# Greenpeace Research Laboratories Analytical Results 2023-04

# Microplastic fibres and fragments in nearshore surface waters of 7 lakes in Austria in May and June 2023

**David Santillo, August 2023, 19 pp.**

## Introduction

A total of 12 (approximately 2.9 litre) composite samples of surface water were collected from the shores of 7 different lakes in Austria (two samples per lake from 5 lakes, collected at different locations, plus single samples from two additional lakes) during May and June 2023. Samples were returned to the Greenpeace Research Laboratories a 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.

Details of the samples received, including the location, date and time 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. Sampling equipment is illustrated in use in Figure 2.

On return to our laboratory, samples were filtered through fresh 5µm silver filters (pre-verifed as being free from fibres or fragments by inspection under a dissecting binocular light microscope), 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.

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| GRL sample code | Original sample code | Date/Time | Location | Surroundings | Visible plastic-waste |
|---|---|---|---|---|---|
| AT23001 | 1 | 22.05.2023 11:15 | Alte Donau I<br><br>48.23808, 16.42951 | The sample site is a public jetty for bathers. Right next to it are several roads with heavy traffic and densely built-up residential areas. A bridge with heavy traffic is 50 m away. The water was clear and calm. Beforehand, underwater algae had been "mown".<br>Sample drawn between 10-20 cm water depth | styrofoam, PU-foam, packaging remnants, labels, cigarette butts, |
| AT23002 | 2 | 22.05.2023 11:40 | Alte Donau II<br><br>48.21416, 16.43800 | Public bathing jetty in the middle of the built-up settlement area (former Schreber. The motorway is 50 m away. The shore is lined with reeds and rows of trees.<br>Sample drawn between 10-20 cm water depth | Plastic cutlery, lighter, labels, caps, plastic straws |
| AT23003 | 3 | 22.05.2023 14:15 | Neusiedler See I<br><br>47.92828, 16.83382 | Trial pull took place from the jetty. Surroundings are developed with tourist infrastructure. Right next to the local sailing harbour and a restaurant. Next to it is a lido. The sample was very turbid.<br>Sample drawn between 10-20 cm water depth | No pollution visible |
| AT23004 | 4 | 22.05.2023 15:15 | Neufelder See I<br><br>47.86607, 16.38600 | Trial pull took place in a lido from a jetty. The lakeshore is lined with single-family houses. Few lido visitors in the water. The surrounding area is residential. The lake is a former coal mine. | No pollution visible |
| AT23005 | A | 28.06.2023 11:15 | Wörthersee Krumpendorf<br><br>46.61827, 14.18993 | Private bathing jetty near property. Surrounded by a Natura 2000 protected area. Car road and railway line 200 metres away as the crow flies. Ship and boat traffic in the area. | In the nearby reeds (about 20 metres) there were corks, several pieces of packaging, PET bottles and various foils. |
| AT23006 | B | 28.06.2023 12:15 | Wörthersee Klagenfurt Ostbucht<br><br>46.62225, 14.25325 | Public bathing jetty, boat landing stage, public lido, road and railway approx. 200 metres away, shore is a park with footpaths and cycle paths, gastronomy in the vicinity. | Lake bottom: pipes, tarpaulins, packaging remains, rubber hoses |
| AT23007 | C | 28.06.2023 16:50 | Wolfgangsee St. Gilgen | Public bathing beach, pebble beach, in the middle of the tourist village of St. Gilgen, hotel and private holiday | Cigar butts, tablet cases, foils, crown corks, |

| | | | | | |
|---|---|---|---|---|---|
| | | | Ortschaft<br><br>47.76657, 13.36861 | accommodation next door, shipping traffic in the vicinity, public lido approx. 200 metres away. | cutlery, labels, remains of bottles, parts of facades, snus, cable ties |
| AT23008 | D | 28.06.2023<br>18:00 | Wolfgangsee Wasserbad<br><br>47.71720, 13.45343 | Surrounded by agricultural land, nature reserve, lido with jetty, pedal boat rental and gastronomy, road about 250 metres away. | various plastic scraps lay at the bottom of the lake Wrappings, hair bands lay on the beach<br><br>Very clean |
| AT23009 | E | 29.06.2023<br>07:45 | Attersee Weyregg, Musikpavillion<br><br>47.90162, 13.56628 | Park on the lakeside of the village, no beach, holiday apartments and lidos in the immediate vicinity, lakeside road approx. 250 m away. | Cigarette butts, crown corks, plastic rope, plastic sign, foils |
| AT23010 | F | 29.06.2023<br>09:30 | Attersee SJ-Europabad<br><br>47.79867, 13.53097 | Free bathing place of the socialists, road 50 metres away, gastronomy, sawmill, camping site in the direct vicinity. | Packaging residues, building boards, various small parts, bathing costume residues, PU foam, cigarette packets, cigarette butts, foils, bottles, snus, bags, ettape, insulation material |
| AT23011 | G | 29.06.2023<br>12:45 | Lunzer See Seeterasse<br><br>47.85730, 15.05855 | Bathing jetty, road, rows of concrete seats, toilet block, | Styrofoam, ,etiquettes, pipes, snuns, plastic threads, plastic scraps, cigarette butts |
| AT23012 | H | 29.06.2023<br>13:15 | Lunzer See Badesteg<br><br>47.85259, 15.04010 | Gastronomy, rest area with many benches, boat mooring and boat traffic, rainwater drainage of the concreted forecourt, | Plastic scraps, foils, hard plastic parts, plastic parts, pipes, remains of cups |

Table 1: details of samples received and analysed at the Greenpeace Research Laboratories

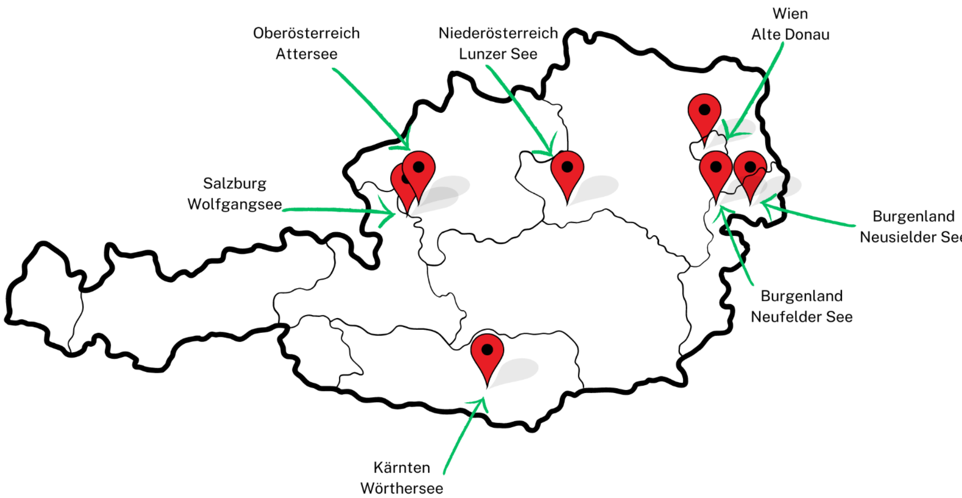



Figure 1: map showing the approximate locations of the 7 lakes sampled in this study

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.

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. As our laboratories are not medical-grade clean-rooms, a separate silver filter was placed in a petri dish on the bench adjacent to the areas in which samples were handled to verify that there was minimal or no deposition of fibres or fragments from the air during those procedures.

For 11 of the 12 samples, it was possible to filter the entire volume of the sample through a single filter (total volumes between 2760 and 2950 ml), with this process taking no more than a few minutes in each case. During those times, no visible fibres or fragments were deposited on the control filter in the fume cabinet.


Figure 2: sampling equipment used, showing sample pole, 500ml sampling bottle and 2.9 litre composite sample container (Winchester)

*[NB: In the case of the sample labelled no. 3 from the Neusiedler See (assigned our laboratory code AT22003), the very high turbidity caused by suspended minerals made it impossible to filter more than 300ml in total through a single filter, and this itself took many hours to complete, increasing the risk of contamination from laboratory air despite all the precautions taken to protect the filter during filtration. Efforts continue to try to clear the mineral turbidity of the remainder of the sample to enable filtration of the remaining volume without the risk of removing or destroying any microplastics present, but this will take more time to resolve. In the meantime, whereas the numerical data for the other 11 samples can be considered to be fully quantitative of what was in the samples at the time of collection, those for sample AT23003 should be considered as qualitative and indicative only, given that it is not possible to ensure that any microplastics present were evenly distributed through the entire volume of the sample before the 300ml subsample was filtered (especially as different microplastics vary significantly in density, with some rising rapidly to the surface of the sample).]*

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 four small fibres that were deposited on different filters during the period of FT-IR analysis of all 12 samples.

Individual candidate materials (fibres and fragments) retained on each of the silver filters were subsequently examined using a PerkinElmer Spotlight 400 FT-IR Imaging System (MCT detector, KBr window) operating in reflectance mode across a wavenumber range from 4000 to 750 $cm^{-1}$ and with a resolution of 4 $cm^{-1}$. A total of 16 scans were collected for at least two sections of 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 were excluded from analysis.

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 initially recorded as “unidentified”. In these cases, where it was possible physically to remove the fibre or fragment from the filter surface using fine forceps, each was then transferred to a diamond compression cell (pre-cleaned with ethanol and lint-free tissues) and compressed between the diamond windows to enable FT-IR analysis on the same microscope in transmittance rather than reflectance mode (i.e. passing the infrared light through the sample rather than detecting reflected infrared wavelengths from the sample surface). Although a slower and more complex procedure, the recording of transmittance spectra can often help achieve higher match qualities than reflectance spectra because of the light scattering and interference from surface contamination that can influence reflectance mode. The same acceptance criteria as for reflectance spectra were applied in all cases.

Although it was not possible to collect field blank samples in Austria, two such samples were collected in an urban area of the UK (two locations on the edge of open areas of water at the University of Exeter, close to residential buildings and roads) as a verification for the sampling method. In each case, 2900 ml of pre-filtered deionised water contained in a pre-cleaned Winchester bottle were transferred to a separate pre-cleaned empty Winchester bottle by repeatedly filling and emptying a 500ml pre-cleaned glass bottle, so as to replicate as closely as possible the procedure used in the field in Austria. These “field blank” water samples were then returned to our laboratory and filtered in the same way as the samples themselves. Only 3 small (<20 µm diameter) irregular transparent fragments were found on one of the two field blank filters, and these were found by FT-IR analysis to be dust particles of organic origin (two of them proteinaceous, possibly pollen clumps or skin fragments, and one showing cellulose/lignin-like characteristics).

Figure 3 illustrates some of the equipment used for sample processing and analysis, including the filtered samples in pre-cleaned glass petri dishes, the marking of candidate materials on the filters with a sharp needle immediately after filtration, the PerkinElmer Spotlight 400 FT-IR microscopy system used for the identification of all fibres and fragments, initially in reflectance mode, and a diamond compression cell used with the same system where it was necessary to confirm identities using transmittance FT-IR.

## Results

A total of 241 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 12 samples (see Table 2). Of these, 163 were fibres and 78 were fragments. All were subjected to FT-IR microscopy in reflectance mode, as described above, with a subset also analysed in transmittance mode to try to verify identity in the case of initial low match qualities. The identities of those fibres (other than modified cellulose fibres) and fragments confirmed to be synthetic in nature are summarised in Table 3.


Figure 3: a) silver filters after sample filtration, with positions of candidate materials for FT-IR analysis marked, b) marking of the filters with a sharp needle under the dissecting binocular light microscope, c) the PerkinElmer Spotlight 400 FT-IR microscopy system used for FT-IR analysis and d) a diamond compression cell used for transmittance FT-IR microscopy where necessary to confirm identities.

FT-IR analysis confirmed that 80 of the 163 fibres were of “natural” origin, primarily transparent or pale brown fibres identified as cellulosic, lignin-based (wood fibre) or proteinaceous material which are likely to have arisen from plants or animals living in or around or upstream from the lakes. 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 also present in the samples and which could not have arisen from natural sources (see below).

A total of 82 fibres were confirmed as synthetic materials, characterised by very uniform diameters and smooth surfaces 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, green & purple).

- Of these, 49 fibres were identified as being cellulose-based, despite their clearly synthetic nature and often strong colouration, most probably being fibres of processed dyed cotton or materials such as viscose or rayon.

- A further 20 fibres were confirmed as synthetic polymers (i.e. plastic microfibres), primarily polyester (both transparent or coloured) or polyamide, but also including fibres of acrylic, PVC and in one case, PTFE. One glass fibre was also identified. These identities are summarised in Table 3.

- In the case of 13 fibres, reliable identification was not possible, even for those cases for which transmittance FT-IR microscopy was conducted. 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 microns (μm) and the identifications determined from their infrared spectra, are shown in Figure 4.

Of 78 fragments analysed, 23 were determined to be of natural origin (including proteinaceous material, inorganic mineral particles and small fragments of charcoal).

- 35 were confirmed to be synthetic materials (microplastics), including polyethylene (PE, 6 fragments); polypropylene (PP, 5 fragments); polyvinyl chloride (PVC, 4 fragments); PTFE (3 fragments); polyacrylamide (3 fragments); strongly coloured cellulose (3 fragments); synthetic rubber (3 fragments) and acrylic (2 fragments). Single fragments of chlorinated polyethylene, epoxy resin, formaldehyde resin, polystyrene, strongly coloured paraffin wax and metallic foil (possibly aluminium) were also found. These identities are also summarised in Table 3.

- For a further 20 fragments, while the infrared spectral characteristics were very similar to those of microplastics, the match qualities either in reflectance or transmittance mode were not high enough to verify their identities with sufficient confidence.

Examples of the synthetic fragments identified, again photographed using the FT-IR microscope camera and identified from their infrared spectra, are shown in Figure 5.

For the 11 samples for which the complete volume was filtered, it is possible to present the data as abundance of fibres and fragments per litre of water (see Table 4), though it must be noted 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. 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.

Of those 11 samples, sample AT23007 (original sample code C, collected from the Wolfgangsee at St. Gilgen Ortschaft) contained the highest combined abundance of fibres and fragments (just over 12 per litre), dominated by fibres, the majority of which were determined to be of natural origin (i.e. mainly irregular fibres of cellulosic or lignin-based material). Sample AT23001 (original sample code 1, collected from Alte Donau, location I) contained a slightly lower combined total of fibres and fragments (around 11.5 per litre), though in this case fragments were more prominent, and the majority of those were confirmed as microplastics (including two fragments of polypropylene, one orange and one red, 2 of blue polyacrylamide, 2 of transparent PTFE and three small black fragments of elastic material which could be identified only as synthetic rubber, and which could be particles from tyres).

The sample containing the highest abundance of synthetic microfibres, at around 4.8 per litre, was AT23005 (original sample code A, collected from the Wörthersee at Krumpendorf). Half of the fibres identified were of modified cellulose (highly processed cellulose fibres, often strongly coloured), and the other half were synthetic polymers (acrylic, PVC or in some cases not able to be identified with sufficient confidence but showing many spectral characteristics suggestive of polyester fibres).

As noted above, in the case of sample AT23003, it was possible to filter only a fraction of the total volume of the sample because of the inorganic material that is naturally so abundant in the water, and it is not possible to conclude that the 300ml filtered was a quantitatively representative subsample of the whole because of the discrete nature of microplastics as contaminants and their different densities relative to water, despite every effort being made to homogenise the sample before filtering. On the basis of the 300ml subsample we were able to filter and analyse so far, conversion to fibres and fragments per litre would suggest that this was the most contaminated sample by far, but for the reasons given above it would not be fair to draw this conclusion. Efforts will continue to be made to filter and analyse the entire volume of this sample such that the totals can be updated in due course.

| Sample code | Sample location | Volume filtered | Total fibres & fragments | Fibres | | | | Fragments | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total fibres | Natural fibres | Synthetic fibres | | Total fragments | Natural fragments | Synthetic fragments |
| | | | | | | *cellulose* | *other* | | | |
| **AT23001** | Alte Donau I | 2940 | 34 | 16 | 10 | 6 | 0 | 18 | 4 | 14 |
| **AT23002** | Alte Donau II | 2900 | 21 | 11 | 5 | 4 | 2 | 10 | 2 | 8 |
| **AT23003** | Neusiedler See I | 300 | 15 | 12 | 3 | 6 | 3 | 3 | 2 | 1 |
| **AT23004** | Neufelder See I | 2760 | 11 | 6 | 4 | 1 | 1 | 5 | 0 | 5 |
| **AT23005** | Wörthersee Krumpendorf | 2950 | 25 | 17 | 3 | 7 | 7 | 8 | 5 | 3 |
| **AT23006** | Wörthersee Klagenfurt Ostbucht | 2790 | 19 | 13 | 6 | 2 | 5 | 6 | 3 | 3 |
| **AT23007** | Wolfgangsee St. Gilgen Ortschaft | 2890 | 35 | 22 | 14 | 5 | 3 | 13 | 5 | 8 |
| **AT23008** | Wolfgangsee Wasserbad | 2920 | 7 | 1 | 1 | 0 | 0 | 6 | 1 | 5 |
| **AT23009** | Attersee Weyregg, Musikpavillion | 2910 | 18 | 13 | 2 | 6 | 5 | 5 | 1 | 4 |
| **AT23010** | Attersee SJ-Europabad | 2770 | 13 | 13 | 5 | 5 | 3 | 0 | 0 | 0 |
| **AT23011** | Lunzer See Seeterasse | 2860 | 19 | 17 | 11 | 2 | 3 | 2 | 0 | 2 |
| **AT23012** | Lunzer See Badesteg | 2780 | 24 | 22 | 16 | 5 | 1 | 2 | 0 | 2 |
| **TOTALS** | | | **241** | **163** | **80** | **49** | **33** | **78** | **23** | **55** |

Table 2: overview of total numbers of fibres and fragments (both natural and synthetic) found in the 12 samples

| Sample code | Sample location | Identities of non-cellulosic synthetic fibres | Identities of synthetic fragments |
| --- | --- | --- | --- |
| **AT23001** | Alte Donau I | none | Polypropylene (PP) - 1 x orange fragment, 1 x red sphere<br>PTFE - 1 x transparent fragment, 1 x transparent film<br>Polyacrylamide - 2 x blue fragments/gel<br>Synthetic rubber - 3 x black fragments<br>Cellulose - 1 x blue fragment ('cellophane')<br>Unidentified* - 2 x white fragments, 2 x blue fragments |
| **AT23002** | Alte Donau II | Unidentified* - 1 x black fibre, 1 x brown fibre | Polyacrylamide - 1 x blue fragment/gel<br>Metal foil - 1 x fragment<br>Unidentified* - 3 x white fragment, 2 x black fragment, 1 x grey fragment |
| **AT23003** | Neusiedler See I | Polyester - 1 x blue fibre, 1 x transparent fibre<br>Polyamide - 1 x transparent fibre | Polyethylene (PE) - 1 x orange fragment |
| **AT23004** | Neufelder See I | Glass - 1 fibre | Polypropylene (PP) - 1 x white fragment<br>PTFE - 1 x transparent fragment<br>Chlorinated polyethylene (Cl-PE) - 1 x yellow fragment<br>Unidentified* - 1 x black fragment, 1 x white fragment |
| **AT23005** | Wörthersee Krumpendorf | Acrylic copolymer (modacrylic) - 1 x blue fibre<br>Polyvinyl chloride (PVC) - 1 x blue fibre, 1 x brown fibre<br>Unidentified* - 2 x transparent fibres, 2 x brown fibres | Cellulose - 1 x blue fragment, 1 x transparent fragment<br>Unidentified* - 1 x dark green |
| **AT23006** | Wörthersee Klagenfurt Ostbucht | Polyester -1 x transparent fibre, 1 x purple fibre, 1 x orange fibre<br>Acrylic copolymer (modacrylic) - 1 x black fibre<br>Polyamide - 1 x green fibre | Polyethylene (PE) 1 x white fragment<br>Polyvinyl chloride (PVC) - 1 x white fragment, 1 x black fragment |
| **AT23007** | Wolfgangsee St. Gilgen Ortschaft | Polyester - 1 x transparent fibre<br>Unidentified* - 2 x transparent fibres | Polypropylene (PP) - 1 x blue fragment<br>Polyethylene (PE) - 1 x transparent fragment<br>Epoxy resin - 1 x brown fragment<br>Unidentified* - 3 x brown fragments, 1 x white fragment, 1 x black fragment |

| | | | |
|---|---|---|---|
| **AT23008** | Wolfgangsee<br>Wasserbad | none | Polyethylene (PE) - 2 x black film<br>Polyvinyl chloride (PVC) - 1 x yellow fragment<br>Acrylate polymer - 2 x red/orange fragments |
| **AT23009** | Attersee<br>Weyregg,<br>Musikpavillion | Polyester - 1 x black, 1 x red fibre<br>Polyamide - 1 x black fibre<br>Unidentified* - 1 x transparent fibre | Polyethylene (PE) - 1 x transparent fragment<br>Polyvinyl chloride (PVC) - 1 x white/transparent fragment<br>Formaldehyde resin - 1 x black fragment<br>Chlorinated polyethylene (Cl-PE) - 1 x blue fragment |
| **AT23010** | Attersee<br>SJ-Europabad | Polyester - 1 x transparent fibre<br>Unidentified* - 2 x black fibres | none |
| **AT23011** | Lunzer See<br>Seeterasse | Polyester - 1 x blue fibre<br>Polyamide - 1 x red fibre<br>Unidentified* - 1 x black fibre | Polypropylene (PP) - 1 x transparent fragment<br>Polystyrene (PS) - 1 x light brown fragment |
| **AT23012** | Lunzer See<br>Badesteg | PTFE - 1 x transparent fibre cluster | Cellulose - 1 x blue fragment<br>Paraffin wax - 1 x yellow sphere |

Table 3: summary of the identities of non-cellulosic synthetic fibres and of synthetic fragments as determined by FT-IR microscopy analysis (combination of reflectance and, where necessary and practicable, transmittance FT-IR).

*NB: the term "unidentified" is used here to denote fibres and fragments that were clearly distinguishable from natural materials according to their morphology, surface characteristics and/or colour but for which the quality of the match to FT-IR spectral libraries fell short of the criteria for reliable confirmation of the polymer type (either <70% match quality or rejection by analyst on inspection of spectral quality). For example, in the case of the fibres listed as unidentified, the majority showed many characteristics similar to either polyester or polyamide, but with insufficient quality to confirm the polymer type with confidence.*

| Sample code | Sample location | Volume filtered | Total fibres & fragments/l | Fibres per litre | | | | Fragments per litre | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total fibres/l | Natural fibres/l | Synthetic fibres/l | | Total fragments/l | Natural fragments/l | Synthetic fragments/l |
| | | | | | | *cellulose* | *other* | | | |
| **AT23001** | Alte Donau I | 2940 | 11.56 | 5.44 | 3.40 | 2.04 | 0.00 | 6.12 | 1.36 | 4.76 |
| **AT23002** | Alte Donau II | 2900 | 7.24 | 3.79 | 1.72 | 1.38 | 0.69 | 3.45 | 0.69 | 2.76 |
| **AT23003** | Neusiedler See I | 300 | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| **AT23004** | Neufelder See I | 2760 | 3.99 | 2.17 | 1.45 | 0.36 | 0.36 | 1.81 | 0.00 | 1.81 |
| **AT23005** | Wörthersee Krumpendorf | 2950 | 8.47 | 5.76 | 1.02 | 2.37 | 2.37 | 2.71 | 1.69 | 1.02 |
| **AT23006** | Wörthersee Klagenfurt Ostbucht | 2790 | 6.81 | 4.66 | 2.15 | 0.72 | 1.79 | 2.15 | 1.08 | 1.08 |
| **AT23007** | Wolfgangsee St. Gilgen Ortschaft | 2890 | 12.11 | 7.61 | 4.84 | 1.73 | 1.04 | 4.50 | 1.73 | 2.77 |
| **AT23008** | Wolfgangsee Wasserbad | 2920 | 2.40 | 0.34 | 0.34 | 0.00 | 0.00 | 2.05 | 0.34 | 1.71 |
| **AT23009** | Attersee Weyregg, Musikpavillion | 2910 | 6.19 | 4.47 | 0.69 | 2.06 | 1.72 | 1.72 | 0.34 | 1.37 |
| **AT23010** | Attersee SJ-Europabad | 2770 | 4.69 | 4.69 | 1.81 | 1.81 | 1.08 | 0.00 | 0.00 | 0.00 |
| **AT23011** | Lunzer See Seeterasse | 2860 | 6.64 | 5.94 | 3.85 | 0.70 | 1.05 | 0.70 | 0.00 | 0.70 |
| **AT23012** | Lunzer See Badesteg | 2780 | 8.63 | 7.91 | 5.76 | 1.80 | 0.36 | 0.72 | 0.00 | 0.72 |

Table 4: nominal abundance per litre of fibres and fragments (both natural and synthetic) found in the 12 samples

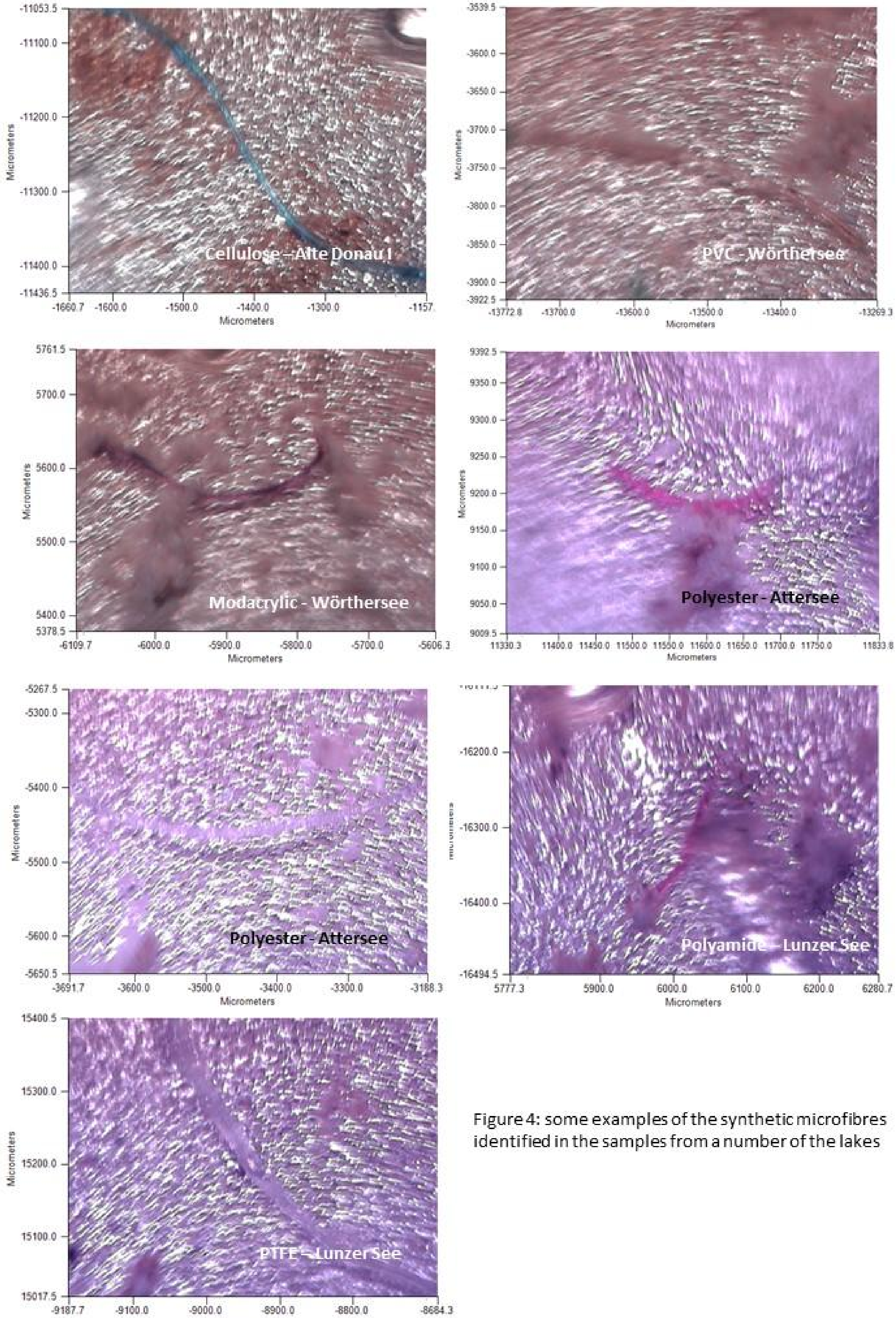
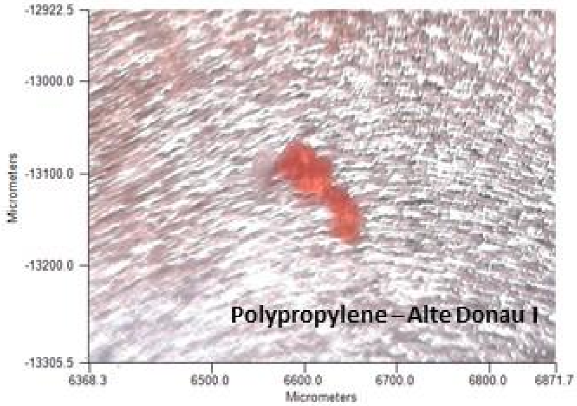
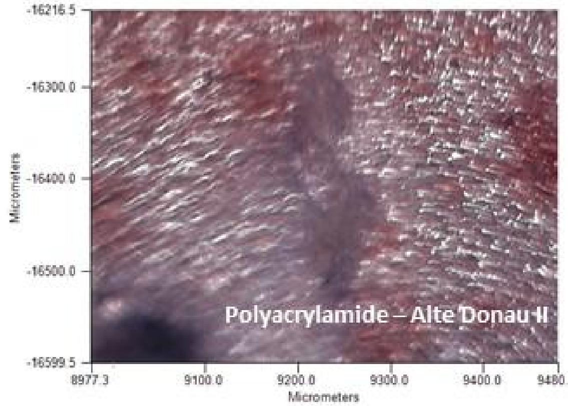
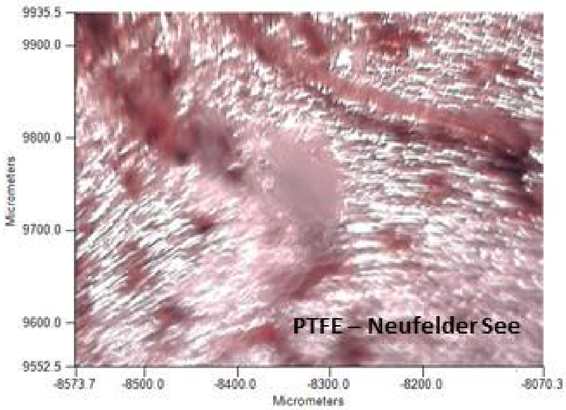
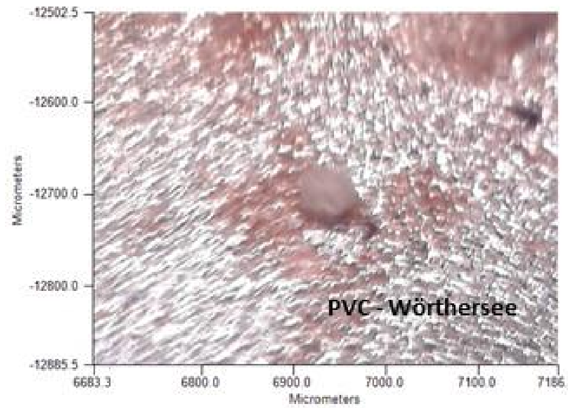


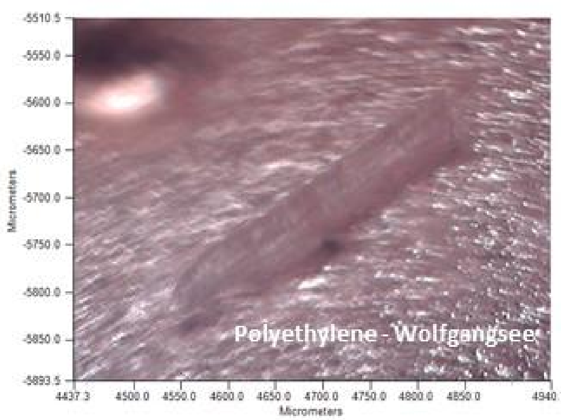

Figure 4: some examples of the synthetic microfibres identified in the samples from a number of the lakes

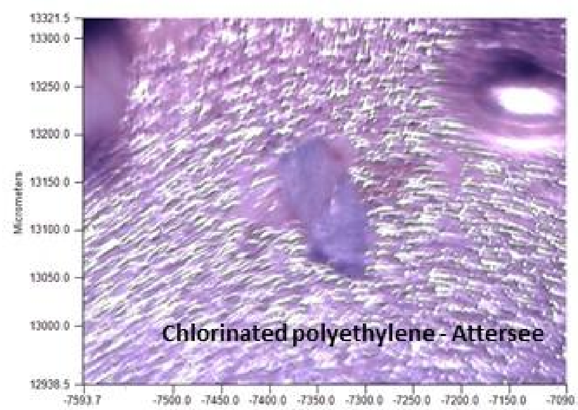


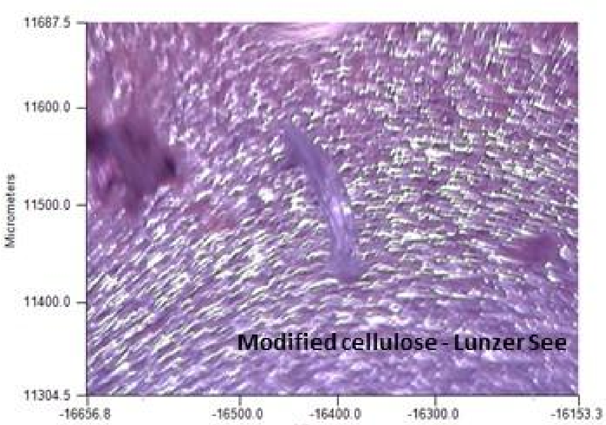




Figure 5: some examples of the synthetic fragments identified in the samples from a number of the lakes

## 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 give an insight into the range of abundances of microplastics in such water bodies in Austria and represent the first known measurements for microplastics in the majority of the lakes sampled. With the exclusion of sample AT23003 for the reasons set out above, abundances of non-cellulosic microfibres (which may be considered as "true" microplastic fibres) across the other 11 samples ranged from 0 to 2.37 per litre (average 0.95 per litre), while abundances of synthetic microplastic fragments ranged from 0 to 4.76 per litre (average 1.7 per litre).

Despite the large and growing literature on the presence of microplastics (fibres and fragments) in the environment, especially in marine systems and the rivers flowing into them, so far remarkably few studies have focused on documenting microplastics as contaminants in lakes. Although several studies and review articles have been published since Wagner *et al.* (2014) noted that there were "immense gaps in knowledge regarding freshwater microplastics", they nonetheless still remain limited in number and geographical scope, with the majority of lakes sampled to date being located in Asia (Pan *et al.* 2023). Furthermore, the majority of studies conducted in lakes to date have relied on collecting microplastics using nets towed at the surface (Dusaucy *et al.* 2021). Although this enables far larger volumes of water to be sampled than the whole water sampling and filtration protocol used in our study (and by a minority of others), arguably providing more representativity for comparative purposes, such nets nonetheless collect data only for microplastics in larger size ranges, often only down to a nominal minimum size of 250µm. They therefore fail to capture fragments smaller than this (which made up the majority of fragments found in our study, for example) and likely also miss a proportion of the microfibres considerably larger than this as they are less likely to be caught in the net than fragments because of their narrow diameter.

For example, in one of the most recent studies, and also the most geographically widespread, Nava *et al.* (2023) used a 250µm mesh to collect samples from 38 lakes and reservoirs, including the Lunzer See and several others in Europe. However, as a result of the larger mesh size, the abundances recorded for microplastics were approximately 1000 times lower than we determined in our study. This was similarly the case for the study conducted by Tanentzap *et al.* (2021) of 67 European lakes, in which the same mesh size and sampling protocol was used. Indeed, based on the size and morphology of the fibres and fragments identified in our whole water samples filtered through the 5µm mesh of the silver filters, it is doubtful that any of those we found would have been retained by the 250µm mesh of a plankton net employed in those studies.

This does not undermine the significance of the findings of those and other studies in which plankton net tows have been used to document microplastics in lakes; microplastics in those larger size ranges are clearly also of significance in terms of their presence and potential ecological impacts, as well as acting as contributors to smaller fractions as they further degrade and fragment. Nonetheless, data from studies such as ours, in which much smaller filter sizes have been used (albeit with much smaller volumes of water sampled as a result), indicate that studies employing net tows at higher mesh sizes are underestimating the full extent of microplastic pollution in numerical terms, and missing the much higher abundances of small fragments and fibres that may nonetheless fall in the size range of relevance to filter-feeding and scavenging organisms in the plankton. Some of the studies of lakes in Asia, such as those of Wang *et al.* (2017) and Yin *et al.* (2019) in urban lakes in China and of Gopinath *et al.* (2020) on the Red Hills lake in Chennai (India), in which smaller mesh sizes have been used (50µm, 40µm and 120µm respectively in those studies), have yielded abundances of microplastics recorded per litre of water that are more comparable to those found in our study, in the order of several fibres and/or fragments per litre of water rather than per cubic metre. The value of greater standardisation of methodologies used for sampling and analysis, in order to improve the validity of geographical and temporal comparisons, has often been highlighted (e.g. D'Avignon *et al.* 2022), but it will remain important to document the nature and extent of microplastic pollution at all relevant scales.

## References


D'Avingon, G., Gregory-Eaves, I. & Ricciardi, A. (2022) Microplastics in lakes and rivers: an issue of emerging significance to limnology. Environmental Reviews 30: 228–244. https://doi.org/10.1139/er-2021-0048

Dusaucy, J., Gateuille, D., Perrette, Y. & Naffrechoux, E. (2021) Microplastic pollution in worldwide lakes. Environmental Pollution 284 (2021) 117075: 13 pp. https://doi.org/10.1016/j.envpol.2021.117075

Gopinath, K., Seshachalam, S., Neelavannan, K., Anburaj, V., Rachel, M., Ravi, S., Bharath, M. & Achyuthan, H. (2020) Quantification of microplastic in Red Hills Lake of Chennai city, Tamil Nadu, India. Environmental Science and Pollution Research (2020) 27:33297–33306. https://doi.org/10.1007/s11356-020-09622-2

Nava, V., Chandra, S., Ahern, J. *et al.* (2023) Plastic debris in lakes and reservoirs. Nature 619, 13th July 2023: 317-332. https://doi.org/10.1038/s41586-023-06168-4

Pan, T., Liao, H., Yang, F., Sun, F., Guo, Y., Yang, H., Feng, D., Zhou, X. & Wang, Q. (2023) Review of microplastics in lakes: sources, distribution characteristics, and environmental effects. Carbon Research (2023) 2:25: 19 pp. https://doi.org/10.1007/s44246-023-00057-1

Tanentzap, A.J., Cottingham, S., Fonvielle, J., Riley, I., Walker, L.M., Woodman, S.G., et al. (2021) Microplastics and anthropogenic fibre concentrations in lakes reflect surrounding land use. PLoS Biol 19(9): e3001389. https://doi.org/10.1371/journal.pbio.3001389

Wagner, M., Scherer, C., Alvarez-Muñoz, D. *et al.* (2014) Microplastics in freshwater ecosystems: what we know and what we need to know. Environmental Sciences Europe 2014, 26(12): 9 pp. https://doi.org/10.1186/s12302-014-0012-7

Wang, W., Wairimu Ndungu, A., Li, Z. & Wang, J. (2017) Microplastics pollution in inland freshwaters of China: A case study in urban surface waters of Wuhan, China. Science of the Total Environment 575 (2017) 1369–1374. http://dx.doi.org/10.1016/j.scitotenv.2016.09.213

Yin, L., Jiang, C., Wen, X., Du, C., Zhong, W., Feng, Z., Long, Y. & Ma, Y. (2019) Microplastic Pollution in Surface Water of Urban Lakes in Changsha, China. International Journal of Environmental Research and Public Health 16, 1650: 10 pp. https://doi.org/10.3390/ijerph16091650