Stimulated Raman microspectroscopy as a new method to classify microfibers from environmental samples

Sergey P. Laptenok, Cecilia Martin, Luca Genchia, Carlos M. Duarte, Carlo Liberale

Abstract

Microfibers are reported as the most abundant microparticle type in the environment. Their small size and lightweight allow easy and fast distribution, but also make it challenging to determine their origin. Vibrational microspectroscopic methods as infrared and spontaneous Raman microscopy have been widely used for the identification of environmental microparticles. However, only a few studies report on the identification of microfibers, mainly due to difficulties caused by their small diameter. Here we present the use of Stimulated Raman Scattering (SRS) microscopy for fast and reliable classification of microfibers from environmental samples. SRS microscopy features high sensitivity and has the potential to be faster than other vibrational microspectroscopic methods. As a proof of principle, we analyzed fibers extracted from the fish gastrointestinal (GIT) tract, deep-sea and coastal sediments, surface seawater and drinking water. Challenges were faced while measuring fibers from the fish GIT, due to the acidic degradation they undergo. However, the main vibrational peaks were still recognizable and sufficient to determine the natural or synthetic origin of the fibers. Notably, our results are in accordance to other recent studies showing that the majority of the analyzed environmental fibers has a natural origin. Our findings suggest that advanced spectroscopic methods must be used for estimation of the plastic fibers concentration in the environment.

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1. Introduction

Exponential growth in the use of synthetic polymers (Geyer et al., 2017) has raised concerns due to exposure of marine life and humans to these prevalent plastic particles in the environment (Bellingeri et al., 2020; Law, 2017; Levermore et al., 2020; Lv et al., 2019; Pauly et al., 1998; Rochman et al., 2015; Schwabl et al., 2019). In the marine environment, plastic particles occur in a range of sizes, with large particles fragmenting into microplastics (<5 mm), which are the most abundant size class in the ocean (Cozar et al., 2014; Eriksen et al., 2014). In addition to microplastic particles, synthetic fibers represent a very abundant class of polymers in the environment. Fibers typically outnumber microplastic particles by a factor of 10 across habitats, from freshwater to marine (Anderson et al., 2017; Barrows et al., 2018; Horton et al., 2017). Fibers are prevalent in the environment, reported in drinking water, industrial drinks, table salt, honey, sugar, wastewater effluents, rivers, and the ocean (Browne et al., 2011; Hernandez et al., 2017; Kosuth et al., 2018; Liebezeit and Liebezeit, 2014, 2013; Taylor et al., 2016; Yang et al., 2015). Environmental fibers are typically small, with characteristic dimensions of up to a few mm in length and <50 μm, often <15 μm, in diameter (Carr, 2017; Liu et al., 2019; Mishra et al., 2019). Their small size allows for easy transport, including Aeolian transport (Dris et al., 2017, 2016), but makes it hard to characterize the fibers, compared to other microplastic particles. The synthetic nature of particles classified as putative synthetic polymers is typically confirmed using FT-IR and Raman spectroscopy (Kappler et al., 2016; Lüder and Gerdz, 2015; Shim et al., 2017). This routine verification should be applied also to environmental fibers, as fibers within the this size range can also be of natural origin (Lüder and Gerdz, 2015; Remy et al., 2015). For instance, production and processing of timber, plant waste, paper, and building materials are sources of plant-derived fibers to the environment (Elanthikkal et al., 2010; Galaska et al., 2017; Kvavadze et al., 2009).

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Lacorte et al., 2003; Reichert et al., 2015), along with manufacturing, processing, and washing of textiles of vegetal and animal origin (e.g., cotton, linen, wool, silk) (Galaska et al., 2017). However, whereas the classification of microplastic particles is becoming straightforward using commercial FT-IR and spontaneous Raman spectroscopy systems, the analysis of environmental fibers remains challenging mainly due to their small diameter (Araujo et al., 2018). In particular, while the classification of environmental fibers has been demonstrated with FT-IR microspectroscopy, mostly in ATR (Attenuated Total Reflectance) mode (Dris et al., 2016; Obbard et al., 2014), this method has a challenging application to the case of individual fibers with small sizes (Araujo et al., 2018; Levermore et al., 2020; Stanton et al., 2019). Spontaneous Raman microspectroscopy is superior to FT-IR in the identification of very small particles (Araujo et al., 2018; Köppler et al., 2016; Renner et al., 2018). More recently, detection methods based on optical tweezers and plasmatic enhancement of the Raman signal have been shown to detect plastic particles with size below 1 μm (Gillibert et al., 2019; Lv et al., 2020). However, conventional Spontaneous Raman microspectroscopy suffers from limitations due to long integration times and to its susceptibility to autofluorescence, and only a few studies have used this technique to classify fibers (Ghosal et al., 2018; Zobkov et al., 2019). As a consequence of these limitations, only a fraction of studies reporting on environmental fibers presents a verification of their synthetic nature (Cincinelli et al., 2017; Hernandez et al., 2019; Le Guen et al., 2020; Miller et al., 2017; Song et al., 2015; Yang et al., 2015). This may lead to an overestimation of the abundance of synthetic fibers present in the environment, because a fraction of fibers found in the environmental samples may be instead of natural origin. Here we show that a Coherent Raman technique, Stimulated Raman Scattering (SRS) microscopy (Laptenok et al., 2019), is a highly sensitive vibrational spectroscopy method that allows the fast and reliable identification of the nature, synthetic or otherwise, of individual microfibers from environmental samples. The intrinsic 3D optical sectioning capability of the multiphoton SRS process allows to analyze small fibers on conventional substrates, like glass slides, without collecting the spurious background signal generated by the substrate itself. As a comparison, in spontaneous Raman microscopes the signal from the substrate can only be minimized using a confocal configuration, but at the cost of longer integration times that sensibly slow down the acquisitions. Moreover, opposite to Spontaneous Raman, the SRS signal has the advantage of being intrinsically insensitive to parasitic auto-fluorescence from the sample.

2. Materials and methods

2.1. Environmental sample collection and preparation

Environmental fibers to be analyzed at the SRS microscope have been randomly subsampled from a pool of fibers obtained from surface seawater, coastal and deep-sea sediments, the gastrointestinal tract (GIT) of fish and drinking water samples (Fig. S1).

Surface seawater samples were collected using manta trawls as described in Marti et al. (2017). In summary, a manta net with a 150-μm mesh size was towed for 15–30 min in the surface of Saudi Arabian Red Sea waters at a speed of 2–3 knots. All the material congregated in the cod-end was collected and resuspended using 150-μm filtered seawater obtained from the same sampling station. The whole sample from each station was carefully screened at a stereomicroscope and fibers were picked and washed with distilled water. All material used was previously rinsed three times with distilled water and kept in aluminum foil until usage. Controls, consisting of Petri dishes with a thin layer of distilled water, were set alongside during the sample processing to account for contamination. Only 0 to 3 fibers were found in each control, representing only 4.2 ± 1.6% of the fibers found in each sample.

Sediment samples were collected using cores as described in Saderne et al. (2018). In summary, 1-m long PVC cores of 70 mm diameter were hammered into the soil of vegetated coastal habitats (mangroves and seagrasses) of the Saudi Arabian coast of the Red Sea. Cores were opened and sliced, using a ceramic knife, in 1-cm thick sediment samples that were oven-dried at 60 °C. Fibers extraction from the sediment sample (<60 g) occurred via density separation using 1.5 g cm⁻² solution of Zinc Chloride into a Sediment-Microplastic Isolation unit as described in Coppock et al. (2017). The supernatant was vacuum-filtered through a 25-μm nylon mesh and carefully screened at the stereomicroscope to pick fibers, later rinsed with distilled water. During the extraction of fibers from sediments, all the material used was rinsed three times with distilled water and kept in aluminum foil until usage and controls were set alongside as described before. Only 0 to 2 fibers were found in each control Petri dish, accounting for 6.5 ± 3.2% of the fibers extracted from each sample.

Deep-sea sediment samples were collected in the Atacama Trench (23° 21′ S 71° 20′ W, at < 5500 m of depth) using a multi core. Sediments inside the cores were extruded using a metal piston, sliced in 1-cm thick sediment samples and oven-dried at 40 °C. Given the small volume of the sample (10 g wet weight, < 5 g dry weight), the whole sample was visually screened at the stereomicroscope and fibers picked. To avoid contamination, the visual screening occurred inside a laminar fume hood, which bench was previously cleaned using distilled water. All the material used was previously rinsed three times with distilled water and kept in aluminum foil until usage. No fibers were encountered in the controls set alongside while processing the sample.

Fish were obtained from commercial fishermen along the Saudi Arabian coast of the Arabian Gulf. The analyzed species include Siganus canaliculatus, Rastrelliger kanagurta, Lethrinus nebulosus, Gerres acinaces. Samples from the fish gut intestinal tract (GIT) were obtained as described in Baalkhuyuur et al. (2020). In summary, the GIT was extracted through dissection of the fish, placed in a 50 mL falcon tube, and oven-dried at 60 °C for 1 h. A 30-mL solution of NaOH (10 M) was added for tissue digestion, that occurred for 3 days at room temperature, followed by filtration through a 200-μm stainless steel sieve. The sample was rinsed out of the sieve using distilled water and collected in a Petri dish where it was visually inspected by use of a stereomicroscope. Fibers were picked and washed with distilled water. All materials used were previously washed with distilled water and kept in aluminum foil until usage and only 1 fiber every 3 samples were found in the controls set alongside. To assess the impact of the tissue digestion procedure, we subjected test fibers of synthetic (i.e. nylon, polyester) and natural origin (i.e. paper, cotton and fibers from a banana leaf) to NaOH (10 M) for 3 days at room temperature. Moreover, we exposed test fibers of the same type to 0.1 M HCl (from 1 day to two weeks) to mimic the acidic environment in the fish stomach and verify if fibers are affected while in the fish GIT.

Drinking water samples were obtained from Elkay ezH2O bottle filling stations, itself connected to a desalinated drinking water supply installed at King Abdullah University of Science and Technology (Thuwal, Saudi Arabia). For each sample, two 1-L glass bottles were previously rinsed with distilled water, by filling, to the point of overflowing, and emptying the bottle three times. The third time, water was let overflow for 1 min. Cleaned glass bottles were immediately capped. Caps were also rinsed three times with distilled water. The faucet of the refilling station was rinsed thoroughly with distilled water and 1 L of drinking water was let flown before filling the two glass bottles. After shaking vigorously,
drinking water from the two glass bottles was filtered through a 1.2-μm polycarbonate filter. The two bottles were repeatedly rinsed with distilled water to ensure collection of all the fibers. The filter was inspected at the stereomicroscope and fibers picked. To prevent contamination, all procedures from filtering to visual inspection occurred inside a laminar fume hood, which bench was cleaned using distilled water. Additionally, a Petri dish with distilled water was set inside the fume hood as a control to verify occurrence of contamination. No fibers were encountered in the Petri dish. All material used was previously rinsed three times with distilled water and kept in aluminum foil until usage. As a control, we repeated the same procedure three times using distilled water instead of drinking water to account for potential contamination during the sample processing. No fibers were found in the procedural controls.

To analyze the samples at the SRS microscope, fibers were carefully picked and compressed between two clean glass coverslips, which were then sealed. This step is particularly important in the study of microfibers since they are easily moved by the airflow and lost, an issue not typically present with non-fiber types of microparticles. This operation occurred inside the laminar fume hood to avoid contamination. Remarkably, SRS microscopy does not require special and expensive substrates as instead needed for infrared and spontaneous Raman microscopy (Levermore et al., 2020).

2.2. SRS microscope

The broadband SRS microscope used in this work was previously described by Laptenok et al. (2019). In short, it is based on a dual-beam femtosecond laser system with an 80 MHz repetition rate (Chameleon Discovery, Coherent Inc., Santa Clara, CA). One of the two output beams has a fixed wavelength (1040 nm) and acts as Stokes beam. This beam is spectrally filtered down to a 0.9 nm bandwidth and modulated at 5 MHz with an acousto-optical modulator. A second, tunable, output of the laser is filtered with an acousto-optical tunable filter down to a 0.5 nm bandwidth and used as a pump beam. The two beams are spatially and temporally overlapped, using a dichroic mirror and a mechanical delay line, and guided to an inverted laser scanning microscope (Nikon Eclipse Ti-E). The combined laser beams are focused to a ca. 350 nm diffraction limited spot on the sample by a water-immersion high numerical aperture objective (Nikon CFI Plan Apo IR SR 60XWI, NA = 1.27). The signal is collected in the forward direction by a second microscope objective (Nikon CFI Apo LWD Lambda S 40XC WI, NA = 1.15). The total power of the excitation was measured at the sample plane and set according to the requirements of the sample. The maximum power of the Stokes beam was set to 45 mW on the sample plane (corresponding to 47 MW/cm²•s intensity) for the samples that could be measured with high power, and 5–15 mW on the sample plane (corresponding to 5–16 MW/cm²•s intensity) for more fragile samples. The pump beam was set at 5–10 mW on the sample plane (corresponding to 5–10 MW/cm²•s intensity). Our SRS microscope features a continuous tuning range (800–3600 cm⁻¹) and acts as a pump beam. The two beams are spatially and temporally overlapped, using a dichroic mirror and a mechanical delay line, and guided to an inverted laser scanning microscope (Nikon Eclipse Ti-E). The combined laser beams are focused to a ca. 350 nm diffraction limited spot on the sample by a water-immersion high numerical aperture objective (Nikon CFI Plan Apo IR SR 60XWI, NA = 1.27). The signal is collected in the forward direction by a second microscope objective (Nikon CFI Apo LWD Lambda S 40XC WI, NA = 1.15). The total power of the excitation was measured at the sample plane and set according to the requirements of the sample. The maximum power of the Stokes beam was set to 45 mW on the sample plane (corresponding to 47 MW/cm²•s intensity) for the samples that could be measured with high power, and 5–15 mW on the sample plane (corresponding to 5–16 MW/cm²•s intensity) for more fragile samples. The pump beam was set at 5–10 mW on the sample plane (corresponding to 5–10 MW/cm²•s intensity). Our SRS microscope features a continuous, rapidly tunable and high-spectral-resolution broadband operation in the vibrational range 800–3600 cm⁻¹ that is critical for the capability to identify materials of unknown chemical composition. The broadband SRS spectra were collected from a single point on each fiber sample. The measurement point was manually selected from an SRS image of the sample, obtained using a single wavenumber in the CH-stretch spectral range (2900 cm⁻¹).

2.3. Data analysis

We first obtained a library of reference Raman spectra from textiles or materials made out entirely (100%) of a single polymer type, including synthetic polymers – namely polyethylene (PE), polypropylene (PP), polystyrene (PS), polyester (PET), polyacrylonitrile (PAN), polycarbonate (PC), poly(methylmethacrylate) (PMMA), nylon – and natural fibers (i.e., cotton, linen, silk, and wool). Additionally, paper, wood from a pencil and fibers from banana leaf were also included as additional reference materials for fibers of vegetal origin. The SRS reference spectra were cross validated using a commercial spontaneous Raman system (XploRA Plus confocal Raman microscope, Horiba).

The SRS spectra were collected in a Raman shift range spanning from 800 to 3200 cm⁻¹ with 7 cm⁻¹ resolution. The total acquisition time for a single spectrum, with an integration time of 5 ms per wavenumber, was about 100 s. After acquisition, the raw oversampled SRS spectra were smoothed using a running average routine with a window size of 3. Thanks to the absence of contributions to the SRS signal from the substrate – a serious problem in spontaneous Raman acquisitions of very small fibers (see Fig. 57) – and from sample auto-fluorescence, there was no need to apply a background subtraction. The identification of the fiber composition was done by manually comparing the position of the main peaks in the measured SRS spectra against the library of the measured reference substances.

We implemented discriminant analysis for the statistical classification of the measured fibers. First, we performed Principal Component Analysis (PCA) of spectra obtained from both the reference materials and the environmental samples using the whole measured spectral range (800-3200 cm⁻¹). Then we performed cluster analysis based on the k-means clustering algorithm using squared Euclidean distance to calculate distance between centroids in multidimensional space; we tested the results of the clustering for significance using the Multivariate analysis of variance (MANOVA) test. Finally, we used sparse partial least squares discriminant analysis, as described by Monteiro et al. (2016), to find the most discriminating wavenumbers. This set of wavenumbers could allow the high-throughput classification of microfibers in large-area SRS imaging experiments, in a similar way as done with microparticles by Zada et al. (2018). All data manipulations were done using Matlab 2019a (The MathWorks).

3. Results and discussion

The SRS spectra obtained for reference synthetic and natural materials measured allowed the creation of a custom library, including eight natural (Fig. 1A and Fig. S2A) and eight synthetic polymers (Fig. 1B and Fig. S2B), comprising the most common types of fibers in the environment.

The natural fibers in the reference set can be divided in two groups, namely cellulosic fibers, which are fibers that derive from cellulose and structurally are polysaccharides, and animal fibers, like silk and wool, which are made of proteins. The SRS spectra of cellulosic fibers were highly variable (Fig. 1A and Fig. S2A) due to changes that can occur in the cellulose conformation and to different interactions with the water content (Agarwal, 2014). However, there are some characteristic features that can be used to identify cellulosic fibers, such as peaks around 1100 cm⁻¹, a group of peaks between 1200 cm⁻¹ and 1500 cm⁻¹, and the main peak at 2900 cm⁻¹ (Agarwal, 2019) (Fig. 1A and Fig. S2A). Other peaks around 1600 cm⁻¹, 2950 cm⁻¹ and 3010 cm⁻¹ can be observed in woody fibers and are associated with the presence of lignin (Fig. 1A). These peaks can also vary between samples due to the high variability of lignins in different plants and lignins compositions (Agarwal, 2019). The SRS spectra of the animal fibers have the main spectroscopic features of the vibrational spectrum of proteins, namely the Amide 1 peak at around 1650 cm⁻¹, the CH deformation
peak at 1450 cm\(^{-1}\), and multiple peaks in the CH stretching region with a maximum at 2930 cm\(^{-1}\) (Edwards and Farwell, 1995; Hogg et al., 1994). Despite similarities, the animal fibers can be clearly distinguished from the cellulosic fibers (Fig. 1 A).

The SRS spectra of the synthetic fibers, differently from those of natural fibers, are characterized by sharper peaks distributed over the fingerprint (800 cm\(^{-1}\) – 1800 cm\(^{-1}\)) and CH stretch (2700 cm\(^{-1}\) – 3200 cm\(^{-1}\)) regions. Moreover, polyacrylonitrile has a unique peak at 2245 cm\(^{-1}\), which is related to a CN vibration (Fig. 1B, light green spectrum). Each of the plastic samples is characterized by a specific set of vibrational frequencies and can be easily identified. Remarkably, the spectra of both types of fibers (natural and synthetic) show an overlap of multiple peaks in fingerprint and CH regions. Therefore, the access to wavenumbers across the whole fingerprint-to-CH-stretch spectral range (800 cm\(^{-1}\) – 3200 cm\(^{-1}\)) is crucial for a reliable classification, especially for implementation of partial least squares discriminant analysis, where measurements on sparse wavenumbers have the potential to provide a fast semi-automated method for fiber classification (Zada et al., 2018).

All fibers retrieved from environmental samples had similar dimensions, with a diameter of 10–20 µm and different lengths, ranging from 100 µm to a few millimeters, regardless of source and type. In Fig. 2 and Fig. S3 we show the optical images and the SRS spectra collected on the fibers retrieved from environmental samples. Most of the fibers that could be classified can be attributed to natural origin materials, with fibers of natural origin accounting on average (±SE) for 71.8 ± 12.0% of the fibers that could be successfully analyzed across sample types (=natural fibers/natural + synthetic fibers), Table 1, Fig. S3). We identified three different synthetic fibers (PP, PAN, PET) in the environmental samples and drinking water. Remarkably, for the smallest fiber found (8 µm diameter, cotton fiber, Fig. 2 E), we also performed a spontaneous Raman measurement and compared it to the SRS spectral acquisition (see SI, Fig. S7). This comparison was done to demonstrate the advantages of SRS when used to identify the smallest fibers, particularly if made of materials with a Raman cross section not as large as that of plastic fibers. It should be noted that in this methodological proof-of-principle study we analyzed only a small and representative subset from the total number of the extracted fibers from environmental samples.

While the majority of measured fibers generated clear SRS spectra (Fig. 2), most of the fibers extracted from the fish GIT were either destroyed during the SRS measurements, even when low laser power (<10 mW on the sample plane, corresponding to a power density of 10 MW/cm\(^2\)) was applied (Fig. S4), or could not be measured due to a significantly high background that has transient absorption behavior (Fu et al., 2007) (Fig. S5). Even with such background, the main cellulose peaks (1100 cm\(^{-1}\) and 2900 cm\(^{-1}\)) are still recognizable; however, we excluded such fibers from the classified fibers. The challenge faced in measuring fibers from fish GIT samples, when compared to other environmental samples, could be attributed to the effect of the acidic environment typical of the fish stomach, that would partially digest fibers of natural origin while maintaining synthetic fibers intact. A digestion test that we made by exposing reference fibers to Hydrochloric Acid (HCl) was in agreement with this hypothesis, suggesting that the destroyed fibers from environmental samples, particularly those from fish GIT, are likely of natural origin. Indeed, the test fibers of synthetic origin (nylon, polyester) were still well measured even after long (2 weeks) treatment with concentrated (12 M) HCl (Fig. 3A); conversely, cellulosic fibers (i.e., cotton, fibers from banana leaves and paper) became much more fragile after just one day exposure to 0.1 M HCl and were easily destroyed with very low intensity laser beam, similarly to what occurred to most of the fibers retrieved from fish GIT (Fig. 3 B, C). Differently, the digestion in NaOH affected neither synthetic nor natural fibers (Fig. S6), demonstrating that the method used to extract them from the fish GIT did not further affect the likelihood of these sample fibers being destroyed.

Based on these results, assuming the 16 destroyed fibers from fish GIT to be of natural origin, as supported by the HCl test, then the average (±SE) proportion of fibers of natural origin retrieved from environmental samples increases to 83.8 ± 3.0% of characterized fibers (=natural fibers/(natural + synthetic fibers), but considering natural fibers from fish sample to be 17, Table 1). Despite this being a small subsample, this agrees with other studies that, characterizing fibers, found that most of the microfibers extracted from environmental samples were of natural origin. In the recent study of Le Guen et al. (2020) (fibers from penguin faecal pellets) and of Wu et al. (2020) (fibers from commercial aquatic species), the majority of the fibers (up to 88%) were attributed to a natural origin. In Song et al., 2015, 85% of the fibers from surface seawater samples, initially considered synthetic after microscopic identification following Noren (2007) criteria, were found to be natural, made of cotton or rayon. In the study of Miller et al. (2017), where 14 fibers were characterized by use of FTIR microspectroscopy, half of them were of natural origin (i.e., cotton and nitrocellulose). The results we obtained (Table 1), and those reported in the cited studies, which represent the few studies where the polymers of environmental fibers were characterized, are consistent in demonstrating that most of the fibers in environmental samples are of natural origin. In light of these findings, estimates reporting on concentrations of fibers in the environment, without characterization of their nature, cannot be assumed to...
represent synthetic fibers without additional verification.

The principal component analysis (PCA) allows separation of the main plastic fibers from the natural-origin fibers. The results of the k-means clustering on the PCA components 1, 2 and 4 of the dataset composed from the reference fibers and the fibers extracted from the environmental samples is shown in Fig. 4. In order to improve the stability of the k-means clustering, the starting centroids for the whole dataset were estimated by doing analysis of the dataset containing only reference spectra. PCA analysis shows a clear separation between natural and synthetic fibers, with only few exceptions (Fig. 4). Particularly, 2 synthetic fibers out of 20 (PAN, Fig. S3, fiber 4 from deep-sea...
sedsiments, and fiber 1 from fish GIT) clustered with natural-origin fibers, and 1 natural fiber out of 45 (wool, Fig. S3 fiber 3 from coastal sediments) clustered with plastic fibers. Moreover, the PCA analysis shows a separation among the main synthetic polymers (Fig. 4). The MANOVA test made on the estimated clusters shows significant difference across the means of the groups (for the vector of resulted P-values see supplementary materials). The main reason for misclassification is the low dimensionality of the data used for classification. In fact, only 3 PCA components were used as a compromise between precision and over-classification.

Table 1
Summary of fiber extracted and characterized in the 5 different sample types.

|                                | Total number of fibers classified | Natural fibers | Synthetic fibers | Destroyed fibers |
|--------------------------------|-----------------------------------|----------------|-----------------|-----------------|
| Drinking water                 | 10                                | 9              | 1               | 0               |
| Surface seawater               | 13                                | 9              | 2               | 2               |
| Coastal sediments              | 17                                | 10             | 3               | 4               |
| Deep-sea sediments             | 11                                | 8              | 1               | 2               |
| Fish GIT                       | 20                                | 1              | 3               | 16              |

"Destroyed fibers" refers to fibers that melted in the laser beam and damaged or partially degraded fibers that did not yield in an analyzable spectrum.

Fig. 3. (A) SRS spectrum of a polyester fiber exposed for 2 weeks to 12 M HCl (in black), compared to the polyester reference spectrum (in red), the raw SRS spectra were smoothed using a running average routine with a window size of 3, and no background correction was performed. Image of fibers from a banana leaf treated with 0.1 M HCl before (B) and after (C) attempt to measure it with a total power of the laser on the sample plane (15 mW). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4. Results of the k-means clustering on the PCA components of the combined dataset (reference spectra and spectra of the environmental fibers). The PCA decomposition was performed using the whole measured spectral range (800-3200 cm⁻¹).

From the partial least squares discriminant analysis, we found that the spectra of the eight plastic polymers in our reference database can be separated by measuring the following wave-numbers (857 cm⁻¹, 1289 cm⁻¹, 1445 cm⁻¹, 1613 cm⁻¹, 1727 cm⁻¹, 2245 cm⁻¹, 2855 cm⁻¹, 2900 cm⁻¹, 2954 cm⁻¹, 3075 cm⁻¹). Additional wavenumbers (1094 cm⁻¹, 1118 cm⁻¹, 1241 cm⁻¹, 1287 cm⁻¹, 2930 cm⁻¹, 2963 cm⁻¹) would allow also to separate natural fibers. The number of the wavenumbers that need to be measured in order to perform the classification depends on the number of species to be classified, with more classes requiring more spectral information. An analysis based on a sparse set of wavenumbers, is crucial to develop fast automatic classification tools of the fibers for SRS-based analyses. The sample preparation process can potentially become easier by using an SRS imaging mode with the direct scanning of the area of the whole filter (Zada et al., 2018) (on the order of centimeters), instead of manually picking up individual fibers and proceeding with a single point measurement of the whole spectrum. With the current microscope configuration, a single wavenumber SRS acquisition on a 1 × 1 cm² area, and with a 2 µm pixel spacing, can be collected in about 40 min. We note that spontaneous Raman analysis of microfibers directly on filters would be difficult and time consuming due to the background signal arising from the filter itself that would reduce sensitivity and eventually prevent material recognition. The intrinsic optical sectioning capability of SRS, on the contrary, selectively avoids signal from the substrate, and potentially allows fast and semi-automated analysis of microfibers directly on filters. For the filter to be used at the SRS microscope, it should have the following characteristics: a pore size on the order of 200 nm—1000 nm — as a compromise between minimum size of the particles to be detected, and force needed to push the solution through the filter — absence of a strong Raman signal that can overlap with the sample of interest, and it should allow a transmission path in the NIR region (700 nm—1100 nm). Aluminum oxide membranes are an example, however available filters with 20 nm pores also put some limitation on the extraction protocol (Zada et al., 2018).
4. Conclusions

Here we demonstrate the use of broadband Stimulated Raman Scattering (SRS) microscopy to analyze and classify fibers retrieved across a range of environmental samples and provide a reference library that led to characteristic wavenumbers suitable for diagnostic purposes. SRS analysis of microfibers of natural origin is shown for the first time. Our first finding from a small and representative subset of the samples suggests that only one in about five fibers were of synthetic origin. This finding emphasizes the need to classify the fibers retrieved from environmental samples using spectroscopic techniques. It also suggests that the estimate for the relative abundance of synthetic fibers in environmental samples probably needs to be revised downwards, as most of these fibers are likely of natural origin and pose no harm to organisms or the environment. However, more samples are needed to ultimately demonstrate that natural fibers outnumber synthetic ones in the environmental samples.

CRediT author statement

Serger P. Laptenok: Conceptualization, Investigation, Formal analysis, Writing - Original Draft Cecilia Martin: Resources, Investigation, Writing - Original Draft Luca Genchi: Investigation, Validation, Writing - Original Draft Carlos M. Duarte: Conceptualization, Supervision, Writing - Review & Editing Carlo Liberale: Conceptualization, Supervision, Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115640.

References

Agarwal, U.P., 2019. Analysis of cellulose and lignocellulose materials by Raman spectroscopy: a review of the current status. Molecules 24, 1659. https://doi.org/10.3390/ma24061659.
Agarwal, U.P., 2014. 1064 nm FT-Raman spectroscopy for investigations of plant cell walls and other biomass materials. Front. Plant Sci. 5, 1–12. https://doi.org/10.3389/fpls.2014.00490.
Anderson, P.J., Warrack, S., Langen, V., Challis, J.K., Hanson, M.L., Rennie, M.D., 2017. Microplastic in the surface waters of the Ross Sea (Antarctica): occurrence, distribution and characterization by FTIR. Chemosphere 175, 391–400. https://doi.org/10.1016/j.chemosphere.2017.02.021.
Copié, B., Cole, M., Lindeque, P.K., Queirós, A.M., Galloway, T.S., 2017. A small-scale, portable method for extracting microplastics from marine sediments. Environ. Pollut. 230, 829–837. https://doi.org/10.1016/j.envpol.2017.07.017.
Cozar, A., Eschevarria, F., Górdillo, J.I., Irginson, X., Ubeda, B., Hernandez-Leon, S., Palma, A.T., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernandez-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. Unit. States Am. 111, 10239–10244. https://doi.org/10.1073/pnas.1407407111.
Dris, R., Gasperi, J., Mirande, C., Gruau, M., Langlois, V., Tassin, B., 2017. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environ. Pollut. https://doi.org/10.1016/j.envpol.2017.09.034.
Dris, R., Gasperi, J., Saad, M., Mirande, C., Tassin, B., 2016. Synthetic fibers in atmospheric fallout: a source of microplastics in the environment? Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2016.01.006.
Eichhorn, K.-J., Voit, B., 2016. Analysis of environmental microplastics by Raman spectroscopy of silks. J. Raman Spectrosc. 26, 901–909. https://doi.org/10.1002/jrs.3250608482.
Elnanhkhal, S., Gopalakrishnapanicker, U., Varghese, S., Guthrie, J.T., 2010. Cellulose microfibres produced from banana plant wastes: isolation and characterization. Carbohydr. Polym. https://doi.org/10.1016/j.carbpol.2009.12.041.
Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiél, M., Moore, C.J., Borero, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world’s oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9, e92836. https://doi.org/10.1371/journal.pone.0092836.
Fu, D., Ye, T., Matthews, T.E., Chen, B.J., Yurtserver, G., Warren, W.S., 2007. High-spectral resolution stimulated Raman scattering microscope. J. Biophot. 12, 440. https://doi.org/10.1016/j.carbpol.2009.12.041.
Koelmans, A.A., Penna, A., Corsi, I., 2020. Impact of polystyrene nanoparticles on microorganisms: a review. Aquat. Microb. Ecol. 8391. https://doi.org/10.1007/s00204-019-09458-6.
Koelmans, A.A., Penna, A., Corsi, I., 2020. Impact of polystyrene nanoparticles on microorganisms: a review. Aquat. Microb. Ecol. 8391. https://doi.org/10.1007/s00204-019-09458-6.
Laptenok, S.P., Rajamanickam, V.P., Genchi, L., Monfort, T., Lee, Y., Patel, I.I., Koelmans, A.A., Penna, A., Corsi, I., 2020. Impact of polystyrene nanoparticles on microorganisms: a review. Aquat. Microb. Ecol. 8391. https://doi.org/10.1007/s00204-019-09458-6.
Laptenok, S.P., Rajamanickam, V.P., Genchi, L., Monfort, T., Lee, Y., Patel, I.I., Koelmans, A.A., Penna, A., Corsi, I., 2020. Impact of polystyrene nanoparticles on microorganisms: a review. Aquat. Microb. Ecol. 8391. https://doi.org/10.1007/s00204-019-09458-6.
e201900028. https://doi.org/10.1080/201900028.

Law, K.L., 2017. Plastics in the marine environment. Ann. Rev. Mar. Sci. 9, 205–229. https://doi.org/10.1146/annurev-marine-010816-080249.

Le Guen, C., Siuara, G., Sherley, R.B., Ryan, P.G., Aliani, S., Boehme, L., Brierley, A.S., 2020. Microplastic study reveals the presence of natural and synthetic fibres in the diet of King Penguins (Aptenodytes patagonicus) foraging from South Georgia. Environ. Int. 134, 105303. https://doi.org/10.1016/j.envint.2019.105303.

Levermore, J.M., Smith, T.E.L., Kelly, F.J., Wright, S.L., 2020. Detection of microplastics in ambient particulate matter using Raman spectral imaging and chemometric analysis. Anal. Chem. 92, 8732–8740. https://doi.org/10.1021/acs.analchem.9b05445.

Lieberzeit, G., Liebezeit, E., 2013. Non-pollen particulates in honey and sugar. Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess. https://doi.org/10.1080/19440049.2013.843025.

Liu, J., Yang, Y., Ding, J., Zhu, B., Gao, W., 2019. Microplastics in honey and sugar. Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess. https://doi.org/10.1080/19440049.2019.1485099.

Lv, L., He, L., Jiang, S., Zhou, C., Qu, J., Lu, Y., Hong, P., Sun, S., Li, C., 2020. In situ surface-enhanced Raman spectroscopy for detecting microplastics and nanoplastics in aquatic environments. Sci. Total Environ. 728, 138449. https://doi.org/10.1016/j.scitotenv.2020.138449.

Lv, L., Qu, J., Yu, Z., Chen, D., Zhou, C., Hong, P., Sun, S., Li, C. 2019. A simple method for detecting and quantifying microplastics utilizing fluorescent dyes - safarine T, fluorescein isothiocyanate, Nile red based on thermal expansion and contraction property. Environ. Pollut. 255, 113283. https://doi.org/10.1016/j.envpol.2019.113283.

Mishra, S., Charan Rath, C., Das, A.P., 2019. Marine microplastic pollution: a review on present status and future challenges. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2018.11.047.

Monteiro, J.M., Rao, A., Shawe-Taylor, J., Moura-Miranda, J., 2016. A multiple hold-out framework for sparse partial least squares. J. Neurosci. Methods 271, 182–194. https://doi.org/10.1016/j.jneumeth.2016.06.011.

Noren, F., 2007. Small plastic particles in coastal Swedish waters. KIMO Sweden 11.

Olbard, R.W., Sadri, S., Wong, Y.Q., Khitin, A.A., Baker, I., Thompson, R.C., 2014. Global warming releases microplastic legacy frozen in Arctic Sea ice. Earth’s Future. https://doi.org/10.1002/2014EF000240.

Pauly, J.L., Stegmeier, S.J., Alataa, H.A., Cheney, R.T., Zhang, P.J., Mayer, A.G., Streck, R.J., 1998. Inhaled cellulosic and plastic fibers found in human lung tissue. Cancer Epidemiol. Biomark. Prev.

Reichert, J.M., Rodrigues, M.F., Bervald, C.M.P., Brunetto, G., Kato, O.R., Schumacher, M.V., 2015. Fragmentation, fiber separation, decomposition, and nutrient release of secondary-forest biomass, mechanically chopped-and-mulched, and cassava production in the Amazon. Agric. Ecosyst. Environ. 204, 8–16. https://doi.org/10.1016/j.agee.2015.02.005.

Remy, F., Collard, F., Gilbert, B., Compère, P., Eppe, G., Lepoint, G., 2015. When microplastic is not plastic: the ingestion of artificial cellulose fibers by macrofauna living in seagrass macrophytodetritus. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.5b02005.

Reine, G., Schmidt, T.C., Schram, J., 2018. Analytical methodologies for monitoring micro(nano)plastics: which are fit for purpose? Curr. Opin. Environ. Sci. Heal. 1, 55–61. https://doi.org/10.1016/j.coesh.2017.11.001.

Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.C., Weerasinghe, S., Teh, S.J., 2015. Anthropogenic debris in seafood: plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci. Rep. https://doi.org/10.1038/srep14340.

Saderne, V., Cusack, M., Almahasheer, H., Serrano, O., Masqué, P., Arias-Ortiz, A., Krishnakumar, P.K., Rabouaï, L., Qurban, M.A., Duarte, C.M., 2018. Accumulation of carbonates contributes to coastal vegetated ecosystems keeping pace with sea level rise in an arid region (arabian peninsula). J. Geophys. Res. Biogeosciences. https://doi.org/10.1029/2017JD024288.

Schwabl, P., Köppler, S., Königshofer, P., Bascics, T., Trauner, M., Reiberger, T., Liebmanna, B. 2019. Detection of various microplastics in human stool: a prospective case series. Ann. Intern. Med. https://doi.org/10.7326/M19-0618.

Shim, W.J., Hong, S.H., Eo, S.E., 2017. Identification methods in microplastic analysis: a review. Anal. Methods. https://doi.org/10.1039/c6ay02558g.

Song, Y.K., Hong, S.H., Jang, M., Han, G.M., Rani, M., Lee, J., Shim, W.J., 2015. A comparison of microscopic and spectroscopic identification methods for analysis of microplastics in environmental samples. Mar. Pollut. Bull. 93, 202–209. https://doi.org/10.1016/j.marpolbul.2015.01.015.

Stanton, T., Johnson, M., Nathanial, P., MacNaughtah, W., Comes, R.L., 2019. Freshwater and airborne textile fibre populations are dominated by ‘natural’, non microplastic fibres. Sci. Total Environ. 666, 377–389. https://doi.org/10.1016/j.scitotenv.2019.02.278.

Taylor, M.L., Gwinnett, C., Robinson, L.F., Woodall, L.C., 2016. Plastic microfibre ingestion by deep-sea organisms. Sci. Rep. https://doi.org/10.1038/srep33997.

Wu, F., Wang, Y., Leung, J.Y.S., Huang, W., Zeng, J., Tang, Y., Chen, J., Shi, A., Yu, X., Xu, Z., Zhang, H., Cao, L., 2020. Accumulation of microplastics in typical commercial aquatic species: a case study at a productive aquaculture site in China. Sci. Total Environ. 708, 135432. https://doi.org/10.1016/j.scitotenv.2019.135432.

Yang, D., Shi, H., Li, L., Li, J., Jabeen, K., Kollandhasamy, P., 2015. Microplastic pollution in table salts from China. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.5b03163.

Zada, L., Leslie, H.A., Vethaak, A.D., Tinnevelt, G.H., Jansen, J.J., de Boer, J.F., Ariese, F., 2018. Fast microplastics identification with stimulated Raman scattering microscopy. J. Raman Spectrosc. 49, 1136–1144. https://doi.org/10.1002/jrs.5367.

Zobok, M.B., Esiukova, E.E., Zyubyn, A.Y., Samusev, I.G., 2019. Microplastic content variation in water column: the observations employing a novel sampling tool in stratified Baltic Sea. Mar. Pollut. Bull. 138, 193–205. https://doi.org/10.1016/j.marpolbul.2018.11.047.