Supporting Information

Facile Detection of Microplastics from a Variety of Environmental Samples with Conjugated Polymer Nanoparticles

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General Procedure and Materials

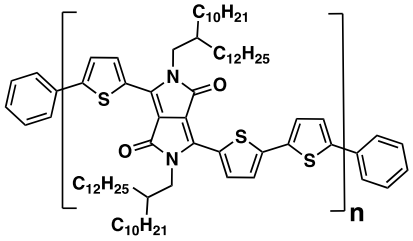
Materials

Tetrahydrofuran (THF), and sodium phosphate were purchased from Millipore Sigma Canada (Oakville, Canada). Fluorescein-labelled hyaluronic acid was purchased from TdB Labs (Uppsala, Sweden). Microplastic pellets polystyrene (PS), polycarbonate (PC), polypropylene (PP), and polyethylene terephthalate (PET) were donated from Dr. Jill Crossman from the University of Windsor (Windsor, Canada). Commercial reactants were used without further purification unless stated otherwise. All the solvents used in these reactions were distilled prior to use. Tris(dibenzylideneacetone)dipalladium(0)-chloroform adduct ($Pd_2(dba)_3$•$CHCl_3$) was purchased from Sigma Aldrich and recrystallized following a reported procedure.[1] 2,5-bis(trimethylstannyl)thiophene and 3,6-bis(5-bromothiophen-2-yl)-2,5-bis(2-decyltetradecyl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione were synthesized according to literature.[2]

Instrumentation

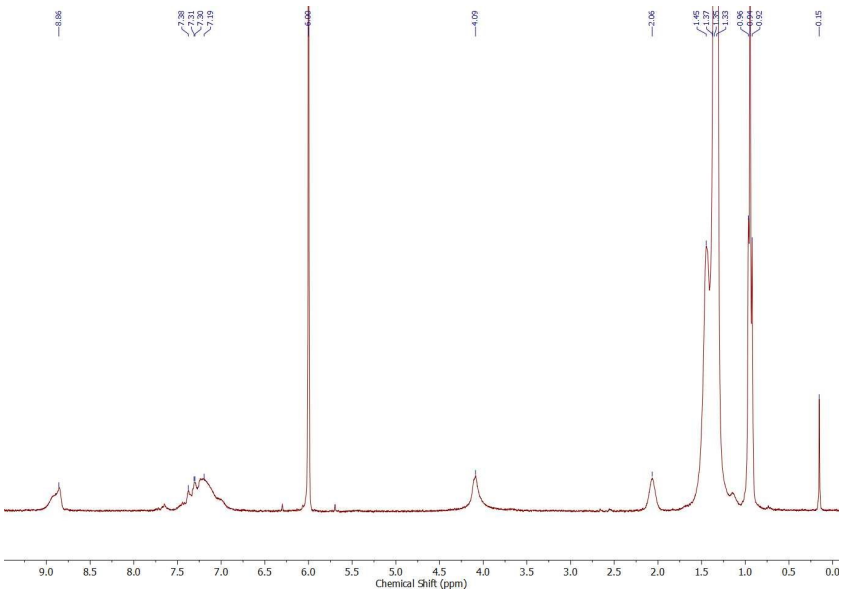
Number average molecular weight (M_n), weight average molecular weight (M_w), and dispersity ($Đ$) were evaluated by high temperature size exclusion chromatography (SEC) using 1,2,4-trichlorobenzene and performed on a EcoSEC HLC-8321GPC/HT (Tosoh Bioscience) equipped with a single TSK gel GPC column (GMHHR-H; 300 mm × 7.8 mm) calibrated with monodisperse polystyrene standards. The samples were prepared using 1 mg/mL of sample in trichlorobenzene (TCB), which were allowed to stir at 80 ^{0}C for 12 h prior to injection. The analysis of the samples was performed at 180 ^{0}C with a flow rate of 1.0 mL/min with injection quantities of 300 µL. The data was collected and integrated using EcoSEC 8321GPC HT software suite. Nuclear magnetic resonance (NMR) spectrum were recorded on a Bruker 300 MHz. The spectra for all polymers were obtained in deuterated 1,1,2,2-tetrachloroethane (TCE-d_2) at 120 °C. Epifluorescence images were obtained on a Zeiss Axiovert 200 with a fluorescein filter set from Chroma (Bellows Falls, VT). Particle concentrations and size distribution were obtained using a nanoparticle tracking analysis (NTA) instrument (Viewsizer 3000, MANTA instruments).

Experimental Procedure



Synthetic procedure for the preparation of P(DPP-T). A microwave vessel equipped with a stir bar was charged with 3,6-bis(5-bromothiophen-2-yl)-2,5-bis(2-decyltetradecyl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione (63.7 mg, 0.052 mmol), 2,5-bis(trimethylstannyl)thiophene (25.0 mg, 0.061 mmol), degassed chlorobenzene (2.1 mL), $Pd_2(dba)_3$ (1.10 mg, 0.001 mmol) and P(*o*-tolyl)$_3$ (1.70 mg, 0.005 mmol) and degassed with N_2 for 30 minutes. The vessel was then immediately sealed with a snap cap and microwave irradiated

under the following conditions with ramping temperature (Microwave Setup: Biotage Microwave Reactor; Power, 300 W; Temperature and Time, 2 minutes at 100°C, 2 minutes at 120 °C, 5 minutes at 140 °C, 5 minutes at 160 °C, and 40 minutes at 180 °C; Pressure, 17 bar; Stirring, 720). After completion, the polymer was end-capped with trimethylphenylstannane (14.7 mg, 0.061 mmol) and bromobenzene (9.60 mg, 0.061 mmol), successively. The reaction was then cooled to room temperature and dissolved in 1,1,2,2-tetrachloroethane. This solution was then precipitated in methanol and the solid was collected by filtration into a glass thimble. The content of the thimble was then extracted in a Soxhlet extractor with methanol, acetone, hexane. The hexane fraction was concentrated and reprecipitated in methanol, followed by filtration and drying under vacuum (yield = 73%). Molecular weight estimated from high temperature GPC: M_n = 13.6 kDa, M_w = 21.1 kDa, PDI = 1.55



^{1}H NMR spectrum of **P(DPP-T)** in 1,1,2,2-tetrachloroethane-d_2 at 100°C.

Synthesis of F-HA-CPNs. Using a commercially available fluorescein-labelled hyaluronic acid (F-HA), fluorescein-labelled hyaluronic acid conjugated polymer nanoparticles (F-HA-CPNs) were made *via* nanoprecipitation. P(DPP-T) (1 mg) and F-HA (2 mg) were dissolved in tetrahydrofuran (1mL) and left to stir for 45 minutes. The solution was injected into deionized H_2O (6 mL at 0°C) under probe sonication (amplitude of 60, power of 55W for 2 minutes). THF was evaporated and the 6 mL aqueous suspension was stored at 4°C prior to use.

Fluorescence measurements. To determine the concentration of the F-HA-CPN solution, the nanoparticle solution was diluted in a 1:2000 ratio in ddH_2O and observed using NTA (MANTA instruments ViewSizer 3000) to obtain particle concentrations. The reported concentration in particles/mL was converted to mol/L using the assumption that one nanoparticle was equivalent to one molecule. The concentration of the stock CPN solution was determined to be ~20-30 pM.

F-HA-CPN labelled pristine MPs. To prepare the microplastics from their pure plastic pellets, either PS, PC, or PET, were frozen using liquid nitrogen (N_2) in a mortar and pestle. The frozen

plastic pellets were then mechanically crushed. The plastics were sorted based on size using a metal sieve. Pieces of plastic that were too larger to pass through were re-frozen with N_2 and re-crushed. The N_2 crushed microplastics were placed on a microscope slide, and covered with 10 pM of F-HA(CPN), in phosphate buffer (0.1M, pH 8.2, 21°C) and left to fully dry. The prepared microscope slides with each probe were viewed on a Zeiss Axiovert 200 epifluorescence microscope with a fluorescein filter set from Chroma using 200ms exposure time, 0% offset, and 0% gain.

Soap Isolation Procedure. A small sample of the slurry (~60mg) was mixed with Sparkleen laboratory soap until bubbles appeared.[3] The soap bubbles were isolated and dried onto a microscope slide.

Raman Spectra of Blanks and Environmental Microplastics from Various Sources

All Raman spectral matches reported are of the highest hit quality index (HQI) in the Wiley Know It All database. For indicated samples, a second HQI match was selected only if it more accurately represented the experimental data based on the number and location of peaks. In addition to single-component matches, 2- and 3-component systems were considered using the Wiley Know It All database when applicable and specified. All y-axis and x-axis are representative of Raman peak intensity (a.u) and wavenumbers (cm^{-1}), respectively.

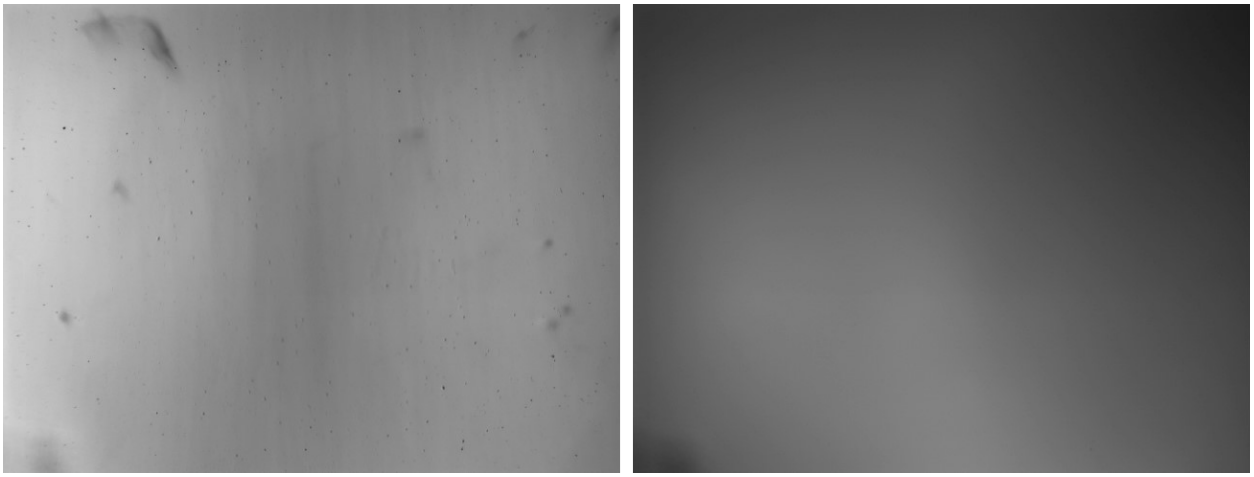


Figure S1. Visible (left) and fluorescent (right) microscope images of CPNs diluted in phosphate buffer.

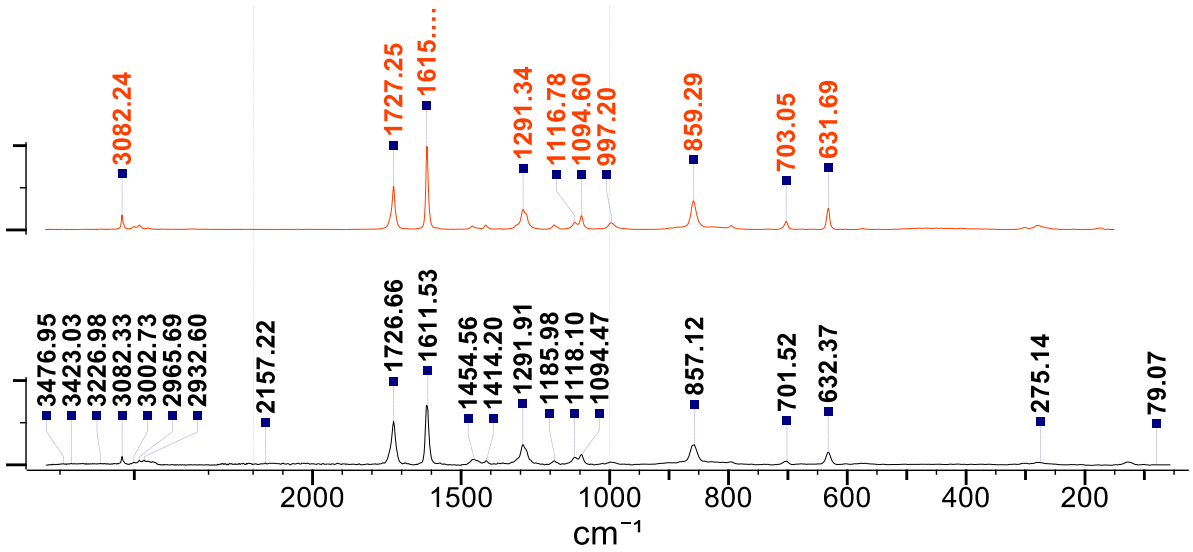


Figure S2. Environmental Harp Lake sample collected at lake outflow was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as polyester with a hit quality index of 85.40 when matched (red spectrum) in the Wiley Know It All Search/Match database.

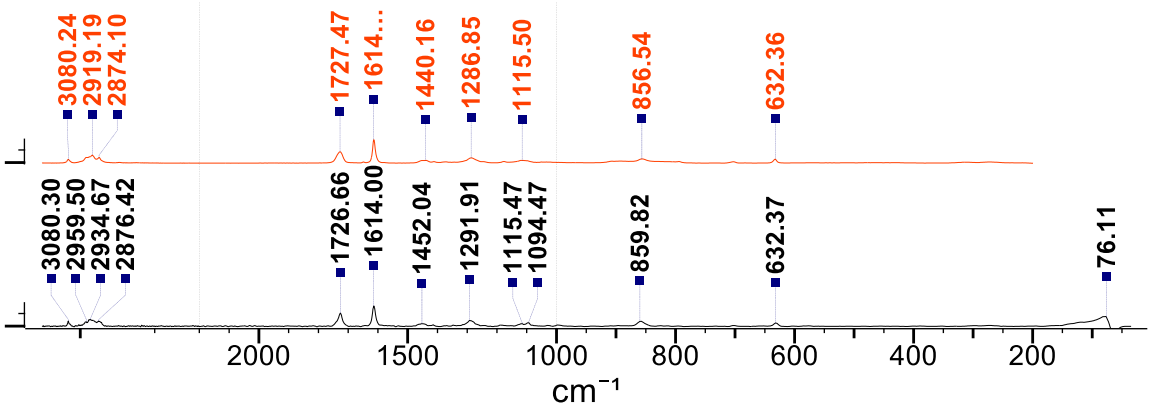


Figure S3. Environmental Harp Lake sample collected at lake outflow was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as a blend of PET and *p*-(vinyl butyral) with a hit quality index of 84.00 when matched (red spectrum) in the Wiley Know It All Search/Match database.

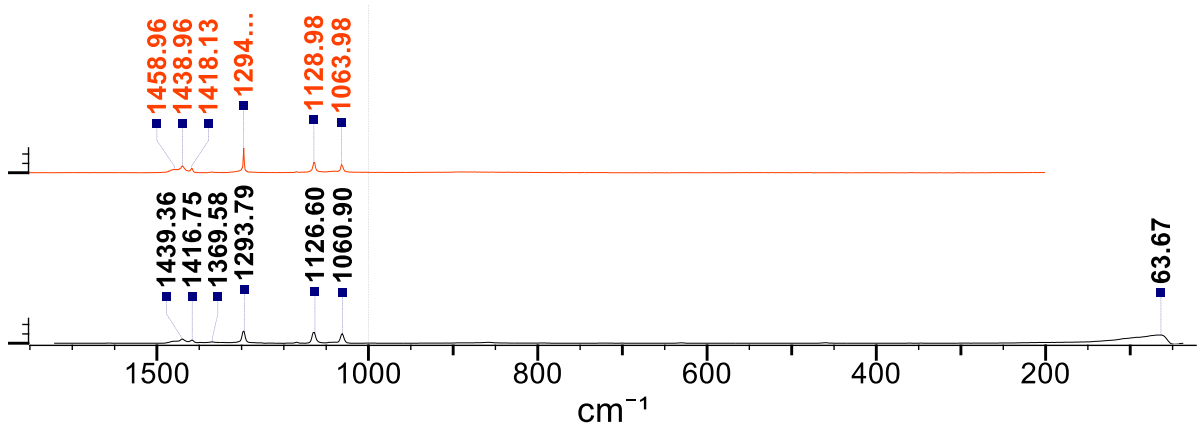


Figure S4. Environmental Harp Lake sample collected at lake outflow was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as low-density polyethylene with a hit quality index of 75.00 when matched (red spectrum) in the Wiley Know It All Search/Match database.

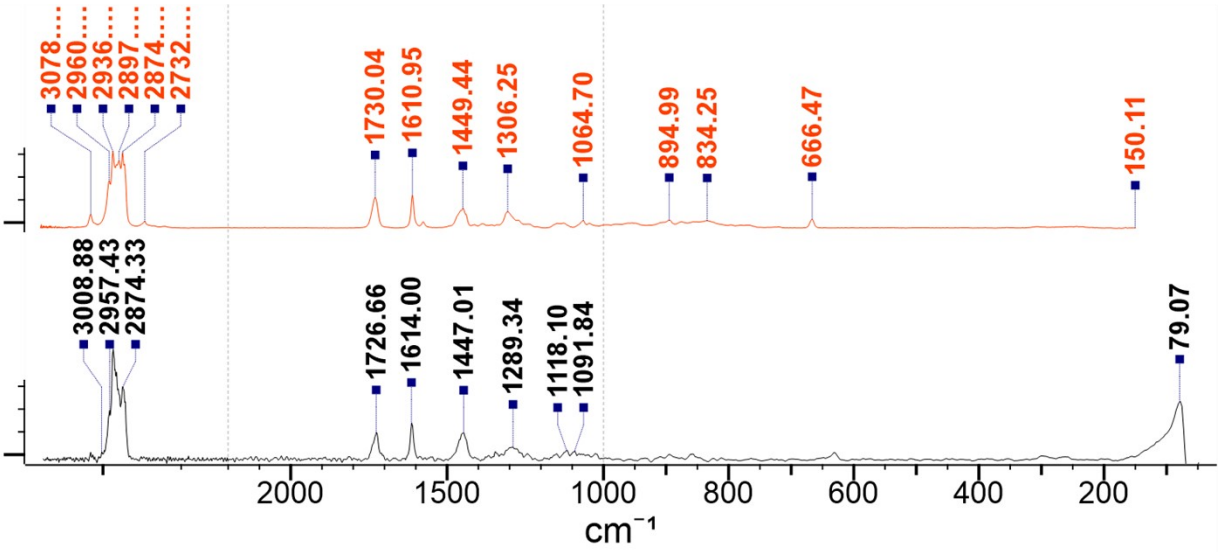


Figure S5. Environmental Harp Lake sample collected from at lake outflow was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as tris(2-ethylhexyl) trimellitate with a hit quality index of 86.35 when matched (red spectrum) in the Wiley Know It All Search/Match database.

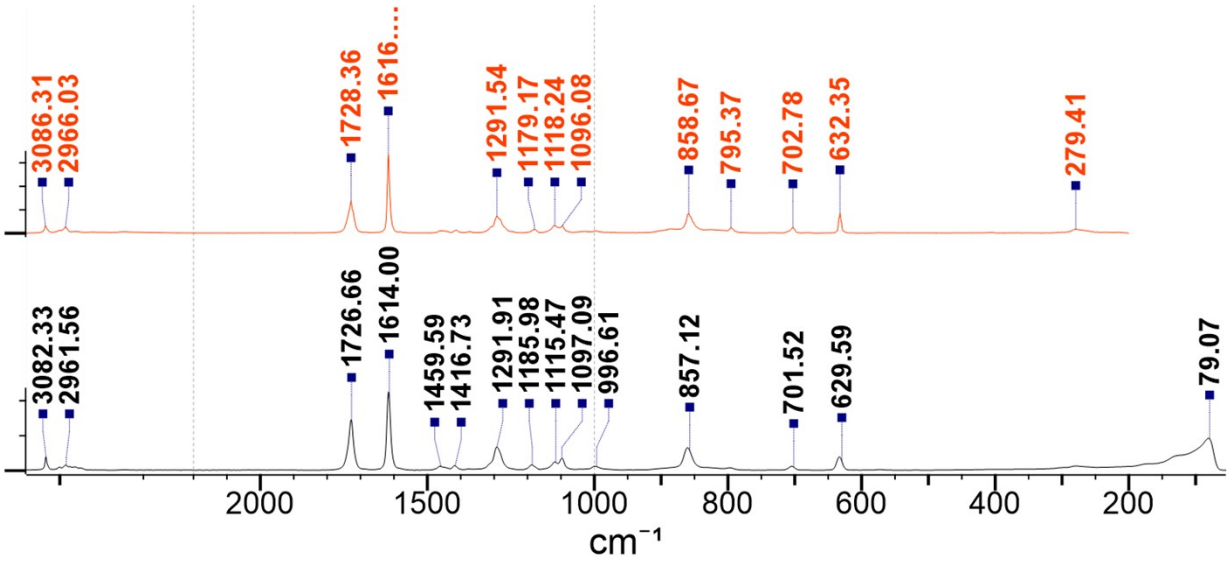


Figure S6. Environmental Harp Lake snow sample collected from frozen surface was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as polyethylene terephthalate with a hit quality index of 85.23 when matched (red spectrum) in the Wiley Know It All Search/Match database.

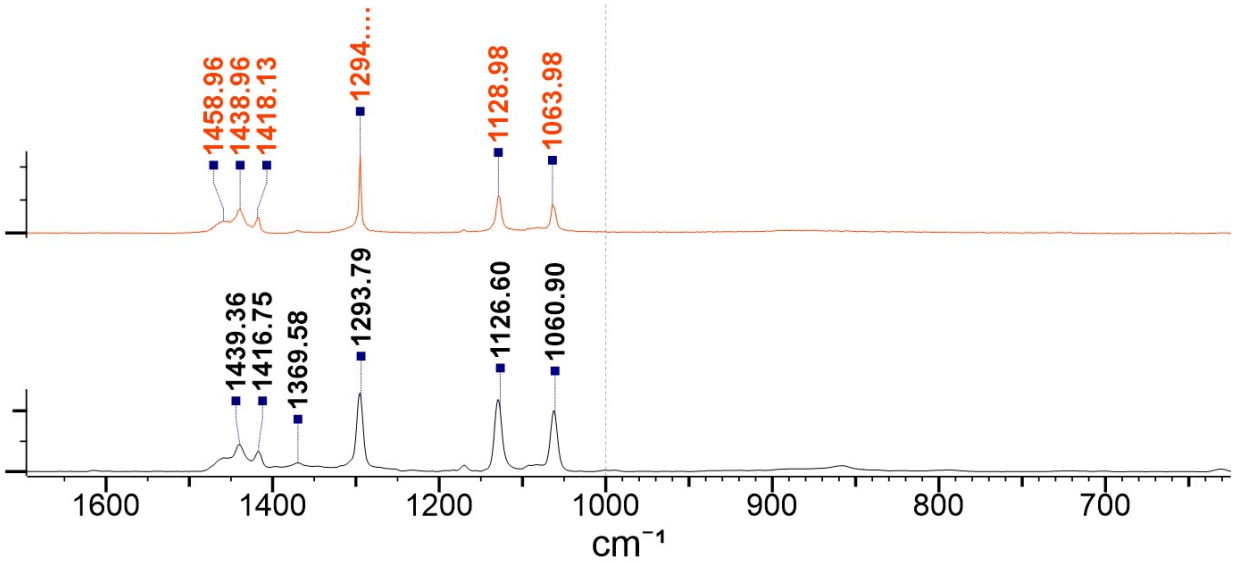


Figure S7. Environmental Harp Lake snow sample collected from frozen surface was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as low-density polyethylene with a hit quality index of 74.19 when matched (red spectrum) in the Wiley Know It All Search/Match database.

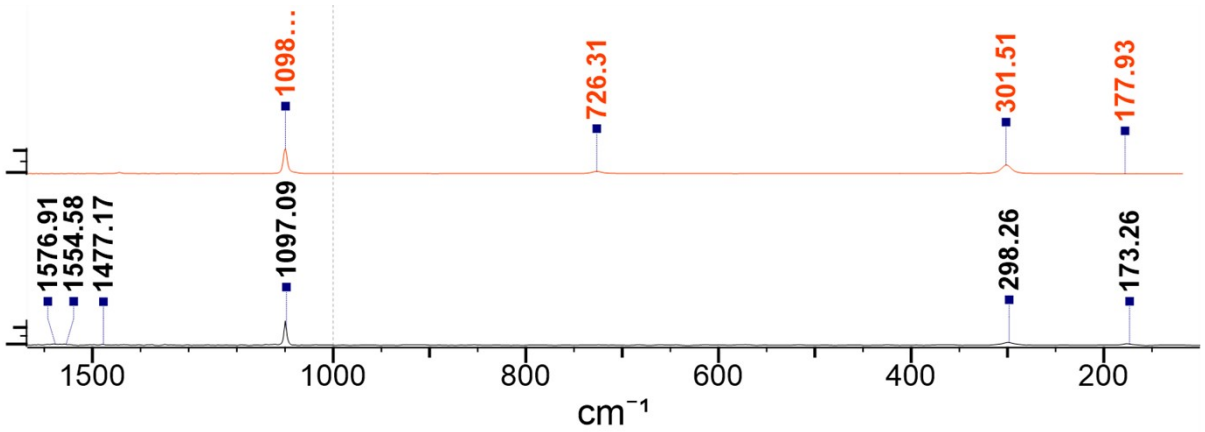


Figure S8. Environmental Harp Lake snow sample collected from frozen surface was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as dolomite with a hit quality index of 76.99 when matched (red spectrum) in the Wiley Know It All Search/Match database.

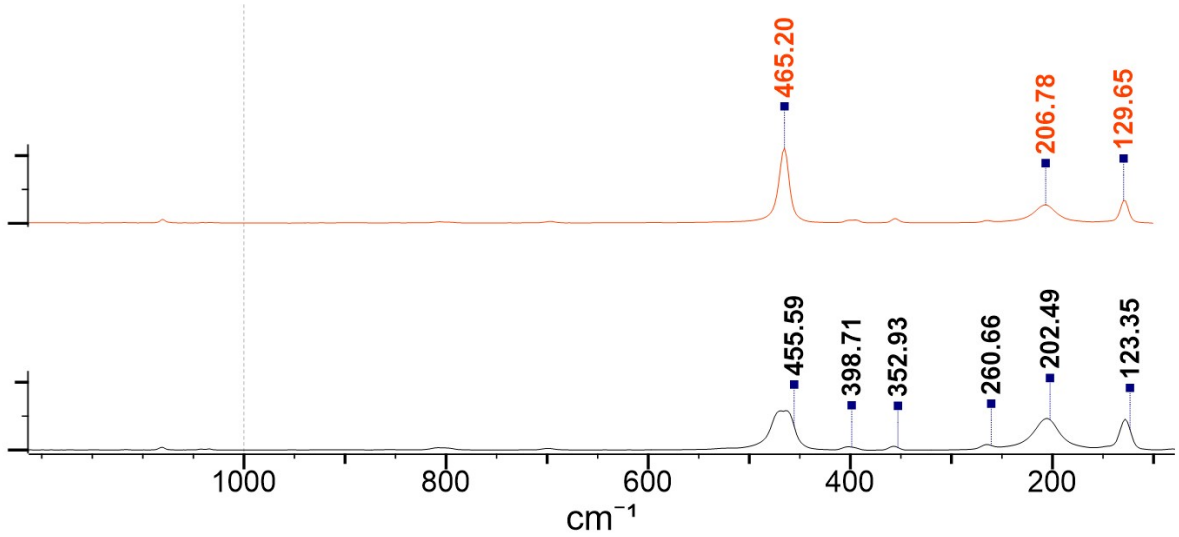


Figure S9. Environmental Harp Lake snow sample collected from frozen surface was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as quartz with a hit quality index of 65.64 when matched (red spectrum) in the Wiley Know It All Search/Match database.

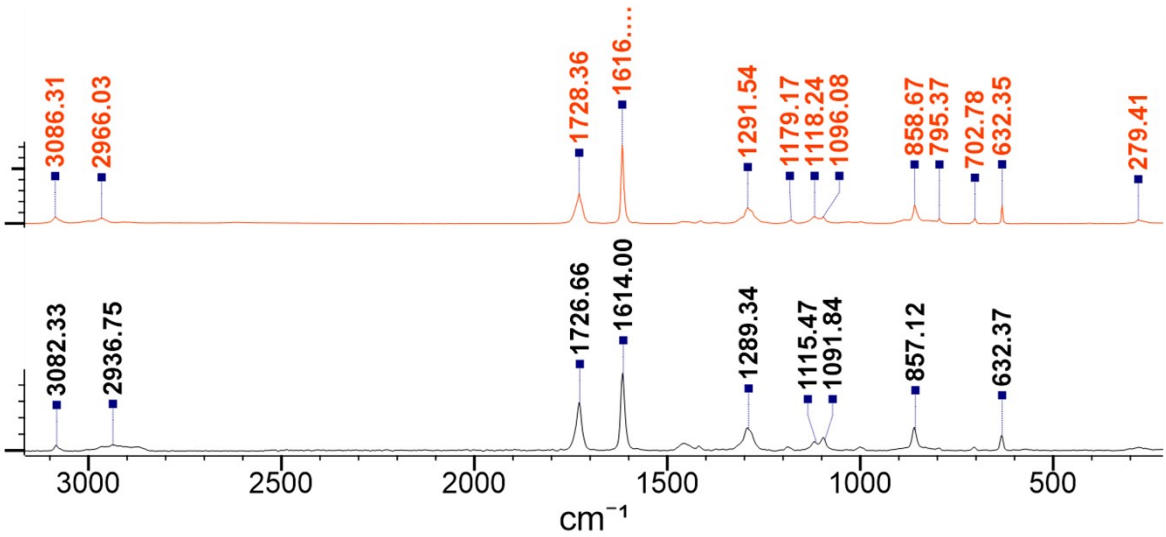


Figure S10. Environmental rain sample collected from Dorest Environmental Science Centre site from a bulk precipitation collector (two-week deployment) was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as polyethylene terephthalate with a hit quality index of 82.96 when matched (red spectrum) in the Wiley Know It All Search/Match database.

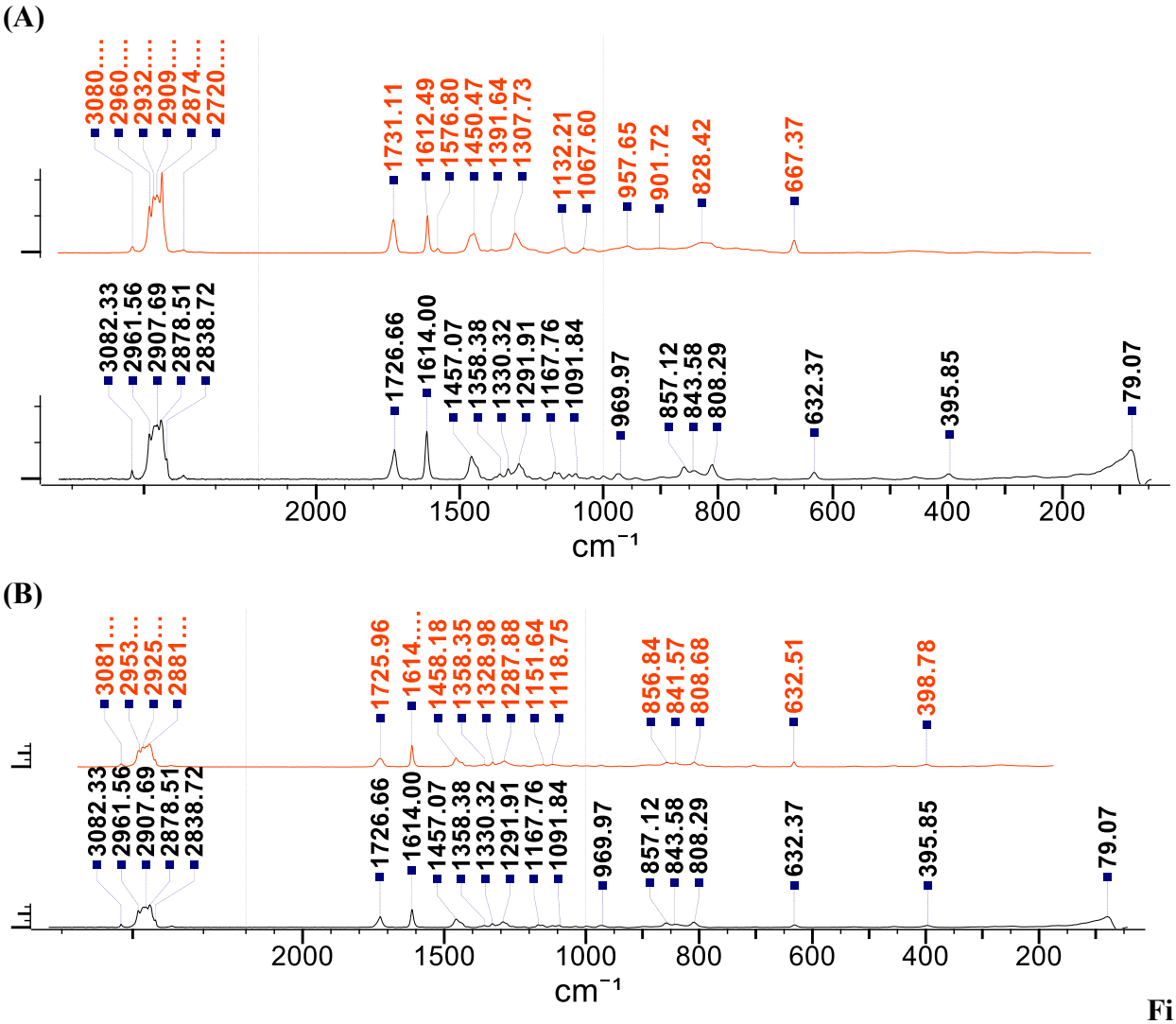


Figure S11. Environmental rain sample collected at from Dorest Environmental Science Centre site from a bulk precipitation collector (two-week deployment) was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). (A) The isolated Raman spectrum was identified as trioctyl trimellitate with a hit quality index of 83.66 when matched (red spectrum) in the Wiley Know It All Search/Match database. (B) The isolated Raman spectrum was identified as a 3-component mixture of PET, PP, and bis(2-ethylhexyl) adipate with a hit quality index of 92.44 with ratios of 40:32:28, respectively when matched (red spectrum) in the Wiley Know It All Search/Match database.

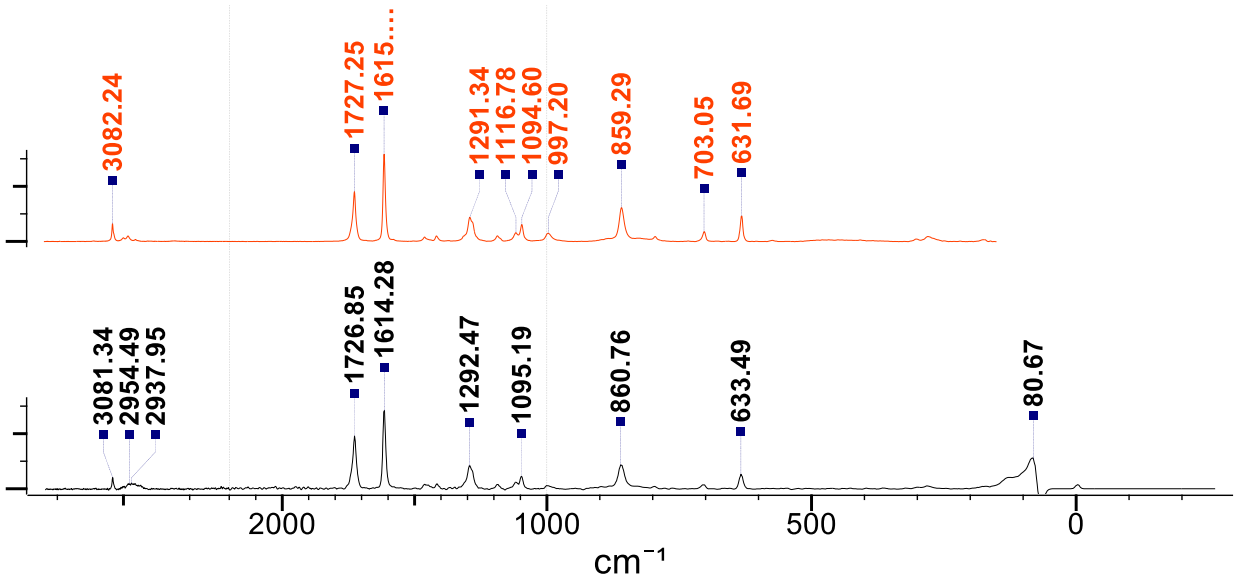


Figure S12. Environmental dirt samples collected from the University of Windsor campus grounds was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as PET with a hit quality index of 84.58 when matched (red spectrum) in the Wiley Know It All Search/Match database.

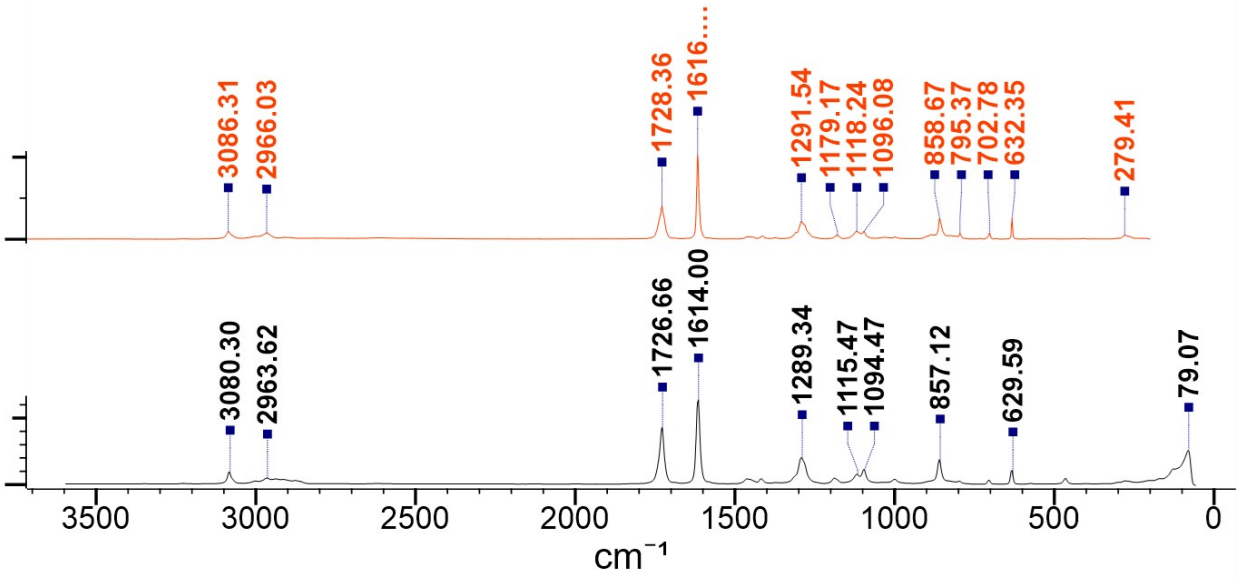


Figure S13. Environmental arctic sediment sample collected from 641-meter depth was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as PET with a hit quality index of 84.49 when matched (red spectrum) in the Wiley Know It All Search/Match database.

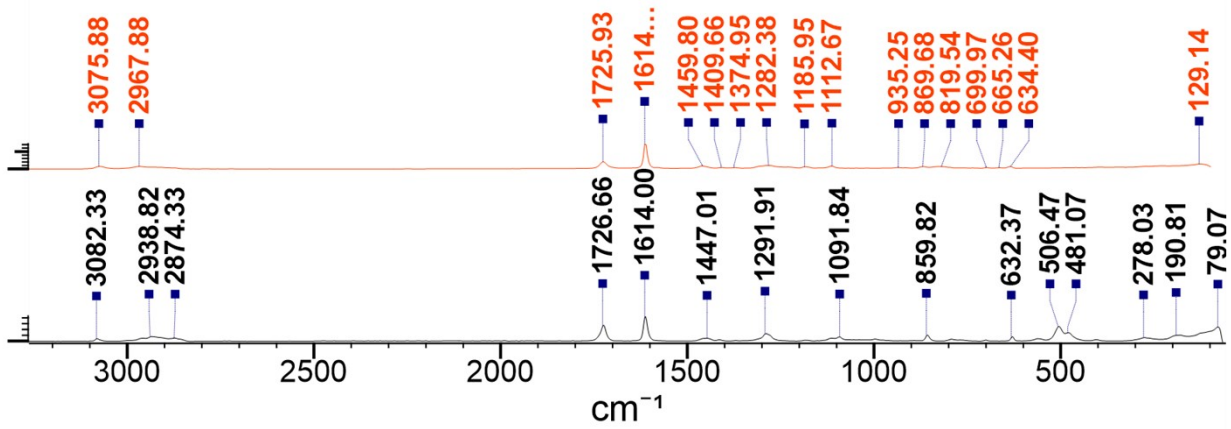


Figure S14. Environmental arctic sediment sample collected from 760-meter depth was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as polyester with a hit quality index of 60.79 when matched (red spectrum) in the Wiley Know It All Search/Match database.

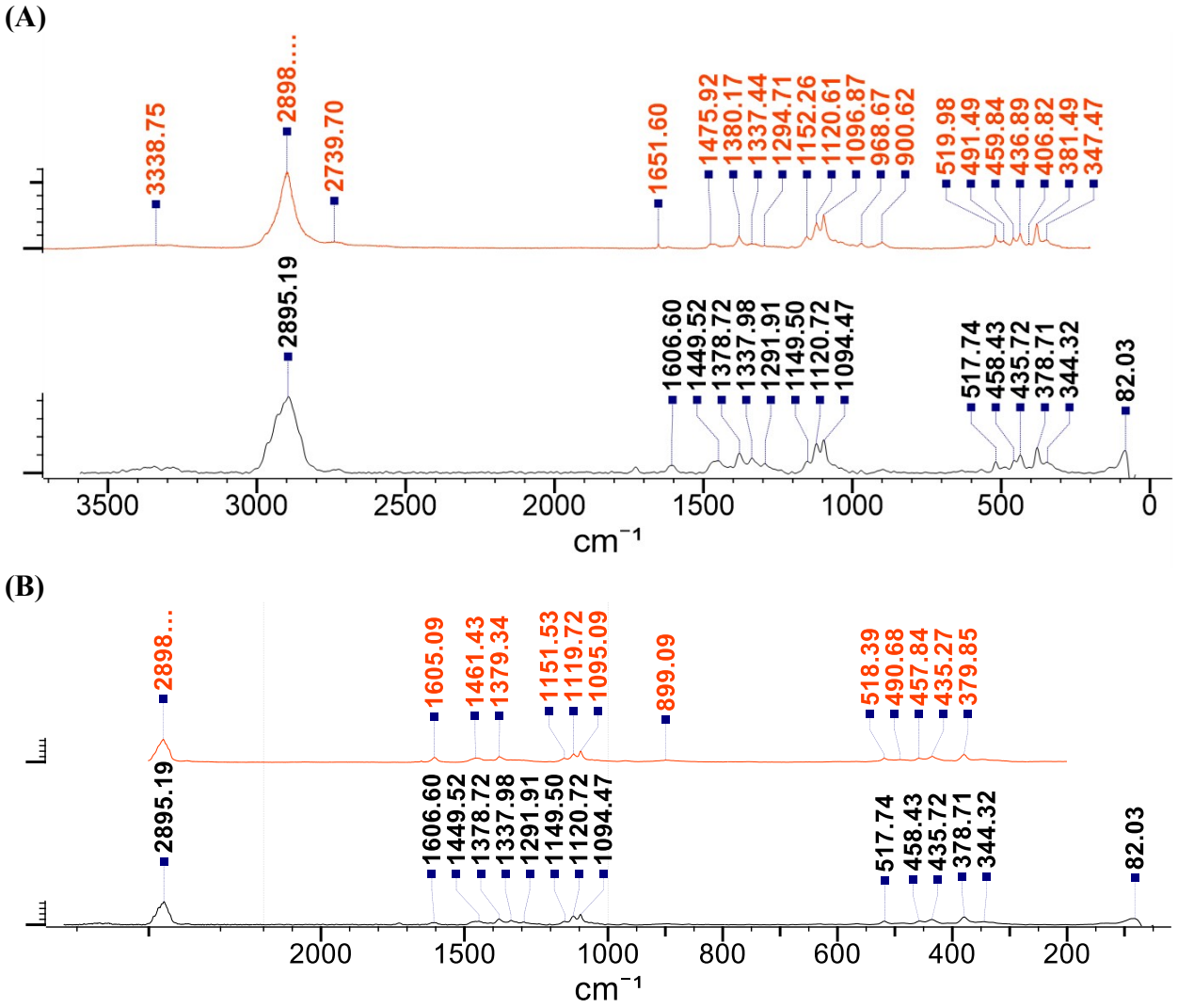


Figure S15. Environmental arctic sediment sample collected from 1372-meter depth was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). (A) The isolated Raman spectrum was identified as cellulose with a hit quality index of 83.83 when matched (red spectrum) in the Wiley Know It All Search/Match database. (B) The isolated Raman spectrum was identified as a 3-component mixture which consisted of cellulose, PA-1121, and methylene blue with a hit quality index of 91.62 in the ratio of 61:27:12 when matched (red spectrum) in the Wiley Know It All Search/Match database.

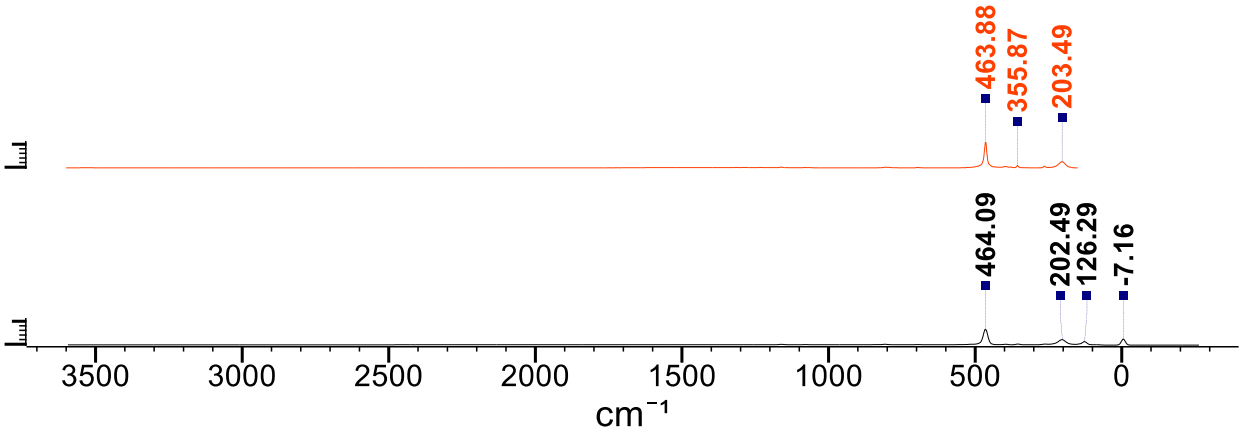


Figure S16. Environmental arctic sediment sample collected from 760-meter depth was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as quartz with a hit quality index of 87.93 when matched (red spectrum) in the Wiley Know It All Search/Match database.

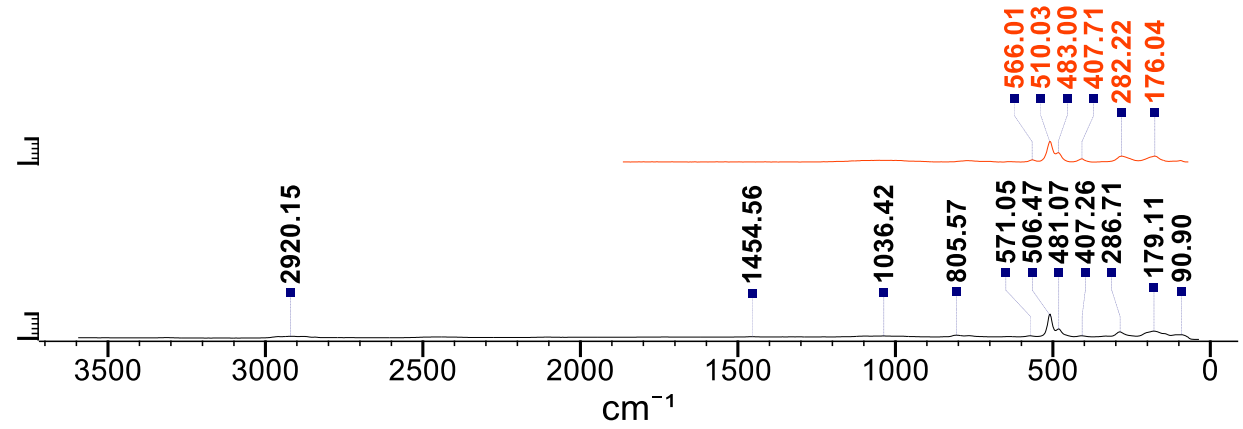


Figure S17. Environmental arctic sediment sample collected from 760-meter depth was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as labradorite with a hit quality index of 83.36 when matched (red spectrum) in the Wiley Know It All Search/Match database.

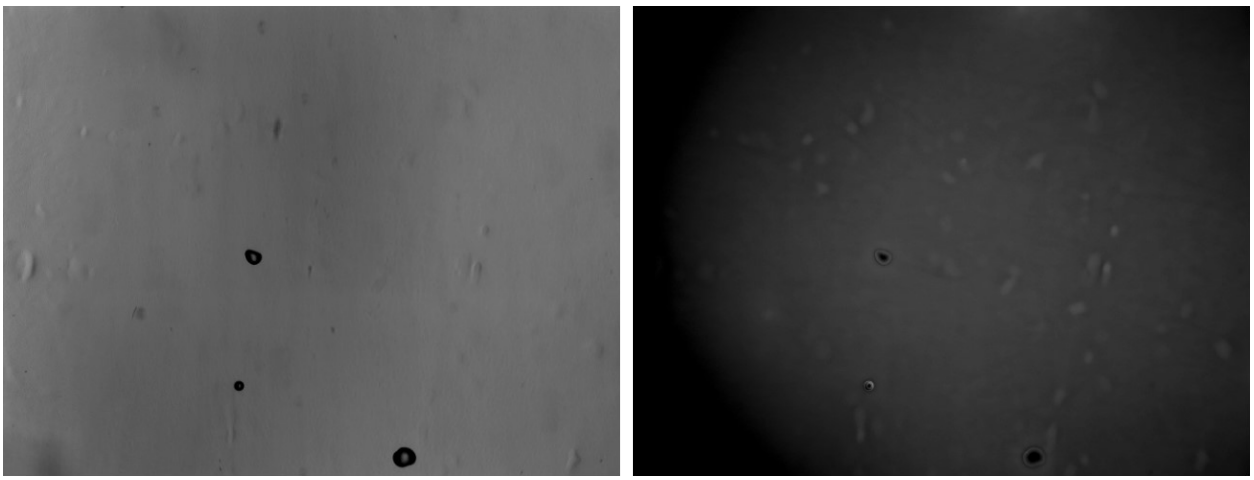


Figure S18. Visible (left) and fluorescent (right) microscope images of CPNs diluted in phosphate buffer on an unused blank air sampling slide. The black dots represent air bubbles that were deposited on the slide.

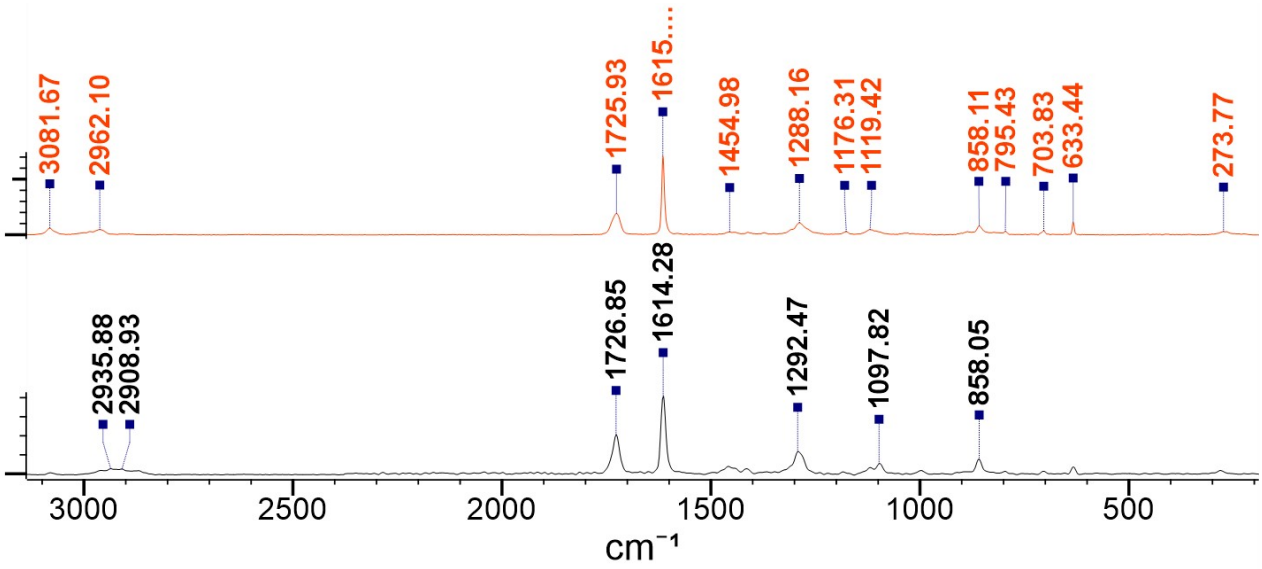


Figure S19. Environmental passive air samples collected from rural areas in the GTA (13–15-day exposure) was subjected to Raman spectroscopy and scanned for microplastic content (black spectrum). The isolated Raman spectrum was identified as PET with a hit quality index of 79.43 when matched (red spectrum) in the Wiley Know It All Search/Match database.

Pristine Microplastics Coated with F-HA-CPN

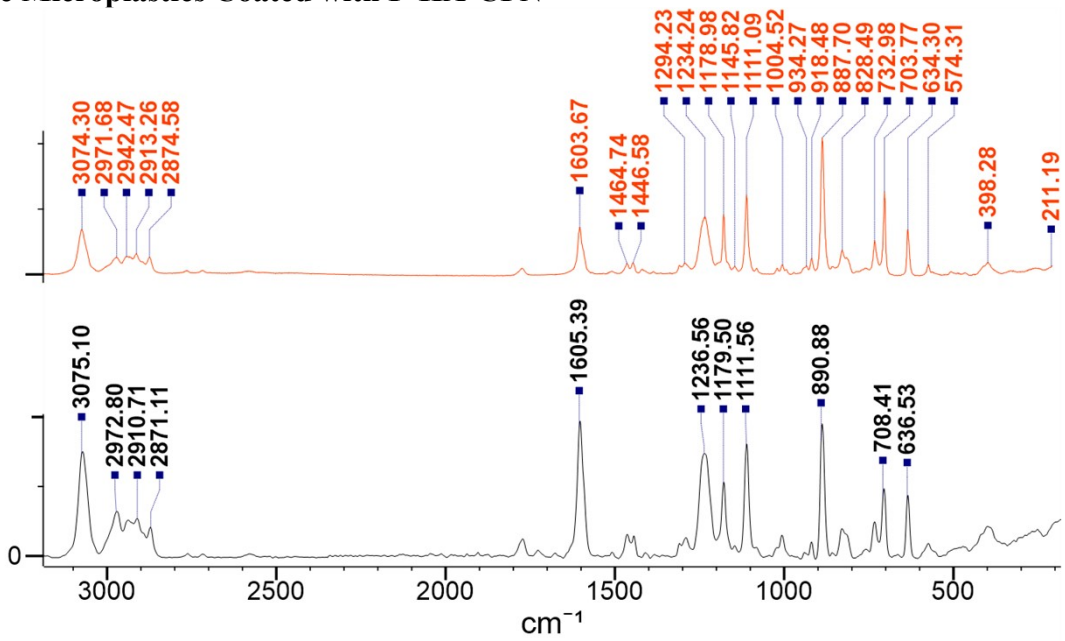


Figure S20. Polycarbonate pristine plastic CPN treatment and Raman identification. Pristine polycarbonate fragments treated with F-HA-CPNs (black spectrum) were successfully identified with a hit quality index of 77.80 when matched (red spectrum) in the Wiley Know it All Search Match Database. Samples were fixed on a glass slide using a double-sided adhesive.

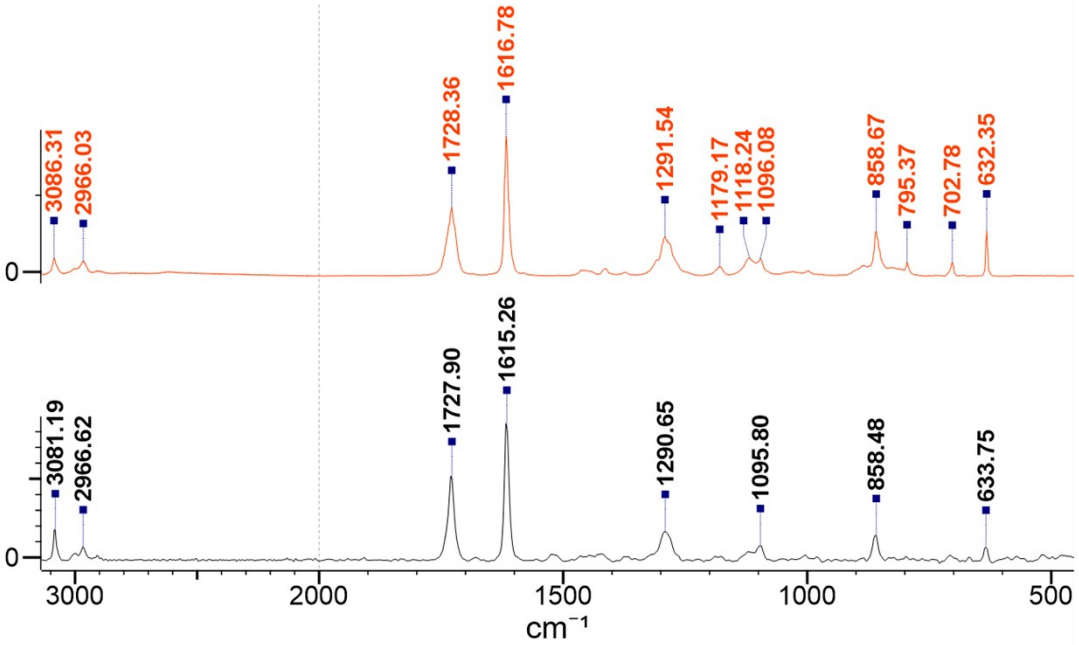


Figure S21. Polyethylene terephthalate pristine plastic CPN treatment and Raman identification. Pristine polyethylene terephthalate fragments treated with F-HA-CPNs (black spectrum) were successfully identified with a hit quality index of 76.35 when matched (red spectrum) in the Wiley Know it All Search Match Database. Samples were fixed on a glass slide using a double-sided adhesive.

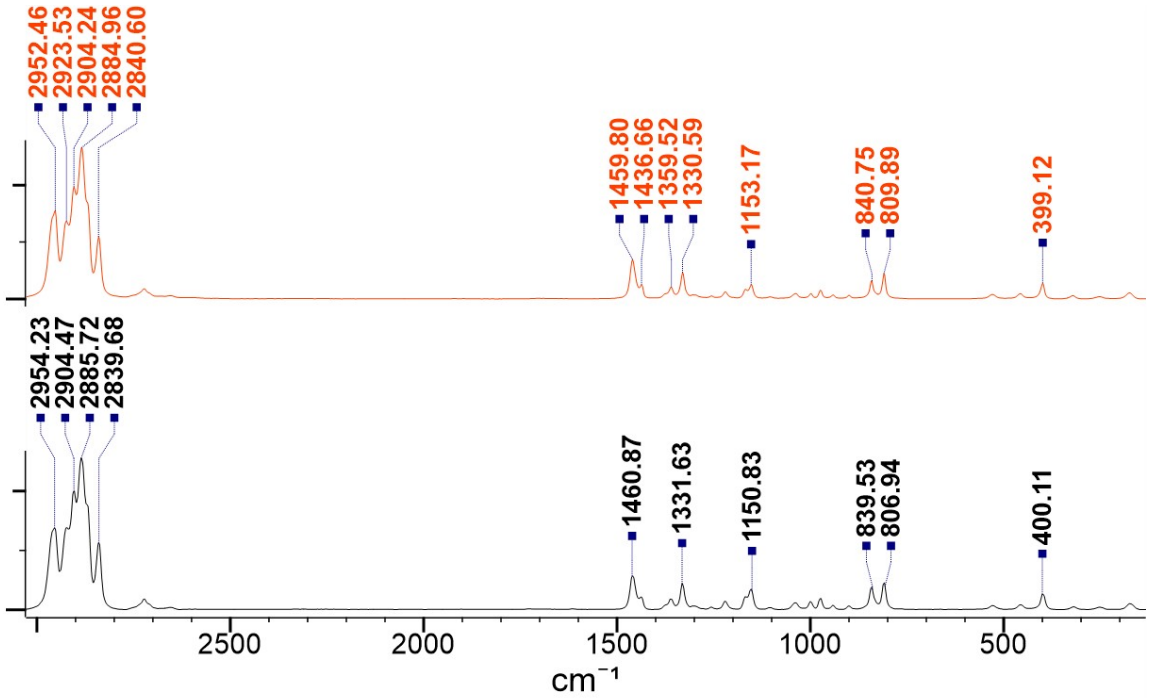


Figure S22. Polypropylene pristine plastic CPN treatment and Raman identification. Pristine polypropylene fragments treated with F-HA-CPNs (black spectrum) were successfully identified with a hit quality index of 95.29 when matched (red spectrum) in the Wiley Know it All Search Match Database. Samples were fixed on a glass slide using a double-sided adhesive.

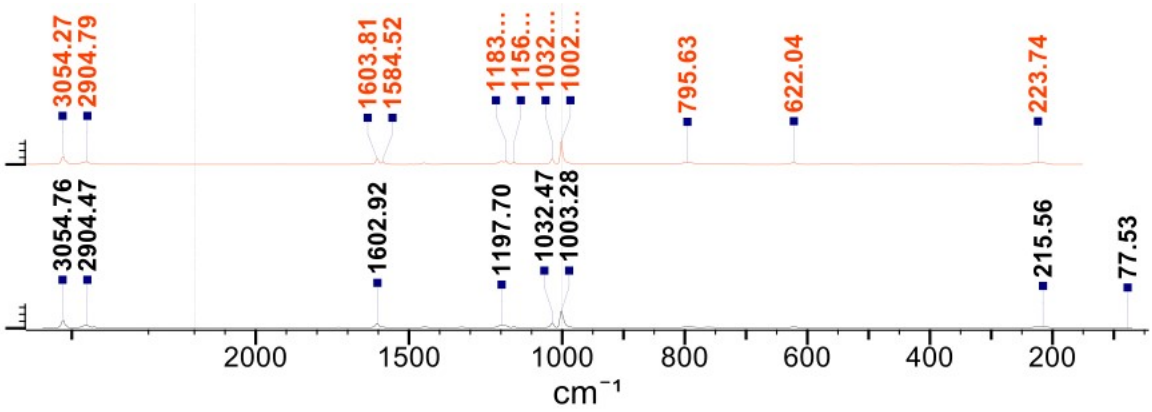


Figure S23. Polystyrene pristine plastic CPN treatment and Raman identification. Pristine polystyrene fragments treated with F-HA-CPNs (black spectrum) were successfully identified with a hit quality index of 87.90 when matched to the (red spectrum) in the Wiley Know it All Search Match Database. Samples were fixed on a glass slide using a double-sided adhesive.

Raman Spectra Controls of Sample Adhesives, CPNs, SDS, and Glass Microscope Slides

Raman spectral controls were taken for the F-HA-CPNs, SDS, and various adhesives used throughout this study. Double sided, opaque and clear tape used were identified as PET (Figure S24A), high ethylene co-polymer (Figure S24B), and PP (Figure S24C), respectively. The air samples adhesive was identified as ditridecyl thiodipropinoate (Figure S25). After spectral processing the F-HA-CPNs showed no significant peaks (Figure S26) from the Raman spectrometer due to their highly fluorescent properties. Additionally, the glass microscope slide and the SDS solution deposited on the glass slide were both identified as glass (Figure S27) and (Figure S28), respectively, these findings were most likely due to used concentrations of SDS, in this study, being too low to be identified via Raman spectroscopy.

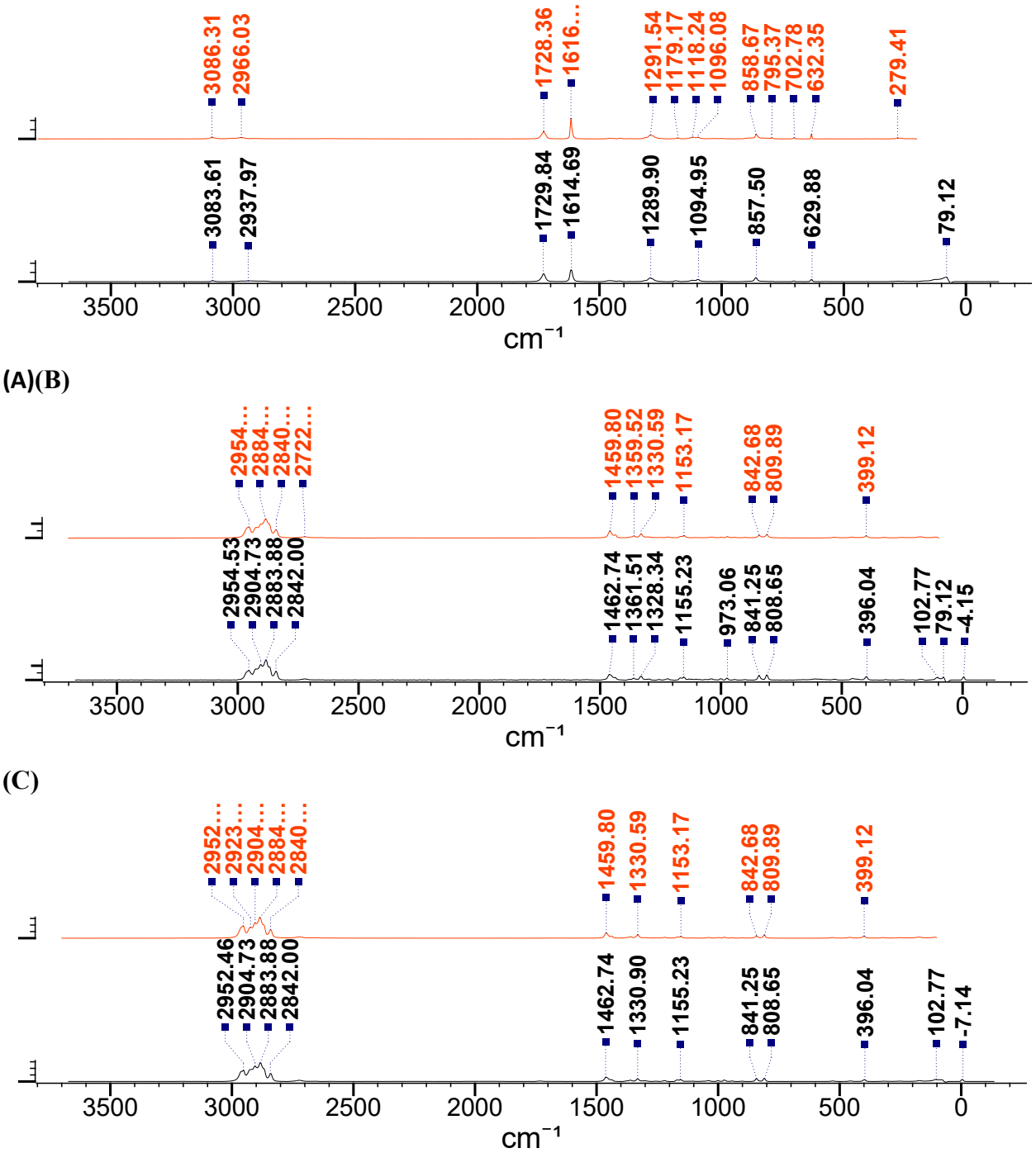


Figure S24. Raman spectra of adhesives used for fixing environmental samples and surveying of MPs. (A) The isolated Raman spectrum of the double-sided tape adhesive was identified as polyethylene terephthalate with a hit quality index of 83.6, (B) the opaque tape adhesive was identified as high ethylene random copolymer with a hit quality index of 90.0, and (C) the clear tape adhesive, was identified as polypropylene with a hit quality index of 92.1 when matched (red spectrum) in the Wiley Know It All Search/Match database.

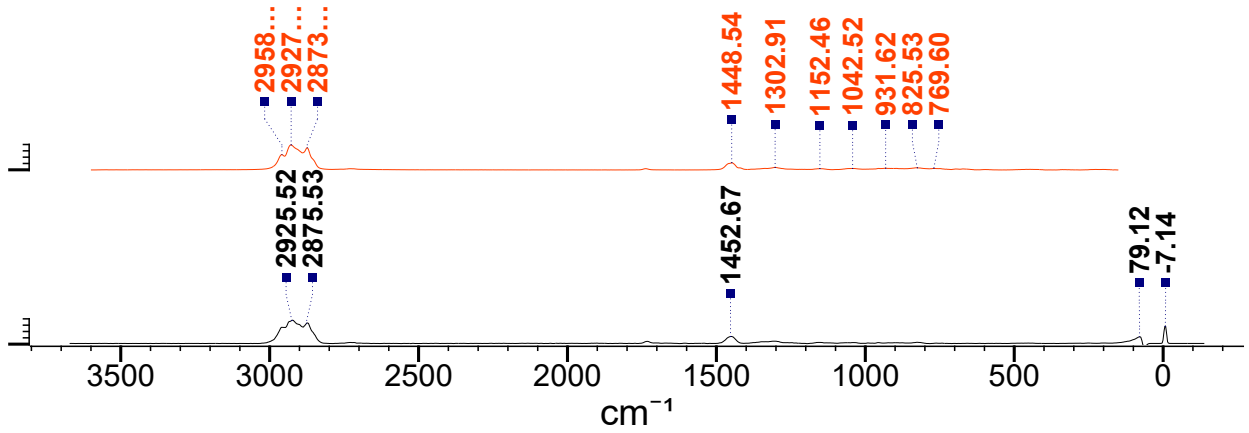


Figure S25. Raman spectra of blank air sample adhesives on glass slides. The isolated Raman spectrum was identified as ditridecyl thiodipropionate with a hit quality index of 92.56 when matched (red spectrum) in the Wiley Know It All Search/Match database.

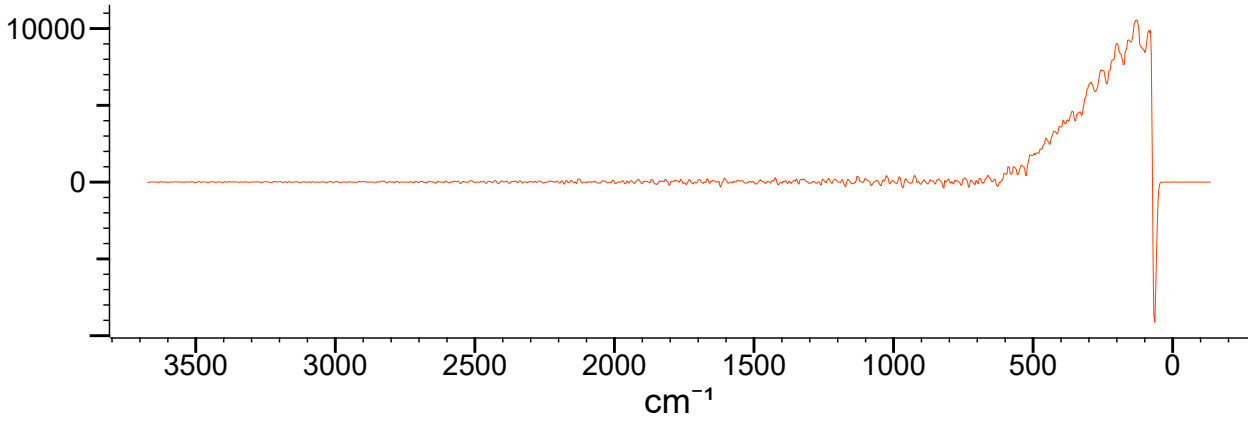


Figure S26. Raman spectrum acquisition of CPNs on a glass slide showing feedback of fluorescence.

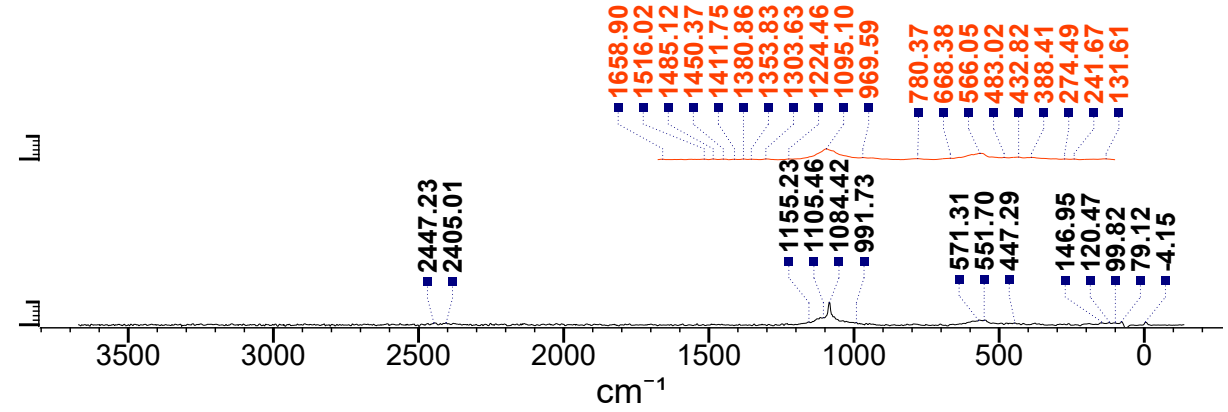


Figure S27. Raman spectrum of glass slides. The isolated Raman spectrum was identified as glass with a hit quality index of 78.44 when matched (red spectrum) in the Wiley Know It All Search/Match database.

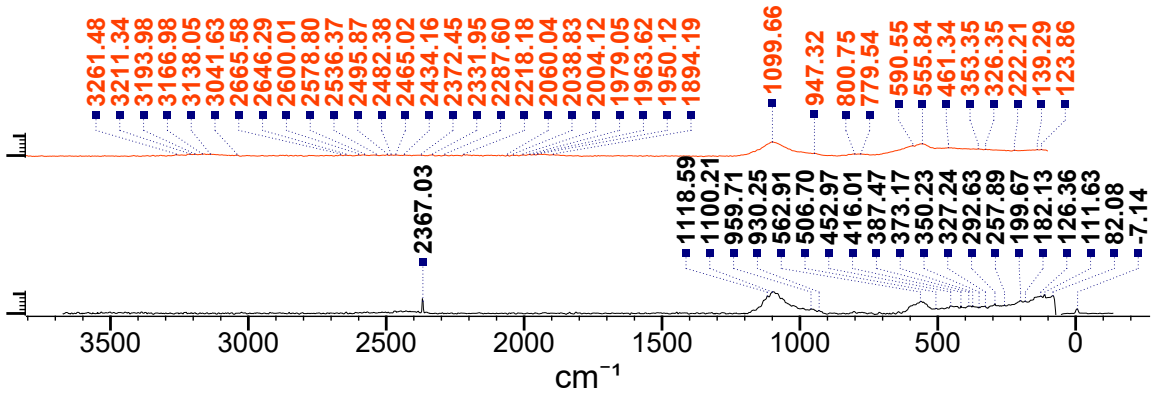


Figure S28. Raman spectrum of SDS on glass slides. The isolated Raman spectrum was identified as glass with a hit quality index of 72.3 when matched (red spectrum) in the Wiley Know It All Search/Match database.

References

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