Supplementary Information (SI) for Environmental Science: Nano.
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Supporting information

Title: Stable staining of microplastics using conjugated polymer nanoparticles

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Experimental

Virgin biodegradable plastics and virgin conventional plastics (Natureplast) alongside polyester fibres were provided by Plymouth Marine Laboratory (PML). Mussel faecal samples also provided by PML as part of a previous study.[1] Cotton, Acrylic and Cotton/Polyester blend fibres obtained from clothing – worn and washed multiple times. Conjugated polymer nanoparticles (CPN 530) were specifically prepared by Stream Bio Ltd for this project, consisting of an emitting conjugated polymer, iron oxide nanoparticles, and without a standard surface polymeric layer.[2] The hydrodynamic diameter of the CPNs was measured at 63 nm using a Malvern Zetasizer Ultra.

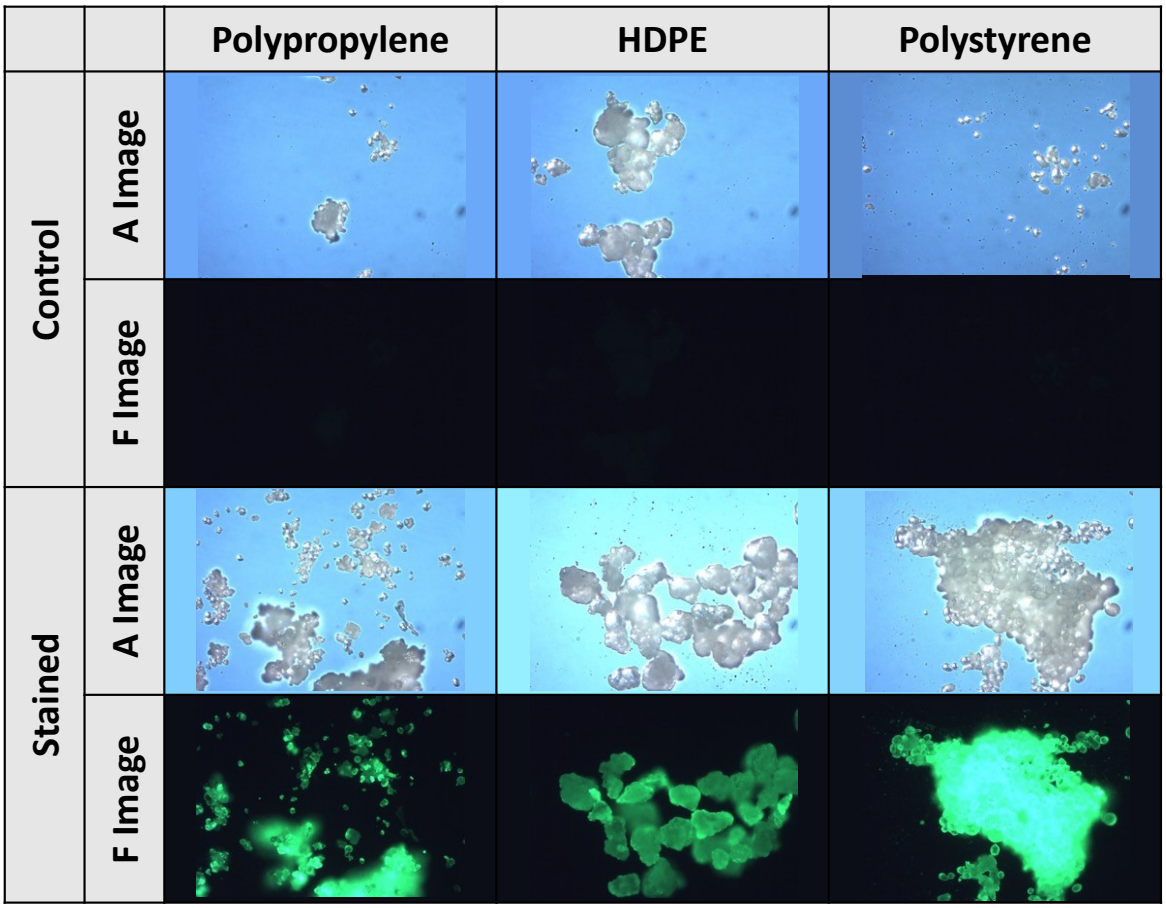
In a typical study, *ca*. 5 mgs of microplastic were incubated with 30 μL of diluted CPN solution (1 x 10^{10} particles/mL). This was vortexed to ensure coating then allowed to stand over night or for 30 minutes in ambient conditions. Fibres and shaving were removed *via* tweezers, dried on filter paper and washed twice with DI water. Powders were transferred by pipette, onto filter paper, and washed twice with DI water. Once dried, placed onto glass slide and imaged using a Nikon Eclipse TS100 optical microscope at x10 magnification, with an epi-fluorescent attachment, and a GFP/FITC filter cube. Excitation of the samples was achieved using a CoolLED pE-200 LED illuminator with a LED excitation source at 470 nm with 3% / 15% / 75% intensities and intensity control between 0 to 100% in 1% increments.

References

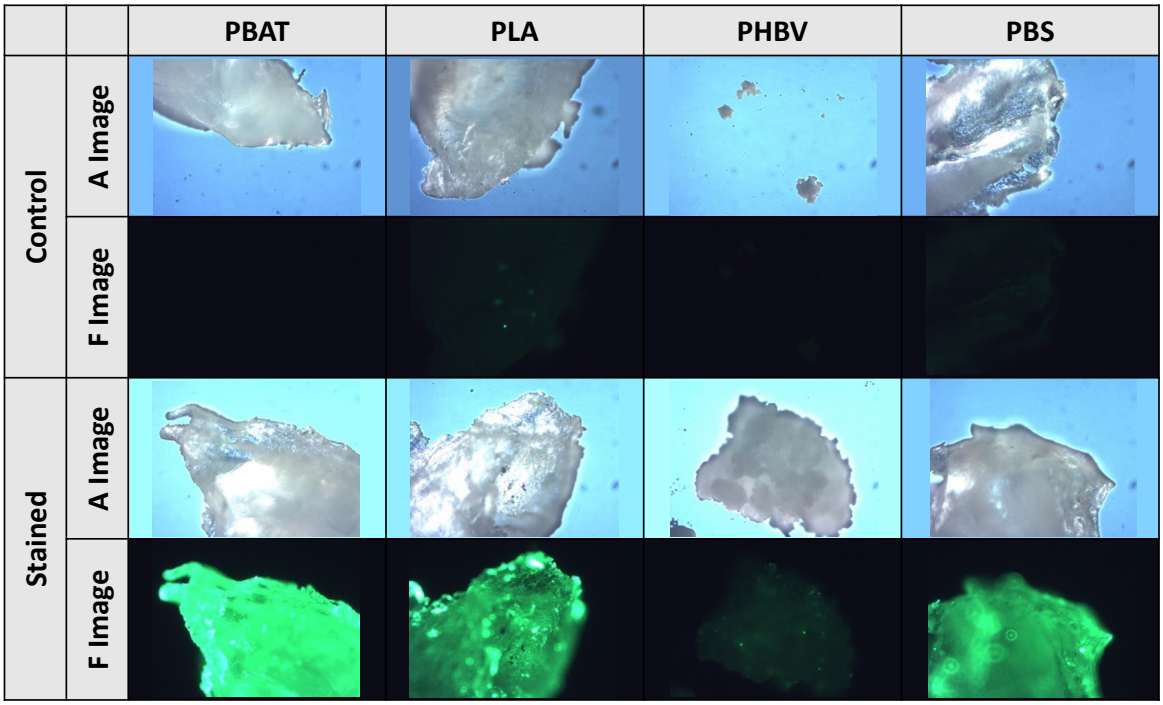
1 – M. Cole, Y. Artioli, R. Coppock, G. Galli, R. Saad, R. Torres, T. Vance, A. Yunnie, P. K. Lindeque, Mussel power: scoping a nature-based solution to microplastic debris. J. Hazard. Mater., 2023, 453, 131392.

2 - Mark Green, Philip Howes. ‘Micellar compositions for use in biological applications.’ WO 2011/039535

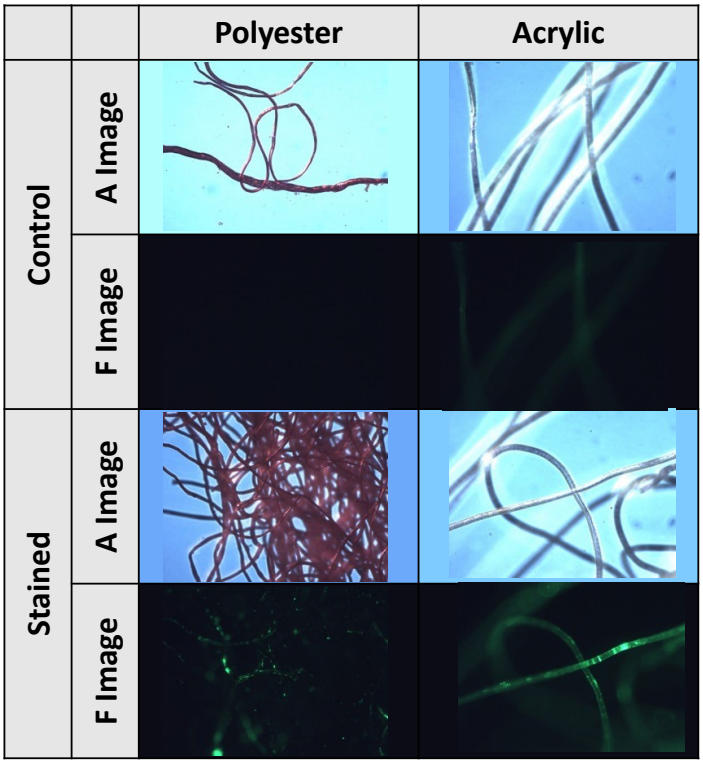
Supporting figures



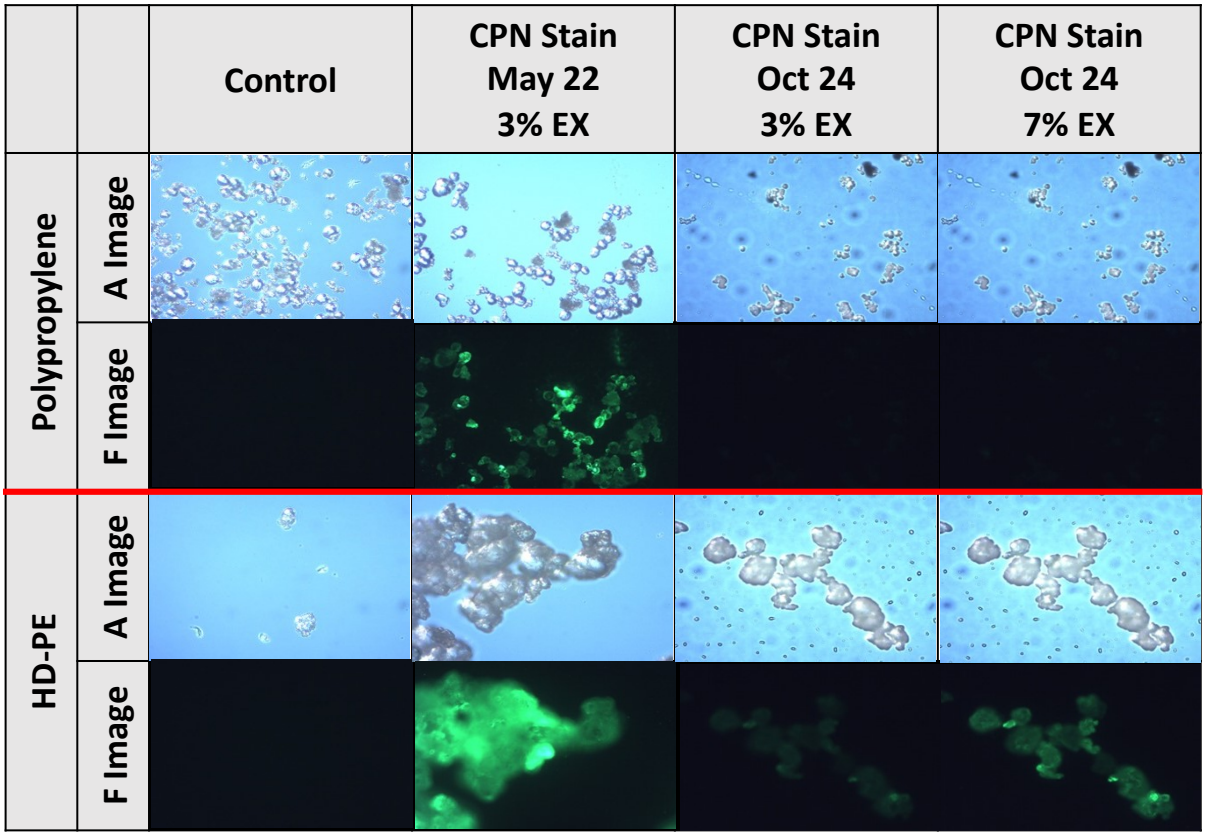
Supporting information figure 1 – optical microscopy and fluorescent images (x 10 magnification) of a range of virgin conventional plastics – control samples and stained with CPNs (excitation wavelength 470 nm, 30-minute incubation and 15 % irradiance power).



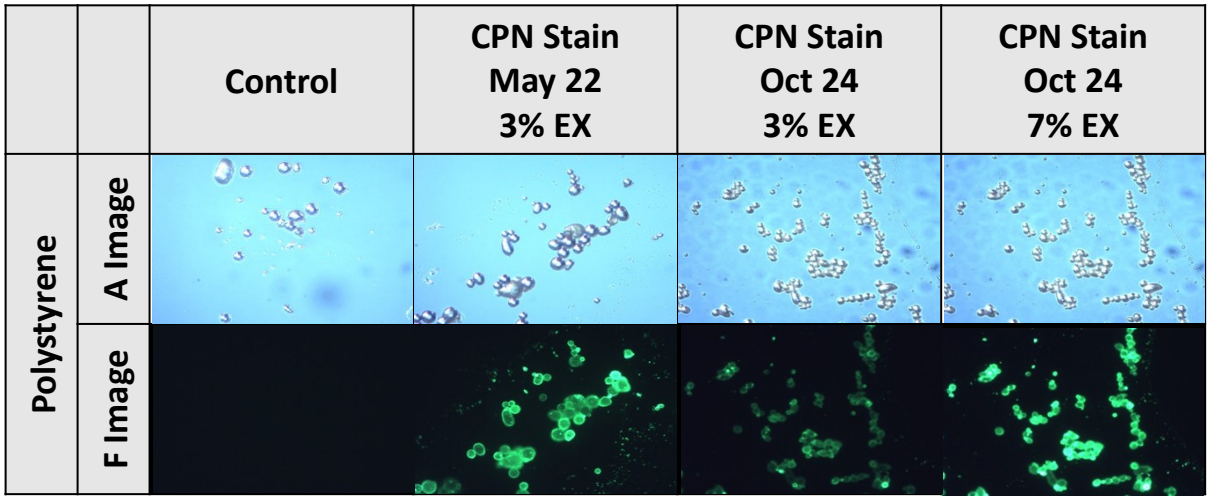
Supporting information figure 2 – optical microscopy and fluorescent images (x 10 magnification) of a range of virgin biodegradable plastics – control samples and stained with CPNs (excitation wavelength 470 nm, 30-minute incubation and 15 % irradiance power).



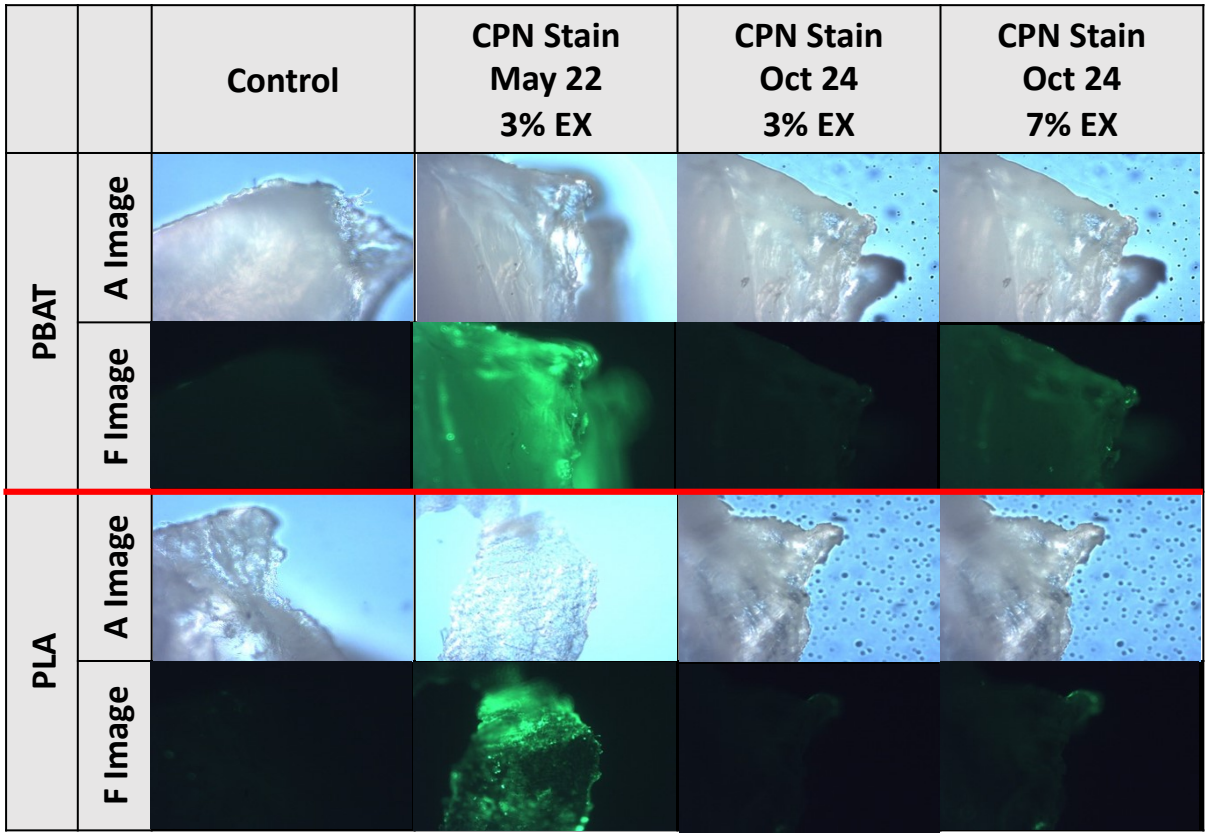
Supporting information figure 3 – optical microscopy and fluorescent images (x 10 magnification) of a range of clothing fibres – control samples and stained with CPNs (excitation wavelength 470 nm, 30-minute incubation and 75 % irradiance power).



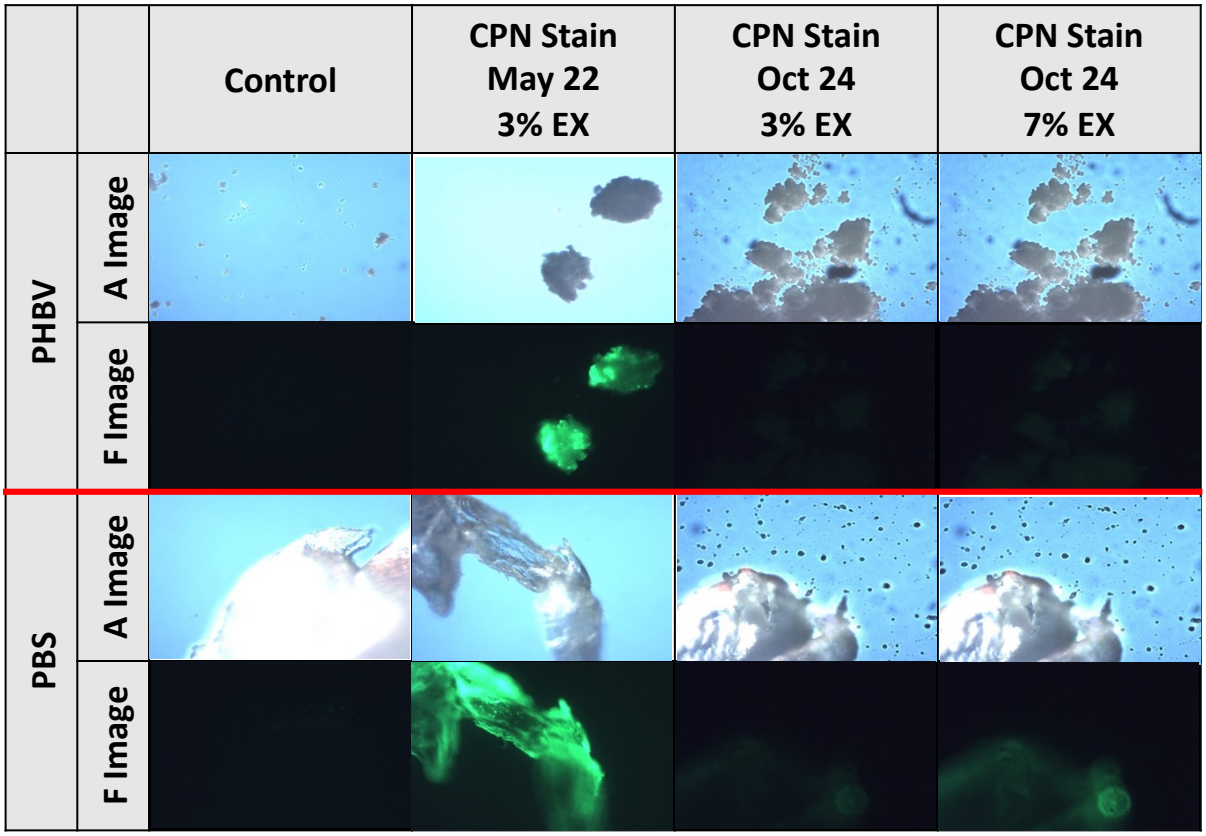
Supporting information figure 4 – optical microscopy and fluorescent images (x 10 magnification) of microplastic samples of polypropylene and HD-PE – stained initially (May 2022) and after ambient storage (October 2024). Control samples and stained with CPNs (excitation wavelength 470 nm, overnight incubation and varying irradiance power).



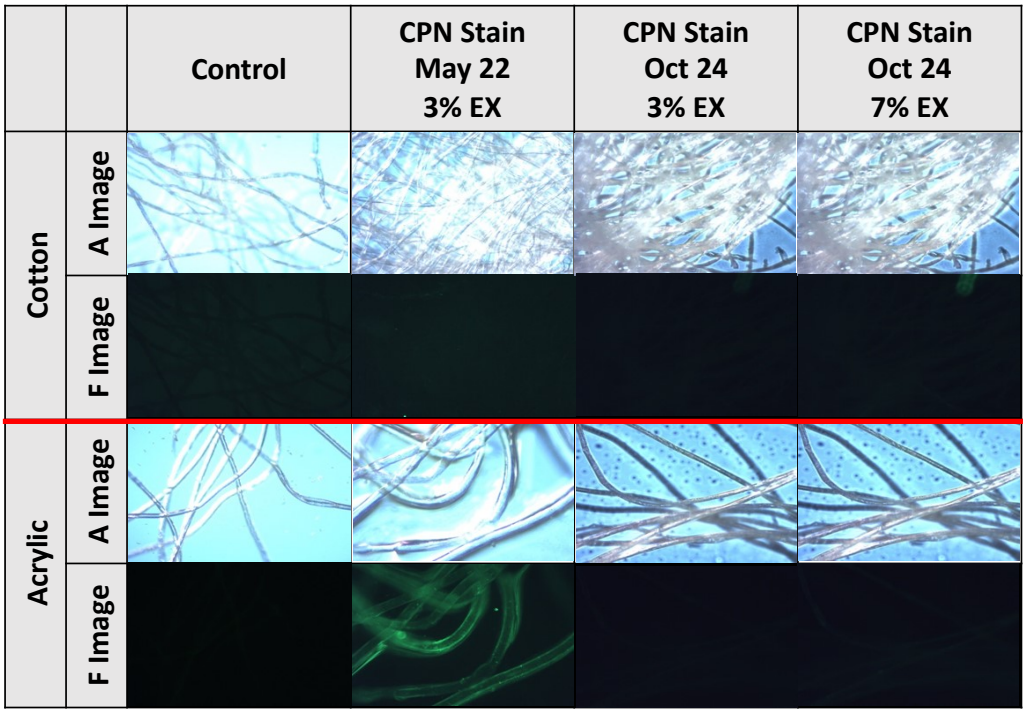
Supporting information figure 5 – optical microscopy and fluorescent images (x 10 magnification) of microplastic samples of polystyrene – stained initially (May 2022) and after ambient storage (October 2024). Control samples and stained with CPNs (excitation wavelength 470 nm, overnight incubation and varying irradiance power).

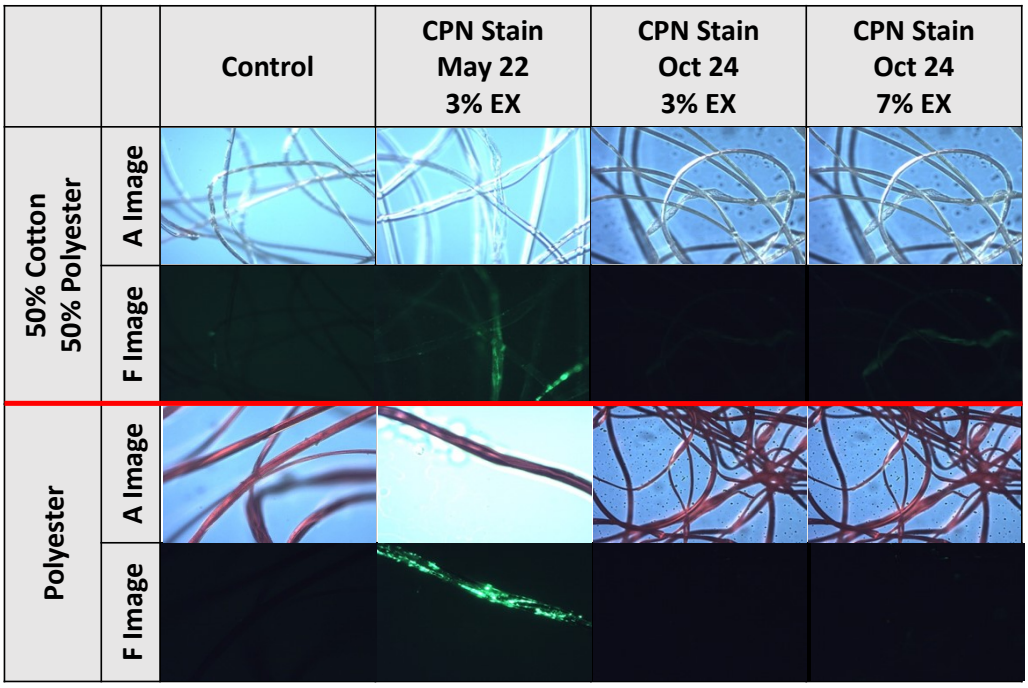


Supporting information figure 6 – optical microscopy and fluorescent images (x 10 magnification) of microplastic samples of PBAT and PLA – stained initially (May 2022) and after ambient storage (October 2024). Control samples and stained with CPNs (excitation wavelength 470 nm, overnight incubation and varying irradiance power).



Supporting information figure 7 – optical microscopy and fluorescent images (x 10 magnification) of microplastic samples of PHBV and PBS – stained initially (May 2022) and after ambient storage (October 2024). Control samples and stained with CPNs (excitation wavelength 470 nm, overnight incubation and varying irradiance power).





Supporting information figure 8 – optical microscopy and fluorescent images (x 10 magnification) of clothing fibres – stained initially (May 2022) and after ambient storage (October 2024). Control samples and stained with CPNs (excitation wavelength 470 nm, overnight incubation and varying irradiance power).