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# COVER PAGE

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# First detection of microplastics in the freshwater of an Antarctic Specially Protected Area

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## Abstract

Microplastics have been found in all environmental compartments investigated so far, even reaching remote areas. However, their presence in Antarctic freshwaters has not been yet reported. Here, we investigated the occurrence of microplastics in a stream from an Antarctic Specially Protected Area (Byers Peninsula, Livingston Island - ASPA No. 126), which is subject to stringent environmental protection measures as a result of which it is considered a pristine international reference site for inland waters research. Our results showed the presence of three types of microplastics in a freshwater seasonal stream, namely four polyester fibers, one black and three transparent; two acrylic fibers, one transparent and one red; and two transparent polytetrafluoroethylene films. The length and width of these fibers and films were in the 400–3546  $\mu\text{m}$  (average 1118  $\mu\text{m}$ ), and 10–1026  $\mu\text{m}$  (average 199  $\mu\text{m}$ ) ranges respectively. The concentration of MP was 0.95 items/1000  $\text{m}^3$  with estimated variability in the 0.47–1.43 items/1000  $\text{m}^3$  range. This is the first report of the presence of microplastics in Antarctic freshwater with the uniqueness that it is an Antarctic Specially Protected Area, meaning that plastic pollution reached even the most remote and pristine environments in the planet.

**Keywords:** Antarctica; Byers Peninsula; Freshwater; Microplastics; Antarctic specially protected area

## 1. Introduction

Small pieces of plastic of less than five millimeters along their largest dimension, the so-called microplastics (MP), have been found in all environmental compartments investigated so far. They have even reached areas distant from human activities, including both marine (Cincinelli et al., 2017; Courtené-Jones et al., 2020; Kelly et al., 2020; Reed et al., 2018) and freshwater remote ecosystems (Ambrosini et al., 2019; González-Pleiter et al., 2020; Zhang et al., 2016). The routes by which MPs reach distant places are still poorly understood. In the case of marine ecosystems, ocean currents and the buoyancy of some plastics appear to play a crucial role (Courtené-Jones et al., 2020). Globally, the Southern Ocean is the less affected region by plastic pollution due to the scarcity of local sources and the barrier effect of Subtropical Front that limits the transport of floating debris from lower latitudes (Suaria et al., 2020). However, the presence of plastic debris in

the Antarctic continent has been documented in seawater, sea ice and marine sediments (Cincinelli et al., 2017; Kelly et al., 2020; Reed et al., 2018). The growing MP pollution in Antarctica is a cause for concern as it may affect marine food webs and threat key species of this fragile ecosystem (Tirelli et al., 2020). In high-latitude freshwater ecosystems, atmospheric circulation (especially jet streams) and the size and shape of MPs seem to be the most important factors explaining their long-range movement. However, the atmospheric transportation seems to be limited to a few hundred kilometers (Zhang et al., 2020), which would limit the presence of MPs in freshwater remote ecosystems.

The Antarctic continent is isolated from any other emerged land on Earth by the surrounding Southern Ocean. We have selected a pristine and remote freshwater network located in Byers Peninsula (Livingston Island, Antarctica) far away from human activities. Byers Peninsula has been

under environmental protecting figures since 1966, when it was declared Specially Protected Area (SPA No. 10). Nowadays, the Committee on Environmental Protection, under the Antarctic Treaty umbrella, designates it as an Antarctic Specially Protected Area ASPA No. 126 and human presence in it is restricted to research activities with the explicit approval of the pertinent authorities (Quesada et al., 2013). Research activities are subjected to stringent environmental protection measures developed in its management plan, which aims to minimize any possible footprint in the area. As a result of this high level of protection, Byers Peninsula is considered as an international reference site for inland waters research (over 60 lakes and ponds and many streams), since it contains highly preserved environmental resources to investigate the dynamics and functions of such ecosystems (Quesada et al., 2009).

In this context, and due to the lack of any previous studies, we tested the hypothesis that MPs have reached the Antarctic freshwater ecosystems, even those less influenced from human activities and located in Antarctic Specially Protected Areas. Up to our knowledge, this is the first report exploring the presence of MP pollution in Antarctic freshwaters (Cera et al., 2020). We also discussed the possible sources of the plastic particles found.

## 2. Materials and Methods

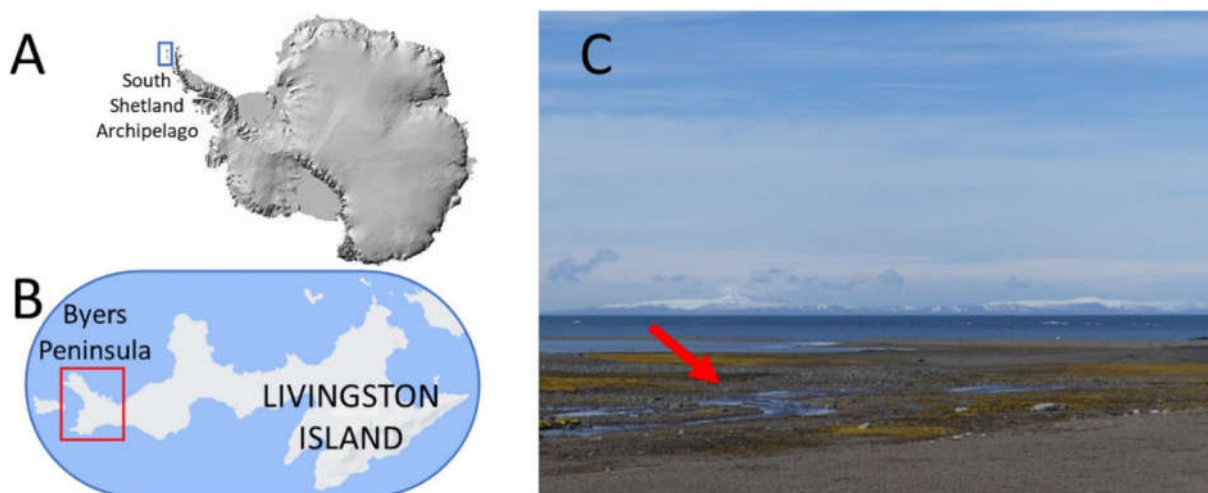
### 2.1. Study area

Byers Peninsula (latitude 62° 34' 35" S, longitude 61° 13' 07" W) is located on the west side of Livingston Island that is part of the South

Shetlands Archipelago, lying about 100 km North of the Antarctic Peninsula (Fig. 1A and B). Byers Peninsula comprises ca. 60 km<sup>2</sup> ice-free area during the summer season and is the largest ice-free area of South Shetland Archipelago. The Peninsula presents a well-developed freshwater network of lakes, ponds, streams, and wetlands across its whole extension. The freshwater stream studied, unofficially known as Ballenas Stream, (62° 39' 38.1" S, 61° 04' 54.7" W) flows about 1 km east of the Byers International Campsite (62°39'49.7" S 61°05'59.8" W). The stream represents the outlet of a water catchment area of 1.10 km<sup>2</sup> at the central plateau of the Byers Peninsula (Toro et al., 2007). Besides, groundwater flows in permafrost soils are important, particularly during late summer season. The camp is a non-permanent setting of two small glass-fiber melon huts (6 m long) and up to 8 camping tents. It accommodates a maximum of 12 people, but during the experiment reported here the camp was inhabited by only 5 people. During sampling, the mean windspeed was 19.5 km/h (maximum gust speed 61.2 km/h); which can be considered low for an area frequently dominated by winds above 100 km/h. The dominant wind direction during sampling days was NW and WE. Liquid precipitation during the experiment was scarce: 2.9 mm in one single day.

### 2.2. Sample collection

Particulate matter dragged by the freshwater stream was collected using two nylon drifting nets (333 µm and 100 µm mesh pore sizes) placed side by side inside the stream (Fig. 1C). The nets were



**Figure 1.** A) General view of Antarctica. The blue rectangle shows South Shetland Archipelago, where Livingston Island is located. B) The red square indicates the location of Byers Peninsula, at the West side of Livingston Island. C) Photograph of the freshwater stream from which the samples were taken.

kept on stream during the sampling period, that lasted eight days, from February 25th, 2018 to March 4th, 2018. The average linear water velocity was  $0.54 \text{ m s}^{-1}$ , measured at the beginning and the end of the experiment by timing the passage of a small spherical float through a measured length of the stream. The measurements were performed using the procedure recommended elsewhere to avoid interferences with wind and to get a good estimate of the average stream velocity in rocky-bottom streams (Gore, 2007). The nets were placed inside the stream, but were not completely submerged, so they received surface water. The submerged area was  $225 \text{ cm}^2$ . Accordingly, and considering the variability recorded during sampling, the total flow through the mesh was estimated as  $21.9 \pm 4.4 \text{ m}^3/\text{h}$ . After sampling, the nets were wrapped with clean aluminum foil and stored at  $4^\circ\text{C}$  for further analysis.

### **2.3. Quantification and identification of microplastics**

Once in the laboratory, the organic matter was digested for 24 h at  $60^\circ\text{C}$  using 33%  $\text{H}_2\text{O}_2$ . The supernatant was filtered through  $25 \mu\text{m}$  stainless steel filters and then through glass fiber filters with  $1 \mu\text{m}$  size particle retention. The sediment from  $\text{H}_2\text{O}_2$  digestion was separated by density using a hypersaline solution (36 g NaCl in 100 mL of solution) and the supernatant was filtered using the same procedure. All filters were placed in glass Petri dishes and sealed to avoid contamination during visual examination.

All particles measuring  $<5 \text{ mm}$  along their larger dimension were photographed with a Euromex-Edubluu stereomicroscope fitted with ImageFocus 4 camera software. ImageJ software was used to measure their projected length and width. The chemical composition of all particles was spectrophotometrically analyzed by micro Fourier Transform Infrared Spectroscopy ( $\mu\text{FTIR}$ ), using a Perkin-Elmer Spotlight 200 Spectrum two apparatus with mercury cadmium telluride detector. For it, the analyzed particles were placed on KBr, which was used as a slide, and their spectra were recorded in micro-transmission mode using the following parameters: spot  $50 \mu\text{m}$ , 32 scans, and spectral range  $550\text{--}4000 \text{ cm}^{-1}$  with  $8 \text{ cm}^{-1}$  resolution. The spectra were compared with Omnic 9 database and with spectra from our own database showing  $>70\%$  matching in all cases, which was considered enough for positive identification of plastic materials (Liu et al., 2019).

### **2.4. Prevention of procedural contamination**

Several measures were taken to avoid sample contamination. Glass and steel material were cleaned with MilliQ water (filtered through a  $0.22 \mu\text{m}$  filter, particle and bacteria free), wrapped with aluminum foil and heated up to  $450^\circ\text{C}$  for 4 h to remove all possible remains of plastics. The aluminum foil used for storing and shipping the drifting nets was also wrapped with aluminum foil and heated to  $450^\circ\text{C}$  for 4 h. During sampling, only one person conducted the experiments without any other person in the vicinity. Nobody walked into the stream's watershed throughout the experiment. During laboratory manipulation, clothing was controlled by using non-typical bright colors (blue, orange and purple) made of 100% cotton. Glass Petri dishes with the collected meshes were opened only to place the meshes after initial storage and during filtering and identification. All types of plastics present during sampling and laboratory experiments were not considered in the results such as those present in the drifting nets (Fig. S1, Supplementary Material, SM) and in personal masks (Fig. S2, SM). A full set of procedural controls was performed to ensure the absence of contamination during sampling and manipulation. Sampling controls consisted of two glass Petri dishes containing a glass fiber filter, which were kept open side-by-side of the stream during the sampling nets deployment and removal. Laboratory controls consisted of another set of glass Petri dishes with a glass fiber filter kept open in the working area of the laboratory. Further negative controls were also arranged during digestion, density separation and filtration.

## **3. Results and discussion**

A total number of 36 particles  $<5 \text{ mm}$  length was recovered from the  $100 \mu\text{m}$  mesh ( $n = 12$ ) and the  $333 \mu\text{m}$  mesh ( $n = 24$ ) after eight days of sampling. 29 particles of presumed anthropogenic origin (8 particles from the  $100 \mu\text{m}$  mesh and 21 particles from the  $333 \mu\text{m}$  mesh) were analyzed by  $\mu\text{FTIR}$ . Eight particles were positively identified as MPs, six fibers and two films. The larger dimension of the films was 869 and  $3546 \mu\text{m}$ . Fiber length were in the  $400\text{--}1327 \mu\text{m}$  range, while fiber width varied in the  $10\text{--}26 \mu\text{m}$  range. Additional details can be found in Table S1 (SM). The MPs identified were: two fibers were identified as acrylic, one transparent (Fig. 2A) and one red (Fig. 2B); four polyester fibers, one black and three transparent (Fig. 2C, D, E and F); and two transparent

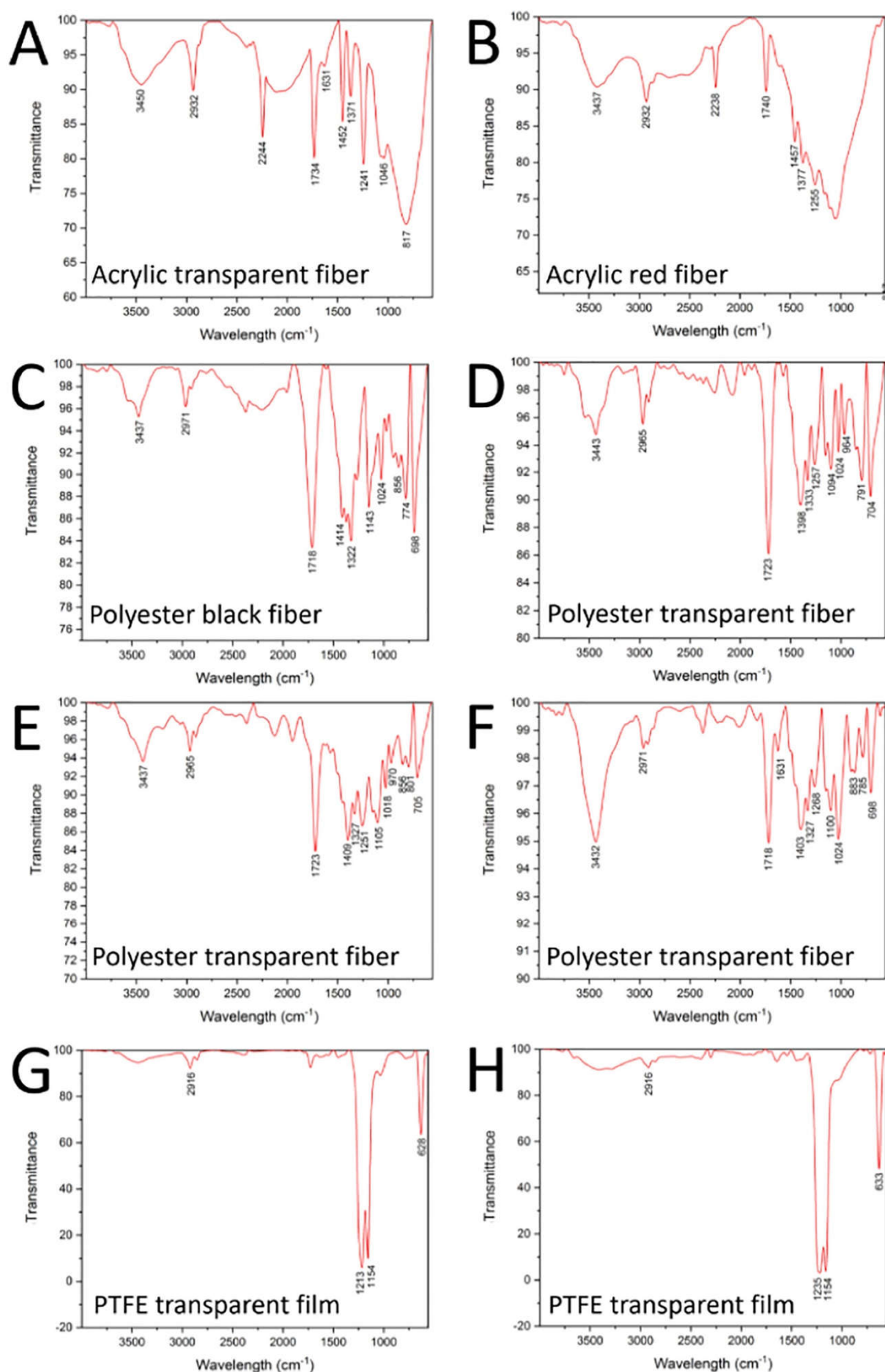
polytetrafluoroethylene (PTFE) films (Fig. 2G and H). It is important to note that the chemical composition of all MP materials identified in this work was different from any other kind of material in the nets used for sampling and from those in laboratory materials (Figs. S1 and S2, SM). In sampling and laboratory controls, no particles of the same composition were detected. In laboratory controls, we detected eight particles identified as cotton with Pearson correlation matching >70%. No contamination was detected that could compromise the origin of the identified MPs. Based on the total flow through the mesh and the number of MPs found, the concentration of MPs in freshwater was 0.95 items/1000 m<sup>3</sup> with estimated variability in the 0.47–1.43 items/1000 m<sup>3</sup> range based on the comparison between both nets. It is important to note the high volume of water filtered, which makes this result reasonably representative of their environmental concentration. The micrographs of all MPs found are shown in Fig. 3 in the same order as in Fig. 2.

The presence of MPs (fibers and films) of polyester, acrylic and PTFE has been demonstrated in a freshwater stream of the Byers Peninsula, a place completely apart from human activities other than strictly limited research campaigns. Apart from the isolation and specific characteristics of the sampling site, this is the first time that MPs are reported in any freshwater network within the Antarctic continent. Even if the concentration is low compared to populated areas, their mere presence in such a remote region is puzzling and a cause for concern.

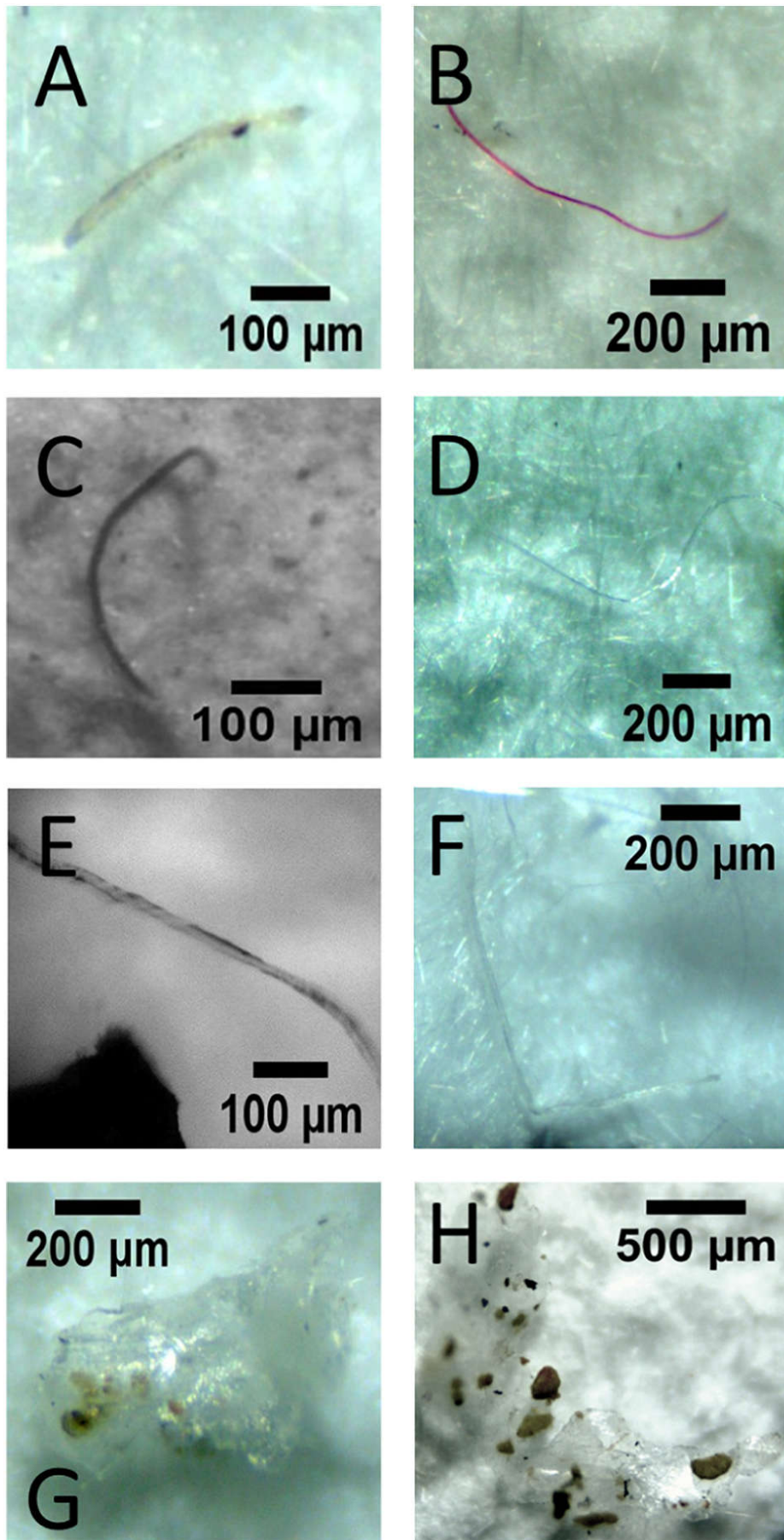
The long-range atmospheric transportation of MPs seems to be a likely explanation of our findings. It has been shown that air masses can transport MPs across relatively long distances and it is an explanation proposed elsewhere to account for the presence of MPs in other remote environments (Allen et al., 2019; Ambrosini et al., 2019; González-Pleiter et al., 2020; Zhang et al., 2016). However, there is some difficulty to explain how MPs could have reached this remote area because atmospheric transport is usually considered improbable if pollution sources are separated by long distances. The recent finding of MP at high altitude demonstrate the potential of the atmosphere for the long-range transport of MP (González-Pletier et al. González-Pleiter et al., 2021). Atmospheric transport and deposition simulations performed using the HYbrid Single-

Particle Lagrangian Integrated Trajectory (HYSPLIT) models clearly showed that MPs may be transported to distant places before being deposited (Brahney et al., 2020; González-Pletier et al. González-Pleiter et al., 2021). Possible local sources are very scarce because human activities in the area are extremely restricted, but meteorological conditions could be compatible with wind transportation of MPs from other Antarctic research stations (Juan Carlos I, Gabriel de Castilla or St. Kliment Ohridsky). King George Island could be another possible origin due to the presence of research stations and some touristic activities, although these potential sources are more than 100 km away from the Byers Peninsula. Noticeably, PTFE, as well as acrylic and polyester fibers are used to produce waterproof winter clothes. Anyway, it should be considered that atmospheric transport of MPs is a new area of atmospheric science and the available data are still very limited. Further research would be needed to further clarify the role of the atmosphere as a dispersion pathway of MPs, including the distance that MPs can travel in order to understand their presence in remote areas such as Byers Peninsula.

An alternative explanation is that MPs could have reached the freshwater ecosystems of Byers Peninsula through the sweeping of the plastic debris from marine origin. The presence of MPs has already been reported in Antarctic marine ecosystems, including marine surface waters (Cincinelli et al., 2017), oceanic zooplankton samples (Absher et al., 2019), marine sediments (Munari et al., 2017; Reed et al., 2018) and sea ice (Kelly et al., 2020). The strong gusts of wind that occur in the Byers Peninsula (100–140 km/h) could drag MPs from landing on the beaches of Byers Peninsula to the freshwater networks on its central plateau. In fact, a similar process has been observed with marine macroalgae. Macroalgal debris are frequently found at the high plateau at Byers Peninsula (80 m above sea level). For example, a debris of about 35 cm long and 4 cm wide was found by us at the shores of limnopol lake located at UTM coordinates 597.1; 3.052.200, in February 2002, which is more than 2 km away from the seaside. Another possible source, although more unlikely, might be bird depositions. MPs have been detected in marine animals in Antarctica, including penguins and, therefore, they could act as a vector for MPs to freshwater (Bessa et al., 2019; Sfriso et al., 2020; Le Guen et al., 2020). In this sense, the stream studied is one of



**Figure 2.**  $\mu$ FTIR spectra of the MPs found in this work. (A) transparent acrylic fiber, (B), red acrylic fiber, (C) black polyester fiber, (D, E and F) transparent polyester fibers, (G and H) transparent PTFE films. (Dimensions given in Table S1, SM.)



**Figure 3.** Images of the MPs identified in this work. A to H are the same as in Fig. 2.

the outlets with the highest conductivity in the area at its mouth in the sea due to the influence of local fauna located upstream (Toro et al., 2007). The concentration of MPs in the sampled area was low compared to those recorded in other places (Rios Mendoza and Balcer, 2019; Wong et al., 2020). Uurasjärvi et al., recorded the presence of  $1.8 \pm 2.3$  ( $>300 \mu\text{m}$ ) and  $12 \pm 17$  ( $100\text{--}300 \mu\text{m}$ ) microplastics/ $\text{m}^3$  in pump filtered samples from a northern European dimictic lake (Uurasjärvi et al., 2020). An investigation performed in two mountain lakes in the northern Apennines in central Italy showed MP concentration ( $>0.3 \text{ mm}$ ) in the  $0.82\text{--}4.42$  particles/ $\text{m}^3$  range (Fischer et al., 2016). This fact reflects the high standards of Antarctic environmental protection but also the difficulty to avoid the contamination due to plastic debris.

#### 4. Conclusions

This research showed the presence of microplastic pollution consisting of polyester and acrylic fibers, and polytetrafluoroethylene films in a freshwater stream of Byers Peninsula, an Antarctic Specially Protected Area far from any human activities. The concentration of microplastics was low when compared with other areas but showed that even the most pristine and protected environments are not free of plastic pollution.

#### Acknowledgements

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## SUPPLEMENTARY MATERIAL

### First detection of microplastics in the freshwater of an Antarctic Specially Protected Area

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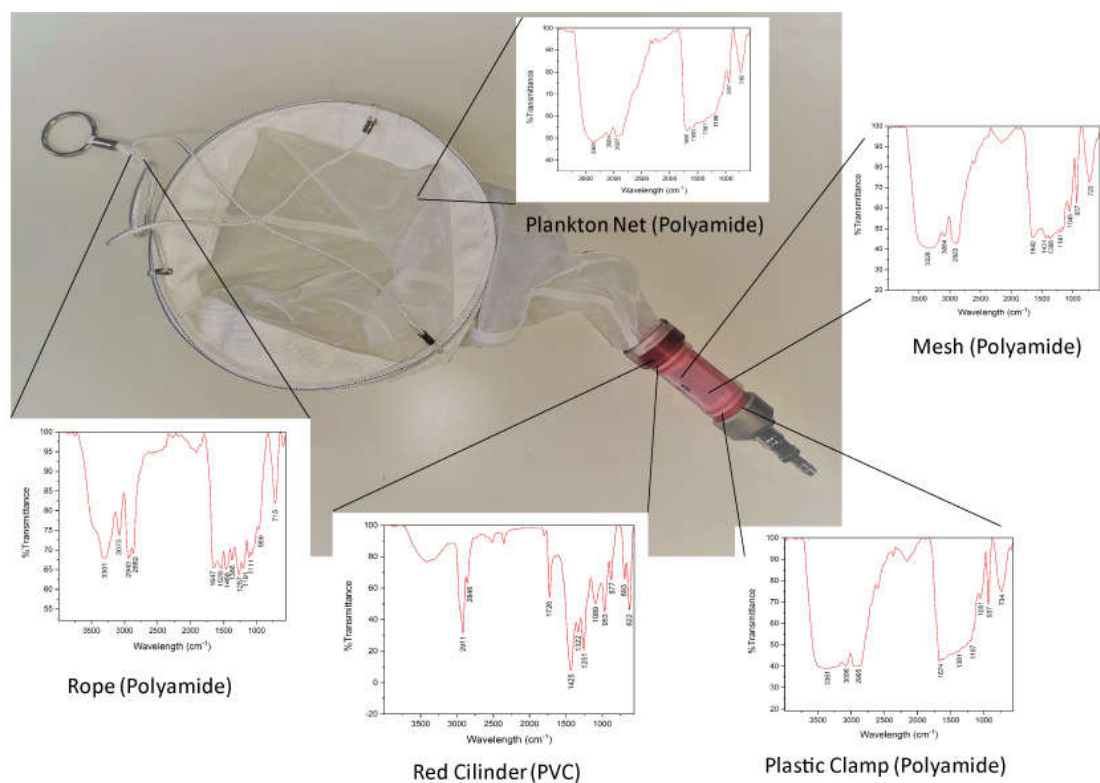
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#### Contents

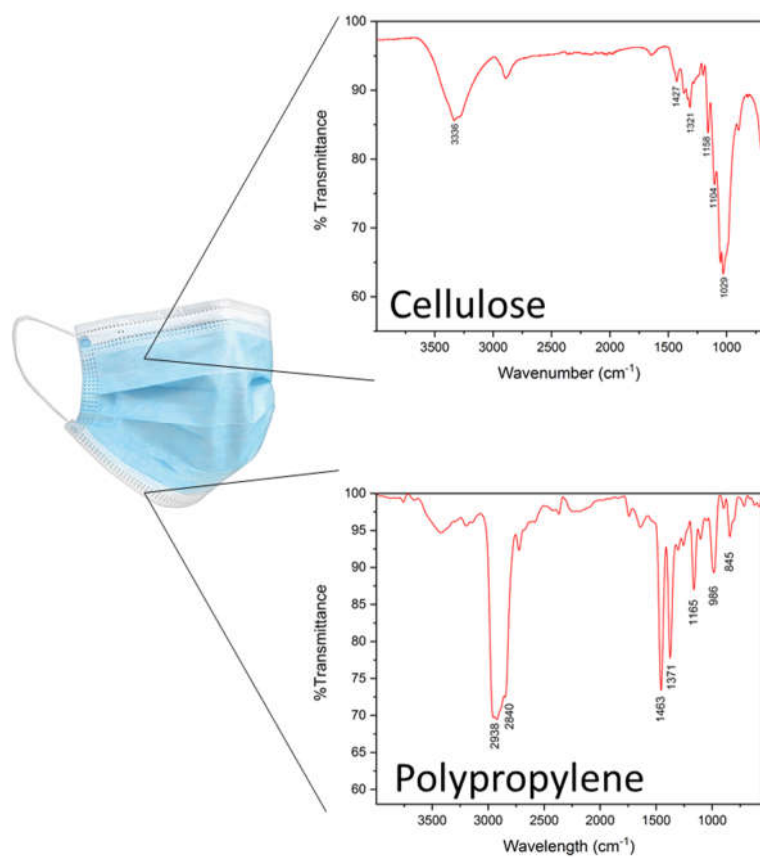
**Figure S1.** Net used during sampling with  $\mu$ FTIR spectra of plastics present in it.

**Figure S2.** Mask with  $\mu$ FTIR spectra of plastics present in it.

**Table S1.** Additional details about MP.



**Figure S1.** Net used during sampling with  $\mu$ FTIR spectra of plastics present in it.



**Figure S2.** Mask with  $\mu$ FTIR spectra of plastics present in it.

**Table S1.** Additional details about MP.

Sample ID	Type of MP	Shape	Color	Length (μm)	Width (μm)
A	Acrylic	Fiber	Transparent	400	20
B	Acrylic	Fiber	Red	1041	13
C	Polyester	Fiber	Black	414	11
D	Polyester	Fiber	Transparent	1327	10
E	Polyester	Fiber	Transparent	509	11
F	Polyester	Fiber	Transparent	835	26
G	PTFE*	Film	Transparent	869	482
H	PTFE*	Film	Transparent	3546	1026

\* Polytetrafluoroethylene