because they were lost in the process of transferring them.

A total of 16 scans were collected for each candidate fibre or fragment. The infrared spectra were acquired, processed and analysed using PerkinElmer Spectrum software (version 10.5.4.738), with polymers being identified by automated matching combined with expert judgement against commercially available spectral libraries (including polymers and additives) and an additional custom spectral library prepared in our laboratory using a range of polymer standards and potential contaminating materials (e.g. tissues, gloves, laboratory coats). Only match qualities greater than 70%, and which could then be cross-checked by the analyst to verify the quality and reliability of the match were accepted as having been positively identified. As noted above, any fibres or fragments appearing on the filters other than in those positions marked immediately after sample filtration (2 fibres in total) were excluded from analysis at the outset.

Fibres or fragments yielding lower match qualities, or for which the analyst rejected the initial >70% identification on visual inspection of the spectral match, were recorded as “unidentified”.

Figure 4 illustrates some of the equipment used for sample processing and analysis, including the filtration of a lake water sample onto a silver filter using glassware freshly rinsed with filtered deionised water, preparing to mark up the position of candidate fibres and fragments on the filters with a sharp needle immediately after filtration, the PerkinElmer Spotlight 200 FT-IR microscopy system used for the identification of all fibres and fragments and a diamond compression cell used to hold each fibre and fragment when conducting the transmittance FT-IR analysis.

Results

A total of 195 fibres and fragments, of maximum dimension 5mm and minimum dimension approximately 20µm (for fragments) or 10µm (for fibres), were detected on filters collected across all 7 samples (see Table 2). Of these, 158 were subjected to FT-IR microscopy in transmittance mode. The identities of those fibres and fragments are summarised in Table 3.

FT-IR analysis confirmed that 71 of the 158 fibres and fragments analysed (across all samples) were of “natural” origin, the vast majority of those (66) being comprised of cellulose. These were transparent, white or pale yellow/brown in appearance, often of irregular cross-sectional diameter, sometimes flattened, often with a relatively rough surface and signs of separation (‘fraying’) of fibre components towards their ends. Some were identified also has having a lignin component, suggesting that they may have been fibres derived from woody plants. In addition to the FT-IR spectral identification, these fibres had a rather irregular appearance under the microscope, being of uneven cross section along their length and often with a rough surface. Although prior treatment of the samples with hydrogen peroxide or enzymes, before or after filtration, would have helped break down and remove these materials from the samples, such treatments may also break down some of the cellulose-based synthetic fibres that were present in the samples (see below) and which because of their colour and morphology could not have arisen from natural sources.

A total of 52 fibres or fragments combined were confirmed as synthetic materials, characterised through high quality matches for their FT-IR spectra, often combined with very uniform diameters, smooth surfaces (e.g. along the entire length of the fibres) indicative of industrial processing, including spinning or extrusion, and being either uniformly transparent in cross section or brightly coloured (including red, white, blue, black or green).

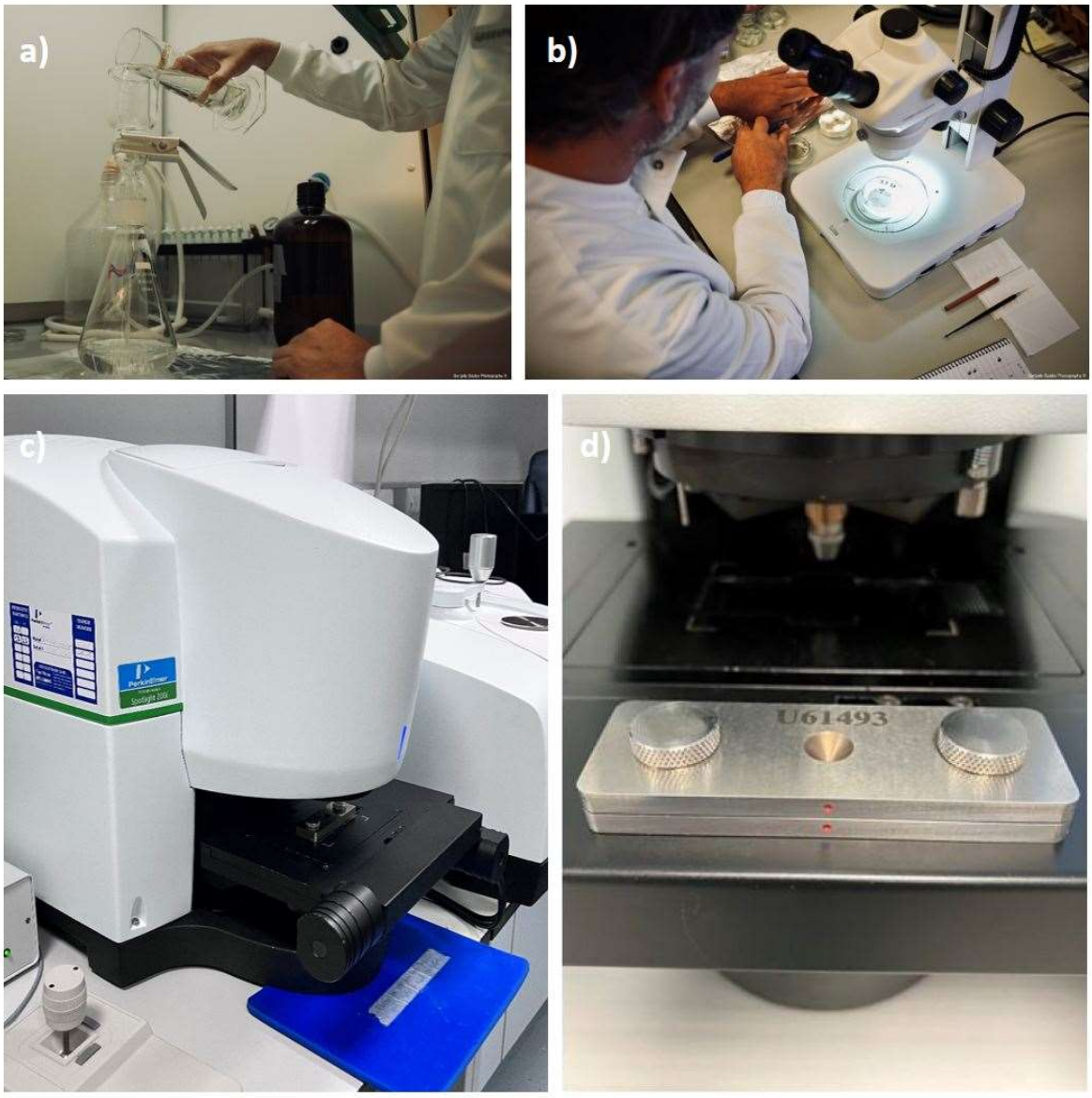

Figure 4: a) filtration of lake water samples in dedicated pre-cleaned fume cabinet with air flow off; funnel was immediately recovered with foil once each volume of sample was introduced, b) preparing to mark the filters with a sharp needle under the dissecting binocular light microscope, to indicate the positions of candidate materials for FT-IR analysis immediately after filtration, c) the PerkinElmer Spotlight 200 FT-IR microscopy system used for FT-IR analysis and d) a diamond compression cell used to isolate and compress each candidate fibre and fragment for transmittance FT-IR microscopy.

Also included in this total were a single black fragment of polyester urethane (polyurethane), one of epoxy resin (black or very dark blue), a fragment of polyethylene (green) and a small section of PTFE film (transparent). Two fragments (one white and one blue) were more tentatively identified as either chlorosulfonated polyethylene (used in synthetic rubbers) or weathered/degraded polyethylene and one black fragment as chlorinated polyethylene (used in some flexible cable coatings, for example). A conspicuous bright orange fragment was tentatively identified as ethyl polysilicate, a material used as

an additive in specialist weatherproof paints and coatings, while a small blue fragment with a gel-like consistency was tentatively identified as polyacrylamide. These identities are summarised in Table 3.

In the case of 35 fibres or fragments, reliable identification was not possible as the spectral match quality was too low. In all cases, these were nonetheless confirmed as being synthetic rather than natural on the basis of their uniform diameter, smooth surface, strong colouration and/or uniform translucency.

Some examples of the variety of synthetic fibres identified, photographed using the camera on the FT-IR microscope and indicating the scale in millimetres (mm) and the identifications determined from their infrared spectra, are shown in Figure 5.

Given that the complete volume of sample was filtered in each case, it is possible to present the data as abundance of fibres and fragments per litre of water (see Table 2). Of the 7 samples analysed, samples 1 (Schlatenkees Glaciertongue), 4 (Großglockner Pasterze) and 11 (Dachstein Kleiner Eissee) contained the highest combined abundances of synthetic fibres and fragments (5.6, 3.8 and 4.8 synthetic fibres or fragments per litre respectively). It must be noted, however, that in each case, the samples represent only a snapshot in time at the particular location sampled and cannot be considered to be more widely representative of levels of contamination in the water bodies as a whole.

This is clearly illustrated, for example, by the differences in type and number of synthetic fibres and fragments identified in samples 10 and 11, collected from the North and South shore respectively of the Kleiner Eissee (Dachstein). Sample 10 yielded the lowest abundance of synthetic fibres or fragments of any of the seven samples analysed in this study, while sample 11 gave the second highest abundance overall, possibly reflecting the effects of wind and/or water motion on the distribution of buoyant microplastics near the water surface within a single water body. Therefore, while the abundance per litre can be a useful indicator of the range of contamination levels across the sample set as a whole, care must be taken to avoid over-interpretation of comparisons between the different lakes sampled on this basis. As microplastics are discrete physical pollutants, in contrast to dissolved chemical pollutants, their distribution within a water body is inevitably likely to be patchy. The collection of larger sample volumes as composites of a number of smaller (500ml) subsamples drawn sequentially at each site in the current study can help to reduce the effect of patchy distribution, but cannot eliminate it as it is a characteristic of the distribution of the microplastics themselves.

Sample code	Sample location	Volume filtered [mL]	Total fibres & fragments	Analysed fibres & fragments	Total synthetic fibres & fragments per litre	Micro-plastic fibres or fragments per litre	Natural origin		Synthetic (anthropogenic) fibres and fragments		Others or unidentified fibres & fragments
							fibres & fragments of natural origin	natural cellulose fibres or fragments	synthetic cellulose fibres & fragments	microplastic fibres & fragments	
1	Schlatenkees Glaciertongue	2.860	52	46	5,6	2,8	0	23	8	8	7
2	Auge Gottes (Schlatenkees)	2.900	14	6	1,0	0,0	0	1	3	0	2
3	Großglockner Pasterze I	2.900	25	13	1,0	0,3	0	5	2	1	5
4	Großglockner Pasterze II	2.870	38	37	3,8	1,7	0	13	6	5	13
8	Kitzsteinhorn	2.900	26	17	1,4	1,0	5	8	1	3	0
10	Dachstein: Kleiner Eissee I	2.900	15	14	0,3	0,0	0	12	1	0	1
11	Dachstein: Kleiner Eissee II	2.900	25	25	4,8	2,4	0	4	7	7	7

Table 2: overview of total numbers of fibres and fragments (both natural and synthetic) found in the 7 samples, along with their nominal abundance per litre

Sample code	Sample location	Cellulosic synthetic fibres	Non-cellulosic synthetic fibres	Synthetic fragments
1	Schlatenkees Glaciertongue	6 x blue, 1 x black, 1 x red	Polyester – 2 x transparent, 1 x white Modacrylic – 1 x blue, 1 x black Polyamide – 1 x blue	Chlorinated polyethylene (tentative i.d.) – 1 x black Chlorosulfonated polyethylene or weathered polyethylene (tentative i.d.) – 1 x white
2	Schlatenkees (Auge Gottes)	2 x blue, 1 x black	none	none
3	Großglockner Pasterze I	1 x transparent, 1 x blue	Polyester - 1 x blue fibre	none
4	Großglockner Pasterze II	2 x black, 1 x blue, 1 x blue & white, 2 x transparent	Polyester – 1 x orange, 2 x blue EVA copolymer – 1 x transparent	Polyester(urethane) – 1 x black
8	Kitzsteinhorn	1 x red	Polyester – 1 x green Polyamide – 1 x blue	Epoxy resin – 1 x black
10	Dachstein: Kleiner Eissee I	1 x blue	none	none
11	Dachstein: Kleiner Eissee II	3 x blue, 2 x black, 1 x red, 1 x transparent	Polyester – 1 x black, 1 x dark blue	Polyethylene (PR) – 1 x bright green PTFE film – 1 x transparent Chlorosulfonated polyethylene or weathered polyethylene (tentative i.d.) – 1 x blue Polyacrylamide (tentative i.d.) – 1 x blue/white Ethyl polysilicate (tentative i.d.) – 1 x bright orange

Table 3: summary of the identities of non-cellulosic synthetic fibres and of synthetic fragments as determined by FT-IR transmittance microscopy analysis.

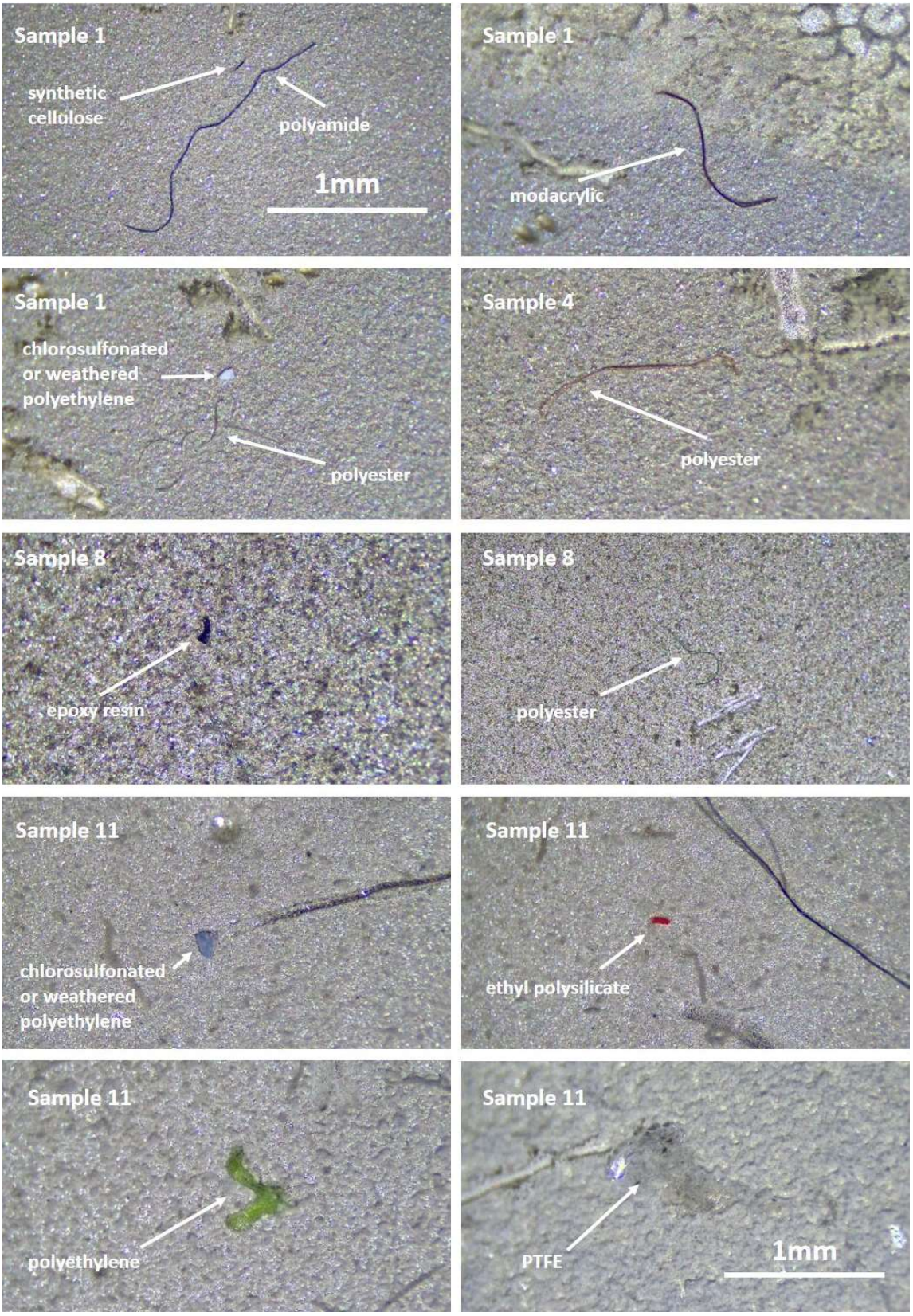

Figure 5: light micrographs of a selection of synthetic microfibers and microplastic fragments identified in water samples collected from the glacier lakes, illustrating the variety of forms and materials identified.

Discussion

As noted above, each sample in this study represents only a snapshot of the levels of contamination in the surface waters of the lakes at the locations and times at which they were collected, and as such they clearly cannot be considered to be representative of levels of contamination in the lakes as a whole, nor used as a basis for detailed comparison of levels of microplastics at the different locations. Levels of microplastic contamination may perhaps be expected to be higher in waters close to the lake shores, given the proximity to sources on land, though it is also possible that winds and currents might lead to quite rapid redistribution across the lake surface. Nonetheless, taken together as a whole set of samples, the results do illustrate the presence of synthetic fibres and fragments, including microplastics, in such water bodies in Austria despite their relative remoteness. Abundances of microfibres identified as synthetic cellulose across the 7 samples ranged from 0.3 to 2.8 per litre (average 1.4 per litre), while abundances of non-cellulosic fibres and fragments that could be identified as microplastics ranged from 0 to 2.8 per litre (average 1.2 per litre).

These results are of a similar order to those we determined for samples collected from nearshore surface waters of 7 lower altitude lakes in Austria in May and June 2023 (Santillo 2023), though the diversity and abundance of microplastic fragments was somewhat lower in the glacial lakes sampled in the current study. Given the origin of the glacial lakes, it may initially seem surprising that microplastics and synthetic cellulose fibres can be found in them at all, as glacial meltwaters may be expected to be pristine and unimpacted by the global spread of plastic pollution. There are, nonetheless, a number of possible routes by which the synthetic fibres and fragments identified could have reached these lakes, including (1) through long distance transport on air currents followed by either wet or dry deposition directly into the lakes or on to the surfaces of the glaciers or (2) from abrasive wear of synthetic clothing and other materials carried to these remote locations by hikers and other visitors.

Although there are still very few studies to date documenting microplastics as contaminants on and within glaciers, research in this field is now expanding. Following one of the earliest of such studies, focused on the Forni Glacier in the Italian Alps (Ambrosini *et al.* 2019), microplastics have since been documented in samples of glacial snow in Austria, close to the Sonnblick Observatory (Materić *et al.* 2020), on the Vatnajokull Glacier in Iceland (Stefánsson *et al.* 2021) and even in Antarctica (Aves *et al.* 2022). Given the remoteness of some of these locations, the most likely route for microplastics to arrive at the glaciers is through long-range atmospheric transport, the significance of which was highlighted by Allen *et al.* (2019) and Evangeliou *et al.* (2020). Indeed, in interpreting their findings in Austria, Materić *et al.* (2021) used atmospheric models to backcast the likely origin of the nanoplastics they detected, noting that a major contributor was the passage of air masses over the more populated regions of Europe. Allen *et al.* (2020) concluded that long-range transport of microplastics from the oceans likely also makes a significant contribution to microplastics subsequently detected on land or in freshwater environments. It is clearly not possible in the case of our study to determine the most likely source for the fibres and fragments we identified, but their presence as contaminants in the waters of these lakes nonetheless provides a further indication of the widespread nature of environmental contamination with microplastics and other synthetic materials, even in what may otherwise be considered to be remote and relatively pristine environments. Gaylarde *et al.* (2023)

provide a useful recent review of the growing evidence for the spread of microplastics within the cryosphere, including within glacier ecosystems, noting that the accumulation of microplastics on and within glaciers, especially coloured microplastics, may (along with black carbon) be contributing to the absorption of solar energy and thereby accelerating glacial melt.

Understanding of the distribution of microplastics as contaminants in freshwater lakes is also growing as more studies emerge, though again from a very low baseline in comparison to the plethora of studies that have been conducted over decades on the marine environment (Wagner *et al.* 2014). Recent reviews by Pan *et al.* (2023) and Chen *et al.* (2023) identified more than 100 studies in total on freshwater systems, covering more than 40 countries. Overall, results reveal a very wide variation in levels of contamination which, in part, correlate with proximity to human activities and sources of plastic use and pollution. At the same time, however, the ongoing lack of standardisation in the methods employed to collect, identify and quantify microplastics inevitably limits the drawing of direct comparisons between studies that employ different approaches (D'Avignon *et al.* 2022). As we noted in our earlier work on a number of lakes in Austria in 2023 (Santillo 2023), the range of concentrations we have determined in both those lakes and in the most recent sampling of the glacial lakes are more comparable with those studies that have employed small mesh sizes and small pore size filters (e.g. Wang *et al.* 2017, Yin *et al.* 2019, Gopinath *et al.* 2020) than to those that have sampled much large volumes of water by towing coarser nets at the surface (Dusaucy *et al.* 2021). Once again, the majority of the microfibers and plastic fragments that we isolated from the filtration of the water samples collected from the glacial lakes would have been too small to have been retained in the 250µm mesh tow nets that have been used for some of the most recent comparative studies of lakes in different regions (e.g. Nava *et al.* 2023, Tanentzap *et al.* 2021).

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