

UC Santa Cruz

UC Santa Cruz Electronic Theses and Dissertations

Title

Spatial Measurement of Microplastics in Lake Tahoe's East Shore Sediment

Permalink

<https://escholarship.org/uc/item/0pz0w52k>

ISBN

9798186336310

Author

Wallner, Madison Mavericks

Publication Date

2026-07-20

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA
SANTA CRUZ

Spatial Measurement of Microplastics in Lake Tahoe's East Shore Sediment

A thesis submitted in partial satisfaction
of the requirements for the degree of

MASTER OF SCIENCE

in

EARTH AND PLANETARY SCIENCE

by

Madison "Madio" Wallner

August 2026

The thesis of Madio Wallner is approved:

Dr. Adina Paytan

Dr. Clive Bagshaw

Dr. Quentin Williams

Donald Smith
Interim Vice Provost and Dean of Graduate Studies

Table of Contents

List of Figures.....	5
List of Tables	10
ABSTRACT	12
Acknowledgements:	13
Land Acknowledgement	14
Personal Motivation for this research:	14
CHAPTER 1 A REVIEW OF MICROPLASTIC RESEARCH IN THE LAKE TAHOE	
BASIN	18
INTRODUCTION:.....	19
Global plastic problem.....	19
Microplastics.....	20
Health effects	21
REVIEW OF MICROPLASTIC RESEARCH IN THE LAKE TAHOE BASIN	22
Site Description	22
Litter in Lake Tahoe.....	25
Litter data	25
Litter mitigation and policy	25
Microplastic Research in Lake Tahoe	27
Motivation for this research	29
CHAPTER 2 SPATIAL MEASUREMENT OF MICROPLASTICS IN LAKE	

TAHOE'S EAST SHORE SEDIMENT	31
Field Methods.....	32
Site Selection	32
Field Methods.....	34
Lab Methods	35
Laboratory Measures	35
Recovery testing.....	36
Laboratory Blanks	39
Sediment sample prep	40
Pretreatment with Hydrogen peroxide and size classing	40
Density Separation using CaCl ₂	41
Secondary Digestion using Hydrogen Peroxide	42
Final filtration and storage	42
Subsampling- Microscopy	42
Analysis Methods	44
Chemical Analysis	44
Raman Spectroscopy	44
QAQC Measures	45
Spectra Analysis.....	46
Quantification using Nile Red	47
Laboratory Method	48
Fluorescence Microscopy.....	48

ImageJ Analysis	50
Statistics	50
Results	51
MP Particle Quantification	51
Particle abundance per gram of sediment.....	51
Blanks.....	52
Particle abundance across sites.....	53
Particle size across sites	55
Morphology and color	59
Morphology.....	59
Color	61
Morphology and Color combined.....	63
Raman Spectroscopy	64
Material Assignments	67
Inconclusive	67
Inconclusive Organic	69
Blue Disperse dye on PET Fiber	70
Dye-Dominated.....	72
Polymer ID	73
Rare assignments	75
Discussion	76
Limitations	81

Preprocessing limitations.....	81
Analysis limitations	82
Conclusion	84
CHAPTER 3: COMPARING THE USE OF DCVJ VS. NILE RED FOR SOLVATOCHROMISM AND EMISSION INTENSITY USING PLASTIC STANDARDS AND ENVIRONMENTAL SAMPLES	87
Introduction	88
Motivation for this research	89
Literature review NR.....	90
Literature Review DCVJ	92
Experimental Design	93
Plastic Standards	93
Materials	93
Staining Procedure	94
Photography	96
ImageJ Analysis	96
Fluorescence Spectroscopy	98
Raman Spectroscopy	98
Results	99
Photography analysis	99
Photograph of the Polymer Standards	99
Photograph of Hawaiian Microplastics	100

ImageJ Results.....	102
RGB Analysis of Polymer standards	102
Fluorescence Spectroscopy Analysis.....	103
Raman Spectroscopy Results of Hawaiian Microplastics	105
Discussion	107
Conclusion.....	111
References.....	112

List of Figures

Figure 1: The Lake Tahoe Basin with sediment sampling sites (red dots) and popular recreation sites (yellow stars). Urban areas are mapped to show relevant land use. The inset map provides situational context for the study site area.

Figure 2: Sediment sampling sites (red dots) along Lake Tahoe’s east shore in relation to the original 2020 clean up dive transects (colored lines). Sediment from three sites along each of the original five transects were sampled in September 2025.

Figure 3: Lab generated recovery testing particles: Polystyrene (pink) and Polyvinylchloride (blue) particles. Imaged on a stainless-steel mesh filter (20µm pore size) overlaid on a gridded MCE filter. Photo credit M. Wallner.

Figure 4: Particles subsampled from filters then staged onto a glass slide covered in double sided tape prior to chemical analysis. All particles are circled in sharpie and numbered. Classifications as follows: (a) tan fragment, (b) red fiber, (c) white fiber, (d) white fiber, (e) white foam (f) black fiber.

Figure 5: Raman spectra and corresponding OpenSpecy reference library matches for polymer standards from the Polykit 1.0. (A) Polypropylene and (B) polystyrene.

Raman spectra were collected using a Horiba LabRAM HR Evolution spectrometer with a 532 nm excitation laser, a 10 s integration time, and three accumulations. The solid dark gray line represents the preprocessed sample spectrum, while the dotted turquoise line represents the best-matching reference spectrum from the OpenSpecy library. OpenSpecy analysis identified the polypropylene standard with a match value of 0.98 and the polystyrene standard with a match value of 0.92, indicating strong agreement between the measured and reference spectra.

Figure 6: (a) Filter B.5 from Site ES2 (Zephyr Cove) imaged using a Zeiss Axio Zoom.V16 fluorescence microscope. The filter was imaged using the DAPI (excitation 365 nm, emission 445/50 nm), GFP (excitation 470/40 nm, emission 525/50 nm), DsRed (excitation 545/25 nm, emission 605/70 nm), and Cy5 (excitation 640/30 nm, emission 690/50 nm) fluorescence channels. (b) The same filter after image processing in ImageJ (Fiji), where a threshold-based mask was

generated to identify and quantify all particles meeting the specified detection threshold.

Figure 7: Mean particle counts measured in procedural blanks ($n = 19$) and environmental samples ($n = 51$). Error bars represent ± 1 standard deviation. Environmental samples contained significantly higher particle counts than blanks (Welch's t -test, $p < 0.001$).

Figure 8: Distribution of particles across all sites not blank corrected. Boxplots show the median (white line), interquartile range (box), and minimum and maximum values (whiskers). Individual points represent count values for each filter. East Incline (ES6) demonstrated the highest mean particle abundance as well as the largest variation.

Figure 9: Percent composition of microplastic particle size classes at sampling sites ES1, ES2, ES4, ES6, and ES7. Stacked bars show the proportion of particles in each size class (5 mm–833 μm , 833–500 μm , 500–250 μm , and 250–63 μm), normalized to 100% of the particles collected at each site. Values within each bar indicate the percentage of total particles represented by each size class.

Figure 10: Distribution of the average Feret diameter measurements for all blank filters. Most blank filters (68.4%) had average Feret diameters within the 500–250

μm size class, while the remaining 31.6% had average Feret diameters within the 250–63 μm size class.

Figure 11: Distribution of particle morphologies identified at each sampling site.

Stacked bars represent the number of particles assigned to each morphology category (fiber, fiber bundle, fragment, film, flake, foam, and cylindrical pellet). Values within each bar indicate the number of particles in each morphology category, and the value above each bar indicates the total number of particles identified for that group.

Figure 12: Morphologies for all sites aggregated compared to all blanks. Stacked bars represent the number of particles assigned to each morphology category (fiber, fiber bundle, fragment, film, flake, foam, and cylindrical pellet). Values within each bar indicate the number of particles in each morphology category, and the value above each bar indicates the total number of particles identified for that group.

Figure 13: Distribution of particle colors identified at each sampling site and in the aggregated laboratory blanks. Bar segments represent the number of particles assigned to each color category, and values within each segment indicate particle counts. Numbers above each bar denote the total number of particles analyzed for each site. White particles were the dominant color at all sampling sites and in the laboratory blanks, followed by blue and black particles. Brown particles were

observed exclusively at ES4, while green and gray particles occurred only infrequently.

Figure 14: Distribution of Raman spectroscopy material identifications by sampling site for environmental particles analyzed from sites ES1, ES2, ES4, ES6, and ES7. Stacked bars represent the number of particles assigned to each material category at each site, including Inconclusive, Inconclusive Organic, Blue Disperse Dye on PET Fiber, Dye-Dominated, PP, PET, PS, PE, Carbonaceous Black Material, and Sulfate Mineral.

Figure 15: Polymer identification results for all particles analyzed, including both environmental samples (ES1, ES2, ES4, ES6, and ES7) and laboratory blanks (n = 179). Slice labels indicate the percentage of particles assigned to each material category. The majority of particles were classified as Inconclusive (69.3%), followed by Inconclusive Organic (10.6%), Blue Disperse Dye on PET Fiber (6.1%), Dye-Dominated (5.0%), PP (3.9%), PET (2.2%), PS (1.1%), and PE, Carbonaceous Black Material, and Sulfate Mineral (0.6% each).

Figure 16: Comparison of Raman spectroscopy material identifications for all analyzed particles (n = 179), environmental samples (n = 118), and laboratory blank samples (n = 46). Material identifications are grouped by unique Raman spectral

matches and shown side-by-side for the complete dataset, environmental samples, and blanks.

Figure 17: Three representative Raman spectra classified as inconclusive due to spectral interference and poor signal quality due to possible diffuse scattering and/or fluorescence. (A) Raman spectra from particle 24 collected at Site ES4, exhibiting very low signal intensity and a noisy, irregular ("scratchy") spectral profile. (B) Three spectra from particle 2 collected on laboratory blank filter 20. The spectra rapidly increase in intensity before saturating at the detector's upper intensity limit, producing clipped signals. This saturation pattern was commonly observed for tubular-shaped particles. (C) Raman spectra from particle 18 collected at Site ES1. Beginning at approximately 1500 cm^{-1} , the spectra display a repetitive sinusoidal pattern characteristic of excessive light from either fluorescence or diffuse scattering interacting with the optics. Fluorescence was the most frequently observed source of spectral interference across all analyzed samples.

Figure 18: Three representative spectra classified as inconclusive organic. (A) Spectra collected from particle ES1 (B) Spectra collected from laboratory blank particle . (C) Spectra collected from laboratory blank particle . All spectra exhibit a broad fluorescent background and only weak but discernible C–H stretching bands in the high-wavenumber region (approximately $2800\text{--}3100\text{ cm}^{-1}$). All spectra show

another weak peak at $\sim 1112\text{cm}^{-1}$, however more information is needed for a confident material ID.

Figure 19: Three representative spectra classified as Blue Disperse dye on PET fiber.

(A) Spectra collected from laboratory blank particle SedBlank5_2. (B) Spectra collected from laboratory blank particle SedBlank5_4. (C) Spectra collected from laboratory blank particle SedBlank14_2. All panels show reproducible peaks at $\sim 980, 1040, 1112, 1200, 1290, 1370, 1490, 1600\text{ cm}^{-1}$; weak C–H stretching bands at approximately $2800\text{--}3100\text{ cm}^{-1}$.

Figure 20: (A) Spectra collected from particle ES7_20. (B) Spectra collected from laboratory blank particle SedBlank18_8. All spectra exhibit a strong fluorescence background with weak, broad Raman features.

Figure 21: Representative Raman spectra of two commonly identified polymer types.

(A) polypropylene (PP) from Site ES1, and (B) polystyrene (PS) from Site ES4.

Figure 22: Raman spectra collected from particle ES2_2. The spectra exhibit the characteristic D and G bands of carbonaceous black material (carbon black), located at approximately 1350 cm^{-1} and 1580 cm^{-1} , respectively.

Chapter 3:

Figure 23: Modified cuvette designed for fluorescence analysis of solid polymer pellets using a Varian Eclipse spectrofluorimeter. Three side walls were removed and an internal mounting platform fitted with double-sided adhesive tape was added to secure pellets within the optical path during measurement. Photo credit: Clive Bagshaw.

Figure 24: Fluorescence responses of reference polymer standards from the Polykit 1.0 under three treatment conditions: NR, DCVJ, and unstained control. Polymers included are polyethylene variants (ULDPE, LDPE, LLDPE, MDPE, HDPE), polypropylene (PP), polyester (PEST), polyethylene terephthalate (PET), ethylene-vinyl acetate (EVA), acrylonitrile butadiene styrene (ABS), expanded polystyrene (EPS), polystyrene (PS), polyamide (PA), and polyvinyl chloride (PVC).

Fluorescence intensity and color variation illustrate differences in dye affinity and staining response among polymer types. Photo credit: Clive Bagshaw, M. Wallner

Figure 25: Fluorescence responses of HMP (Waimanalo Beach, Oahu, HI) and reference polymer standards under three treatment conditions: NR, DCVJ, and unstained control. Polymer standards included are Nylon, Polyethylene (HDPE, LLDPE), cellulose acetate, PVC, Polyamide (PA66), polystyrene and crumb rubber (Tire). The unknown environmental samples are HMP1-8. Photo credit: Clive Bagshaw, Amelia Labbe

Figure 26: Fluorescence emission spectra of selected polymers measured using a Varian Eclipse spectrofluorometer following staining with NR and (DCVJ). Black lines represent NR-stained polymers, blue lines represent DCVJ-stained polymers, and green dashed lines represent unstained controls. The x-axis shows emission wavelength (nm), corresponding approximately to the visible colors indicated by the color bar, and the y-axis shows fluorescence emission intensity. Emission profiles are shown for polyethylene (PE), polypropylene (PP), polyester (PEST), polyethylene terephthalate (PET), ethylene-vinyl acetate (EVA), acrylonitrile butadiene styrene (ABS), polystyrene (PS), polyamide (PA), and polyvinyl chloride (PVC).

Figure 27: OpenSpecy spectral match results for representative polymer particles. (A) Representative spectrum identified as polyethylene (PE) (HMP2). (B) Representative spectrum identified as polypropylene (PP) (HMP3). Spectra were generated in OpenSpecy to assess material identification through spectral matching. The solid black line represents the preprocessed sample spectrum, while the dashed turquoise line represents the corresponding OpenSpecy reference library match. The close alignment between the sample and reference spectra supports the polymer identifications.

List of Tables

Table 1: All sites including the Site ID, the common recreation name and the longitude and latitude coordinates for the beach onshore of the sampled transect as

well as the date and volume of sediment collected. Volume of sediment represents the combination of all three grabs homogenized for each site.

Table 2: Percent recovery for samples processed using the DSD and Beaker methods. The Beaker method exhibited a higher average percent recovery (78.44%) compared to the DSD method (68.76%). However, the number of complete trials differed between methods (DSD: $n = 16$; core: $n = 7$), which should be considered when interpreting variability and overall method performance.

Table 3: Summary of particle abundance for each sampling site. Values include the number of filters analyzed, mean dry sediment mass per filter, mean particle count per filter, blank-corrected particle count per filter, and the resulting blank-corrected particle abundance normalized to sediment mass (particles g^{-1} dry sediment). Blank correction was performed by subtracting the mean particle count observed in procedural sediment blanks from the mean particle count for each sampling site. The summary row shows the average blank-corrected particle abundance across the five sites, with ± 1.69 representing the standard deviation (SD).

Table 4: Summary statistics for particle counts at each sampling site. The number of filters analyzed using fluorescence imaging and Fiji image analysis is reported as n . Mean particle count and standard deviation (SD) are presented for each site.

Table 5: all blank filters with the average ferret diameter for each filter. This average was calculated by taking the ferret diameter of each counted particle then averaging it.

Table 6: Top color–morphology combinations identified across all environmental sampling sites and laboratory blanks. Environmental samples (ES1, ES2, ES4, ES6, and ES7) and laboratory blanks are presented as aggregated datasets. Counts represent the total number of particles assigned to each color–morphology combination, and percentage columns indicate the proportion of particles represented by each combination within the aggregated environmental samples ($n = 132$) and aggregated laboratory blanks ($n = 47$).

Ch 3

Table 7: Red chromaticity and relative fluorescence intensity of dyed polymer samples under three conditions (NR, DCVJ, and control). Red chromaticity quantifies the apparent emission color, where values approaching 1 indicate red emission, values near 0.5 appear yellow, and values approaching 0 indicate green emission. Relative fluorescence intensity values were normalized to EVA dyed with NR, which exhibited the highest fluorescence intensity and was assigned a value of 100. Values in red indicate that the polymer demonstrated relative intensity of values

lower than 20 which are comparable to the background signal from unstained samples meaning that chromacity value is not well defined.

Table 8: Spectral matches for HMP samples 1-7. All exhibit strong match values.

Signal to noise ratio (SNR) is variable among samples.

ABSTRACT

Spatial Measurement of Microplastics in Lake Tahoe's East Shore Sediment

Madison "Madio" Wallner

The Lake Tahoe Basin struggles with litter issues including plastic pollution. Microplastics were first documented in the Tahoe Basin in 2019 and since then have been documented in several environmental compartments in the area. One environmental compartment that is not well understood is Lake Tahoe's lakebed sediments. This thesis examines sediment samples collected from 5 transect sites along Lake Tahoe's east shore to better understand the distribution and chemical composition of microplastic particles in Lake Tahoe's sediments. Using density separation, digestion with hydrogen peroxide and chemical analysis using Raman spectroscopy and microscopy quantification using Nile Red dye, I found that across all sampling sites, the mean blank-corrected abundance was 2.70 ± 1.69 particles g^{-1} dry sediment (mean $\pm$ SD). Particle ($63 \mu\text{m}$ to 5 mm) abundances ranged from 1.01 to 4.96 particles g^{-1} dry sediment across the five sampling sites. The highest abundance was observed at East Incline (ES6 4.96 particles g^{-1}), followed by Zephyr Cove (3.73 particles g^{-1}) and South Sand Harbor (2.65 particles g^{-1}). Lower abundances were observed at Hidden Beach (1.17 particles g^{-1}) and Nevada Beach (1.01 particles g^{-1}).

Acknowledgements:

I would like to thank my advisor, Dr. Adina Paytan, for her continued support and encouragement in allowing me to pursue this passion project. I am grateful to my partner, Miles Schulman, for his unwavering support and thoughtful advice off the clock. I would like to thank Dr. Quentin Williams for his support of the Raman Analysis (for anyone who thinks that Quentin is grumpy they clearly have not seen his eyes light up when presented with a Raman spectra mystery!). I would also like to thank Dr. Clive R. Bagshaw for the time, energy, and dedication he contributed to our collaboration. For their contribution in the lab, and on analytical instruments, I would like to thank my core undergraduate interns: Tatum Taves, Timmy Koehler, Nicole Sawano, and Kate Lau. Their contribution created a community and reminded me that working with a team is so much better than working alone.

My appreciation extends to Dr. Monica Arienzo and her lab, including Rachel Kozolski and Hannah Lucasik, for their generous support over Zoom and for welcoming me into their lab meetings. Special thanks to Dive Master John Peltier and boat captain Dave Hoop and his family for their invaluable assistance during my East Shore sampling campaign. I am also grateful to the Clean Up the Lake team for their support during phase one of sample collection. Technical support from Benjamin Abrams, UCSC Life Sciences Microscopy Center, RRID: SCR_021135. Finally, I would like to acknowledge the many dedicated individuals across organizations in the

Tahoe Basin who work each day to better understand plastic and microplastic pollution and other aspects of Lake Tahoe's environment—with the shared goal of protecting and preserving this remarkable ecosystem.

Land Acknowledgement

I would like to acknowledge that the Lake Tahoe Basin is the ancestral and traditional homeland of the Washoe people. The Washoe Tribe of Nevada and California has stewarded Da ow a ga (Lake Tahoe) and the surrounding lands since time immemorial. The lake and its watershed are central to Washoe history, culture, spirituality, and identity. I recognize and honor the enduring relationship between the Washoe people and this land and water. Despite displacement and ongoing challenges, the Washoe Tribe continues to care for and protect this place. I express gratitude for their stewardship and strive to recognize Indigenous leadership in the protection and preservation of the Lake Tahoe Basin.

Personal Motivation for this research:

While I am not native to the Lake Tahoe Basin, this area feels like the closest thing I have to a homeland. I grew up in a single parent household with my mother who is an addict. Despite the challenges of my homelife I have had the immense privilege of growing up surrounded by clean water and fresh air. Having access to these things as a child inspired my curiosity and made me confident in nature; having access to these

things as a teenager, when my mom relapsed, saved my life. Without a doubt, the influence of growing up in the Lake Tahoe Basin has shaped me into the researcher, environmentalist and woman that I am today.

This influence was clear as early as high school. Observing plastic debris on beaches and trails motivated me to found an environmental club at Incline High School, where I graduated in 2018. The club focused on litter awareness and waste reduction, and initiatives such as campus clean-ups and “Bring Your Own Plate Day” helped build a foundation for my commitment to environmental stewardship in Lake Tahoe. This passion continued to develop during my undergraduate studies at Vassar College. For my senior thesis, titled *Mapping the Distribution of Litter in the Lake Tahoe Basin*, I used the Blue Crew dataset from the League to Save Lake Tahoe to analyze terrestrial litter distribution. My thesis received departmental honors. After graduating, I joined the Tahoe-based nonprofit Clean Up The Lake (CUTL), where I served as the Data Management Coordinator from 2023 to 2024. In this role, I supported volunteer SCUBA teams working to remove submerged litter from Lake Tahoe and other lakes in the Sierra Nevada.

This master’s thesis was inspired by my obsession with litter. Building on my background with terrestrial and aquatic litter data in the basin, I was curious about what happens when plastic litter breaks down into pieces that are too tiny to collect easily by hand. This project was a labor of love, motivated by the idea that this work might add to our understanding of Lake Tahoe’s pollution problem.

CHAPTER 1 A REVIEW OF MICROPLASTIC RESEARCH IN THE LAKE TAHOE BASIN

INTRODUCTION:

Global plastic problem

Plastic is a widespread man-made material with applications in nearly every sector worldwide. The term plastic describes many different polymers- materials derived from fossil fuels and characterized by long chain-like molecular structure. Plastic is versatile, durable, heat resistant and cheap to produce. For these reasons plastic production has increased dramatically since 1945 and continues to increase (Geyer, 2020). Some models predict that global annual primary plastic production will reach 1.1 billion tons in 2050 (Geyer, 2020). As plastic production increases, challenges associated with managing plastic waste also increase (Andrady 2022).

While plastic waste is a global problem, the United States experiences the highest per capita generation of plastic waste at 130 kg/year (Law et al 2020). The ocean serves as the ultimate sink for mismanaged plastic waste, with some estimates projecting that around 250 million metric tons will have entered marine environments by 2025 (Jambeck et al., 2015).

Different plastic polymers behave differently in the environment depending on their chemical composition, size, and depositional context. As a pollutant, plastic debris is

commonly categorized by size: nanoplastics ($<1\text{ }\mu\text{m}$), microplastics ($1\text{ }\mu\text{m}$ – 5 mm), mesoplastics (5 mm – 2.5 cm), macroplastics (2.5 cm – 1 m), and megaplastics ($>1\text{ m}$).

Microplastics

Research has shown that microplastic (MP) pollution is detectable in all compartments on Earth. Microplastics have been detected in the ocean (Carpenter and Smith, 1972; Wang, 2020), freshwater systems (Nava et al., 2023; Negrete Velasco, 2020), aquatic sediments (Cashman et al., 2022), terrestrial environments (Yu and Flury, 2021; Yu et al., 2021; Goßmann, 2021), in the atmosphere (O'Brien et al., 2023) and even in remote regions (Rosso et al., 2024).

MP's are defined as solid, polymeric particles with at least three dimensions greater than 1 nm and smaller than $5,000\text{ }\mu\text{m}$, to which chemical additives or other substances have been added (The Division of Drinking Water, State Water Resources Control Board, State of California, 2022). MP's enter the environment from both primary and secondary sources. Primary sources of MP's are those that were manufactured in a size class between 1 nm and smaller than $5,000\text{ }\mu\text{m}$, such as microbeads in face scrubs or raw resin 'nurdles'. Secondary MPs are largely produced from the degradation and breakdown of larger plastic items in the environment through physical, chemical, or biological processes such as UV radiation, weathering, and mechanical abrasion (Wiesinger et al., 2021).

Health effects

Micro and nano-plastic pollution poses significant environmental and biological risks due to the combined effects of chemical additives present in plastics and the diverse exposure pathways of polymer particles to organisms. Many additives, such as plasticizers, antioxidants, and flame retardants, are not chemically bound to the polymer matrix and can migrate from the polymer resin into surrounding aquatic and terrestrial systems, where they may affect organisms (Megill et al., 2024). Some of these substances are classified as endocrine-disrupting chemicals and, upon ingestion, can interfere with behavioral and reproductive functions (Andrady and Rajapakse, 2019; Lynch et al., 2022). Other plastic additives have been identified as carcinogenic, immunosuppressive, acutely toxic, and, in certain cases, lethal (Lynch et al., 2022; Tian et al., 2022; Wiesinger et al., 2021). Micro and nanoplastics can adversely affect individual organisms through ingestion as well as exert harmful effects across trophic levels (Pastorino et al., 2023).

Micro and nano-plastics are a concern to human health. MP's have been detected in multiple compartments of the human body, including lung tissue (Amato-Lourenço, 2021), placenta (Qin et al., 2024), bronchoalveolar lavage fluid (Chen et al., 2023), sputum (Huang, 2022), and blood (Leslie et al., 2022). As concentrations of MP particles increase, so do their chronic and adverse health effects on organisms (Qin et al., 2024; Wen, 2024).

REVIEW OF MICROPLASTIC RESEARCH IN THE LAKE TAHOE BASIN

Site Description

Lake Tahoe is the largest alpine lake in North America and the second deepest lake in the United States. Famous for its clear waters, Lake Tahoe is situated in the Sierra Nevada Mountain range on the border of Nevada and California. Lake Tahoe has ~120 km of shoreline, sits at an elevation of 1897 meters above sea level and is 501.4 meters deep. Lake Tahoe is considered an oligotrophic lake, and its water has a long residence time of ~650 years (Goldman 1989). The Lake Tahoe Basin refers to the watershed, (813 km²) while the lake itself has a surface area of 497 km². Lake Tahoe receives most of its yearly precipitation in the winter months in the form of rain or snow. Lake Tahoe has 63 inlets but only one outlet, the Truckee River, which flows to the terminal Pyramid Lake located in Northern Nevada.

Lake Tahoe is a popular tourist destination and experiences about 15 million “visitor days” per year (Lake Tahoe Destination Stewardship Council, 2023). The Lake Tahoe Basin is home to ~56,000 year-round residents (United States Department of Transportation, 2022). Tourists and residents enjoy recreation opportunities from skiing in the winter to swimming and boating in the summer.

In 1969 a management compact for Lake Tahoe was formally established in and approved by the U.S. Congress. It created the Tahoe Regional Planning Agency (TRPA), a regional authority responsible for land-use planning and environmental regulation across the entire basin, regardless of state boundaries. Lake Tahoe has regulations that aim to protect water quality. For example, Lake Tahoe was designated as an Outstanding National Resource Water in 1980 under the U.S. Environmental Protection Agency framework of the Clean Water Act (U.S. Environmental Protection Agency, 2011). In 1969 the passing of the Porter Cologne Act mandated that all wastewaters be exported from the basin rather than being treated and returned to the lake as is typical in many other freshwater systems. Wastewater has been documented as a clearly defined pathway for microplastic pollution in freshwater systems (Conley et al. 2019). Despite these unique protections, recent research has shown that anthropogenic pollution, including microplastic, is present in Lake Tahoe's waters and the surrounding basin.

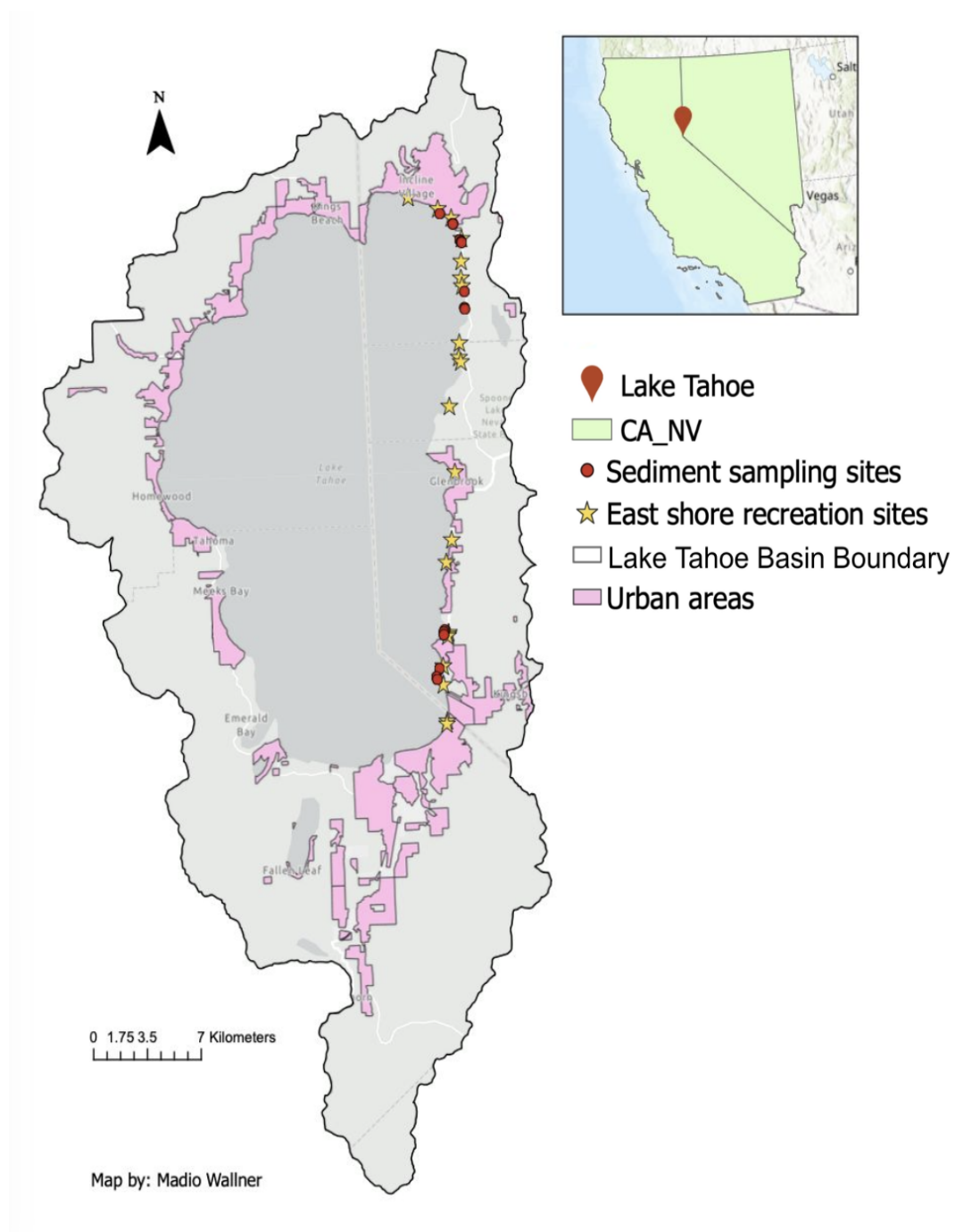


Figure 1: The Lake Tahoe Basin with sediment sampling sites (red dots) and popular recreation sites (yellow stars). Urban areas are mapped to show relevant land use. The inset map provides additional geographic context for the study site area.

Litter in Lake Tahoe

Litter data

The Lake Tahoe Basin is impacted by anthropogenic litter in both its terrestrial and aquatic environments. Since 2014 the League to save Lake Tahoe's blue crew litter program has collected and removed thousands of pounds from the Lake Tahoe Basin terrestrial environment (The League to Save Lake Tahoe n.d.) and since 2020, Clean up the lake (CUTL), a local Tahoe based non-profit has removed 36,162.34 lbs of aquatic litter from Lake Tahoe's lakebed (Clean Up The Lake, n.d.). While litter in Lake Tahoe's terrestrial and aquatic environments are well documented, it is unclear whether degradation of litter is a major pathway for microplastic pollution in the Basin.

Litter mitigation and policy

Pollution awareness and mitigation have a long-standing history in the Lake Tahoe Basin. The phrase "Keep Tahoe Blue" was coined in 1957 by The League to Save Lake Tahoe and is famously seen on bumper stickers around the country and world. This phrase emerged in response to early development that threatened the clarity of Lake Tahoe (The League to save Lake Tahoe, n.d). Today the sentiment of 'Keeping Tahoe Blue' extends to emerging pollution threats including plastic litter and MP. Addressing litter pollution is a clearly stated priority in the 2024 Lake Tahoe Stewardship Destination Plan, with two of the four strategic pillars explicitly addressing litter management. Of the 1,040 surveys from residents, 77% of

respondents “identify stronger enforcement of litter violations as a high priority” (Lake Tahoe Stewardship Destination Plan 2024). Efforts to mitigate anthropogenic litter in the Basin have included recent public campaigns such as the Take Care campaign which began in 2013 and focuses on Tahoe specific litter issues (Lake Tahoe Environmental Improvement Program, n.d.).

State and local legislation in the Lake Tahoe Basin has increasingly focused on reducing litter through restrictions on single-use plastic items. In the City of South Lake Tahoe, single-use plastic bags were initially banned in 2015 and are now regulated under California’s statewide plastic bag law (State of California, n.d.; City of South Lake Tahoe, n.d.). In 2018, the city adopted a “by request only” ordinance requiring that items such as utensils, straws, lids, and condiment packets be provided only when requested by customers (City of South Lake Tahoe, n.d.). That same year, South Lake Tahoe also enacted a ban on polystyrene food containers, prohibiting the sale and use of foam packaging materials (City of South Lake Tahoe, 2018). More recently, in October 2022, the city passed an ordinance banning the sale of single-use plastic water bottles smaller than one gallon, which became fully effective citywide in 2024 (City of South Lake Tahoe, 2022).

Just outside the Lake Tahoe Basin, the Town of Truckee, California, adopted a similar ordinance in April 2025, banning the sale and commercial distribution of unflavored, non-carbonated water in single-use plastic bottles and paper cartons

smaller than one gallon, mirroring South Lake Tahoe's 2024 regulation (Town of Truckee, 2024).

Given that the City of South Lake Tahoe is the Lake Tahoe Basin's largest population center, these policies demonstrate a growing consciousness toward plastic litter mitigation with potential implications for regional microplastic pollution.

Microplastic Research in Lake Tahoe

In 1989, Charles R. Goldman described Lake Tahoe: “With relatively little inflow and outflow, the lake retains most of what enters it”. These characteristics and hydrology make Lake Tahoe the impressive resource that it is, however as Goldman mentions, the lake is vulnerable to pollution due to its long residence time.

Microplastic pollution has been detected in multiple environmental compartments in Lake Tahoe and the surrounding basin. In 2023 MP's were detected in Lake Tahoe's surface waters at quantities higher than some sites in the ocean (Nava et al. 2023).

Nava et al. (2023) found that of the 38 lakes and reservoirs sampled worldwide, Lake Tahoe had the third highest concentration with 5.4 particles per m^{-3} of water. This study focused on particles greater than 250 μm and used trawl-nets to sample surface water.

Recent studies have identified fibers as the dominant microplastic morphology in Lake Tahoe. In 2020, a peer reviewed participatory citizen science study was launched involving seven households within the Tahoe Basin, each of which

installed a dryer vent capture apparatus on their dryer units (Arienzo et al. 2025) . Participating households submitted collected dryer vent samples along with detailed data on dryer usage, including textile types. During the study period, a total of 114 dryer loads were recorded, with 76 loads from households equipped with a dryer vent mesh and 38 from households without one. Microfibers collected from the mesh were subsampled and analyzed using micro–Fourier Transform Infrared spectroscopy (μ FTIR) for seven of the participating households.

The results indicated that the captured textile-derived microfibers were predominantly cellulosic, followed by polyethylene terephthalate (PET/PEST), and other synthetic polymers, and polyolefins. These findings support the conclusion that household dryers are a source of both natural and synthetic microfibers released into the Lake Tahoe Basin.

MP pollution has been documented in Lake Tahoe’s only outflow, the Truckee River (Lukasik, 2024). This thesis research examined MP particle concentrations across different hydrological seasons. Lukasik (2024) found that MP’s are ubiquitous throughout the Truckee River, with concentrations increasing downstream in response to anthropogenic inputs and varying with seasonal hydrology. The highest levels observed were downstream of the city of Reno. MP concentrations ranged from 0.7 to 1.7 MPs/L. Interestingly MP concentrations in the Truckee River (0.7–1.7 MPs/L) were much higher than those reported by Nava et al. (2023) for surface lake water (5.4 particles/m³, or 0.0054 MPs/L). The Truckee River had about 130–

315 times more MP than the Lake, suggesting higher MP pollution in Lake Tahoe's outflow compared to its surface water. This difference may reflect the river's much smaller water volume relative to the lake, resulting in less dilution, as well as additional inputs associated with recreational activities and the river's passage through rural, suburban, and urban landscapes. Lukasik noted that the majority of particles found in the Truckee River system were fragments (73.3%), followed by films (17.3%) and fibers/filaments (9.5%).

One non-peer-reviewed scientific report examined MP concentrations in Lake Tahoe water (Gjeltema et al., 2023). The study used horizontal trawls deployed behind a boat at the surface and at 15 m depth and collected 12 monthly samples from 2020-2021. They found an average of 306,000 ($\pm 417,012$ SD) MP particles per km² at the lake surface and 0.043 (± 0.04 SD) particles per km³ at 15 m depth. The study also analyzed lake biota and one sediment sample. For the sediment, Gjeltema et al. (2023) identified three suspected plastic particles in 1.53 g of sediment; after blank correction, one confirmed particle composed of polypropylene was detected, corresponding to an estimated abundance of 0.65 particles per gram of sediment. The authors emphasize the need for more robust sampling methods and expanded evaluation of MP contamination in lake sediments.

It remains unclear whether litter in the environment is contributing to secondary MP pollution in the region.

Motivation for this research

It is still unclear whether there is MP pollution in Lake Tahoe's sediment compartment (Gjeltema et al, 2023) though litter on the lakebed has been well documented (Harrold et al 2020) and chemically analyzed (Davidson et al 2024). Harrold et al. (2020) sampled litter from the lakebed offshore at six different locations along Lake Tahoe's East shore: South Sand Harbor (427.6 lbs.) , Incline Beach (372.4 lbs.) , East Incline/ NV state park (305.8 lbs.), Hidden Beach (128.3) , Zephyr Cove (290 lbs.) and Elk Point/ Nevada Beach (234.2 lbs.). This litter was then subsampled and chemically analyzed in a different study by Davidson et al (2024) revealing that the most common polymers at the macro scale from the east shore litter loads were polyvinyl chloride (PVC), polystyrene, expanded polystyrene, and polyethylene (Davidson et al., 2024). The Harrold et al. (2020) and Davidson et al. (2024) studies provide a baseline data set for the polymer types at the macro scale removed from lake Tahoe's east shore sediment at ~7.62 m of depth. While the macro scale plastic is well documented in this region, there has been no sampling for investigation at the micro scale for plastic pollution in the sediment. These baseline datasets at the macro scale beg the question of whether macro size lakebed litter is contributing to micro plastic pollution in situ in Lake Tahoe's sediment compartment and motivates this study.

CHAPTER 2 SPATIAL MEASUREMENT OF MICROPLASTICS IN LAKE TAHOE'S EAST SHORE SEDIMENT

Field Methods

Site Selection

Sediment sampling sites were selected based on underwater transects previously sampled for debris removal by Clean Up The Lake, a local Tahoe based non-profit, in 2020. These transects covered 9.3 km across six of the most popular beach locations along the east shore of Lake Tahoe. Using GPS coordinates recorded by CUTL, I returned to five of the east shore transects in September of 2025 and collected sediment grab samples from three points along each of the original ~7.62 m depth transects that were cleaned. From North to South these sites were: East Incline (ES6), Hidden Beach (ES7), South Sand Harbor (ES4), Zephyr Cove (ES2) and Nevada Beach (ES1). Site abbreviations range from ES1 to ES7, with ES3 and ES5 omitted because these identifiers were assigned to sample jars designated for atmospheric blank samples collected during field sampling. As the atmospheric blanks were not processed, these site identifiers were not used in subsequent analyses.

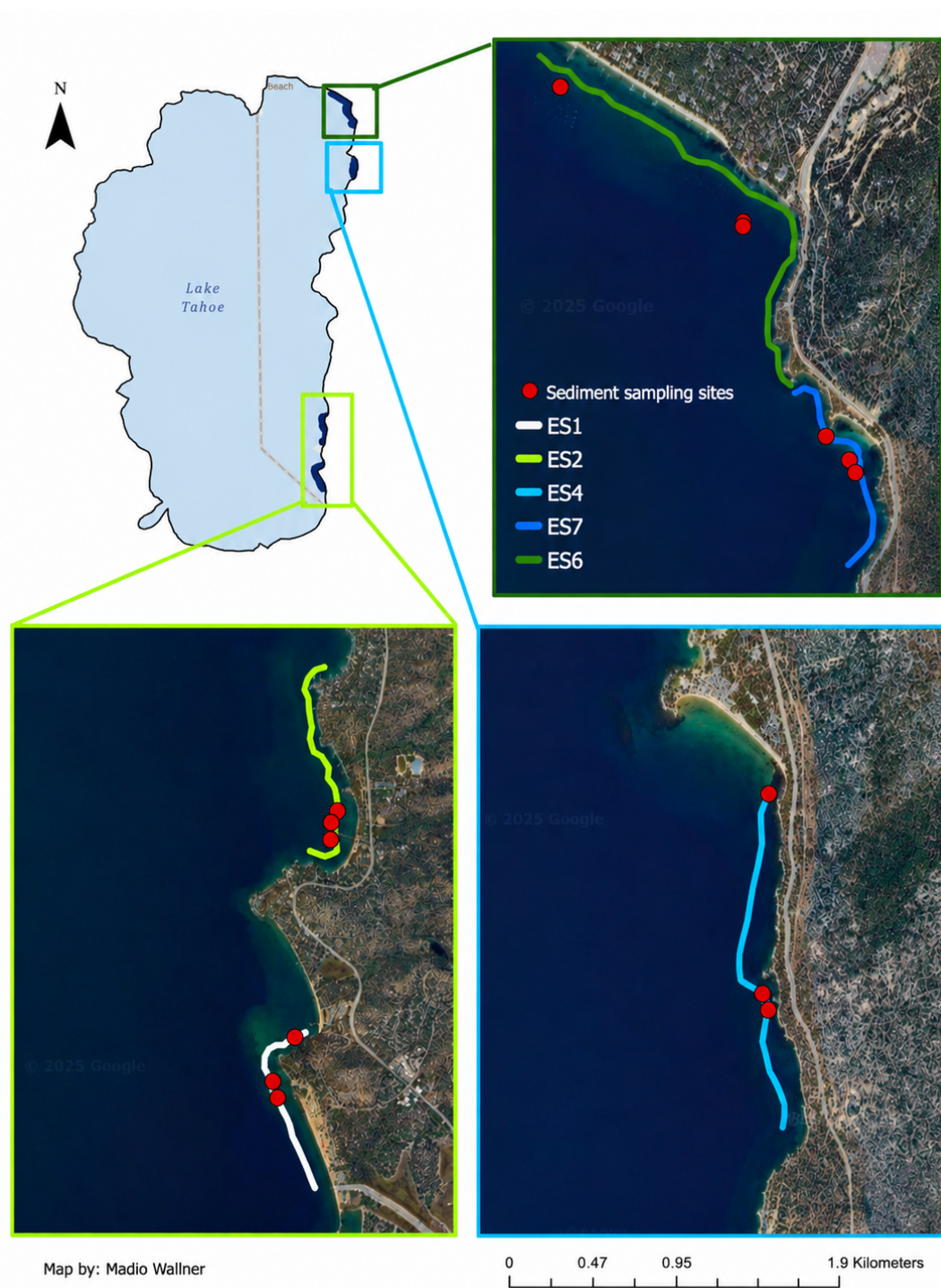


Figure 2: Sediment sampling sites (red dots) along the east shore of Lake Tahoe relative to the original 2020 cleanup dive transects (colored lines). Sediment samples were collected in September 2025 from three locations along each of the five original transects. From south to

north, the transects were: ES1 (Nevada Beach), ES2 (Zephyr Cove), ES4 (South Sand Harbor), ES6 (East Incline), and ES7 (Hidden Beach).

Sampling Method

Sediment samples were collected using a 1 L Wildco Instruments Van Veen grab sampler deployed from a boat and assisted by a SCUBA diver. Three 1L grabs per transect were collected, subsampled using a pre-cleaned metal scoop and homogenized into one pre-cleaned 1 L glass sample jar, totaling five homogenized samples, one per beach site, each in a 1 L mason jar. In total 5.73 kg of sediment was collected across all sites averaging ~1.15 kg per site.

Table 1: All sites including the Site ID, the common recreation name and the longitude and latitude coordinates for the beach onshore of the sampled transect as well as the date and mass of sediment collected. Mass of sediment represents the combination of all three grabs homogenized for each site.

Site number	Site Name	Latitude	Longitude	Date Visited	Mass Collected (kg)
ES1	Nevada Beach	38.988577	-119.95583	9/25/25	1.4438
ES2	Zephyr Cove	39.009424	-119.95099	9/24/25	1.23224
ES4	South Sand Harbor	39.192991	-119.92830	9/24/25	0.99843
ES6	East incline	39.235818	-119.94497	9/23/25	1.05277
ES7	Hidden Beach	39.221198	-119.93073	9/24/25	1.00284

Field Methods

Cleaning procedures of all labware supplies followed the recommendations of Thornton Hampton (2023). Mason jar lids were covered with aluminum foil to create a barrier between the sample and the rubber liner of the lid, minimizing potential contamination from the lid (Chaïb, et al 2025) . The metal Van Veen grab sampler was pre-cleaned with Milli-Q water and wrapped in aluminum foil prior to sampling. A metal scoop was used to transfer sediment from the interior of the Van

Veen sampler into the sample jars following recommendations from Gjeltrema et al 2023. The scoop was triple-rinsed with Milli-Q water using a teflon squirt bottle between each grab sample. Atmospheric blanks were collected for each new sampling event to account for potential airborne contamination. Jars containing sediment samples were stored in a cooler for transport back to the laboratory and upon arrival were stored at 3 °C prior to processing.

Laboratory Methods

Laboratory Measures

To reduce the potential for contamination, measures to reduce blanks were implemented in the laboratory and maintained throughout sample preparation. All glassware was pre-cleaned following recommendations from Thorton Hampton (2023). Briefly, all glassware and tools were washed with soap and water, rinsed three times with tap water, and then rinsed three times with Milli-Q water. After washing all glass ware was left to dry and any openings were covered in aluminum foil. A 100% cotton lab coat dyed pink was worn while working with samples. This allowed for easy identification of potential contaminant pink fibers following recommendations from the Center for Marine Debris Research (Shaw. K, CMDR Marine Debris Circularity Academy Microplastic Quantification Short Course, June 2025) . Teflon squirt bottles were used in both the lab and field to dispense Milli-Q water for rinsing, following recommendations from the Center for Marine Debris

Research. Teflon was chosen because it is a relatively uncommon polymer, reducing the risk of contaminating samples compared to polypropylene squirt bottles.

Recovery testing

Prior to processing environmental samples, two sediment preparation methodologies were evaluated in the laboratory by performing recovery tests. Recovery testing involved spiking clean sediment with a known quantity of dyed plastic particles and then running the spiked material through the sediment preparation protocols. The protocols tested were the Density Separation Device (DSD) method (Shaw et al. 2024) and the Beaker method (adapted from Langknecht et al 2023).

Beach sand from Santa Cruz, CA (Cowell's Beach) and lakebed sediment from Lake Tahoe's West Shore Placer County were treated in a muffle furnace for 2 hours at 400 °C to remove plastic contaminants (Lynch et al. 2025). Plastic particles used for spiking were sourced from the Hawai'i Pacific University Center for Marine Debris Research Polykit 1.0

(https://www.hpu.edu/cncs/cmdr/img/polymerkit1.0_marketingbrochure.pdf).

Polystyrene (1.05 g cm⁻³ (Andrady, 2011; Scientific Polymer Products, n.d.) and PVC.1 (density: 1.38-1.40 g cm⁻³ (Andrady, 2011; Scientific Polymer Products, n.d.) were selected to represent a range of particle densities.

Beads from each polymer type were placed separately into an aluminum coffee grinder and ground into smaller fragments across variable size classes. The ground particles were then dyed using alcohol inks. Dyed particles were wet-sieved at 40 µm

for use in the recovery tests. For initial counting, dyed plastics were filtered onto either stainless steel mesh filters or mixed cellulose ester (MCE) filters. Particles were counted twice under a Leica Dissection Stereo Microscope (Leica S8AP0), and the counts were recorded and averaged for each test.

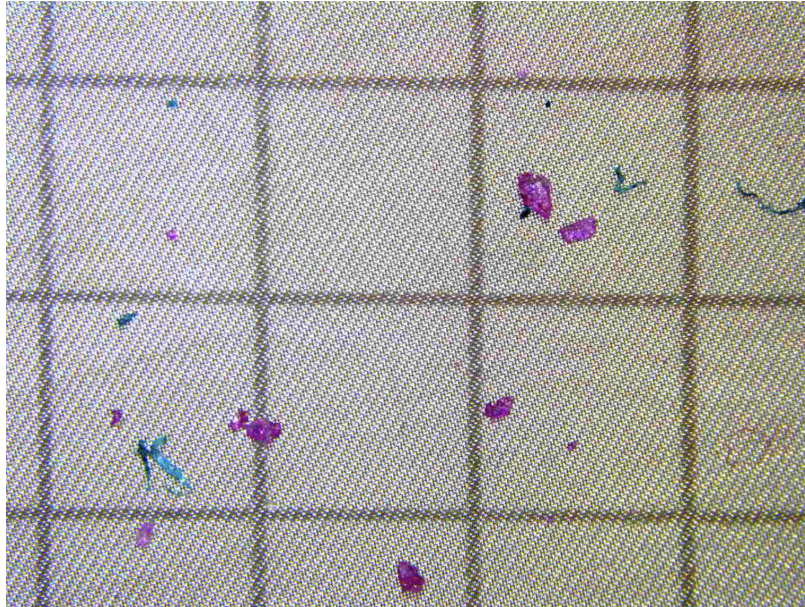


Figure 3: Lab generated recovery testing particles: Polystyrene (pink) and Polyvinylchloride (blue) particles. Imaged on a stainless-steel mesh filter (20 μ m pore size) overlaid on a gridded MCE filter. Photo credit M. Wallner.

Dyed particles of both PS and PVC were added to the plastic free sediment and underwent preparation following either the Density Separation Device method (Shaw et al. 2024) or the Beaker method (Langknecht et al. 2023). After processing following each method, filters were counted twice to determine the number of particles retained through each test and percent recovery was calculated using the following equation:

$$\text{Percent Recovery} = \frac{\left(\frac{\text{Post-count \#1} + \text{Post-count \#2}}{2} \right)}{\left(\frac{\text{Pre-count \#1} + \text{Pre-count \#2}}{2} \right)} \times 100$$

Percent recovery was averaged across all trials for each method. The mean recovery rate was 68.76% for the DSD method and 78.44% for the Beaker method. While both recovery rates were lower than the 94% reported by Lukasik (2024), the Beaker method exceeded the recovery rate of over 70% reported by Cashman et al. (2022). Based on these results, the Beaker method was selected and adopted as the standard operating procedure for sediment sample preparation in this study.

Table 2: Percent recovery for samples processed using the DSD and Beaker methods. The Beaker method exhibited a higher average percent recovery (78.44%) compared to the DSD method (68.76%). However, the number of complete trials differed between methods (DSD: $n = 16$; Beaker: $n = 7$), which should be considered when interpreting variability and overall method performance.

Method	Mean Percent Recovery (%)	Standard Deviation	Number of Samples (n)
DSD	68.76	22.5	16
Beaker	78.44	20.7	7

Laboratory Blanks

To assess background contamination, a laboratory blank was processed for every ~three sediment samples following Langknecht et al. 2023. Blanks were prepared by running clean water through all steps of the sediment preparation procedure and amending the density separation step to mimic that process but conserve the salt solution. Briefly, 1000 mL of Milli-Q water was passed through a pre-cleaned

stainless steel sieve stack (5 mm, 833 μm , 500 μm , 250 μm , 63 μm), collected in a large beaker and left covered with aluminum foil. This solution was periodically subsampled (~100 mL per blank). Subsamples underwent the full preparation protocol, including modified density separation, filtration, hydrogen peroxide digestion, particle isolation, and mounting on double-sided tape for Raman analysis. Samples were then stained with Nile Red, re-filtered, and imaged using fluorescence microscopy.

Of the 19 ($n=19$) laboratory blanks that were run, an average of 197 particles per blank filter ($\text{SD} = 130$) were detected using the same methods for particle quantification described below (staining using Nile Red, fluorescent microscopy, image analysis using Fiji). This average was used to adjust the findings in reporting the particles per gram of sediment for environmental samples.

To calculate blank abundance, the mean particle count of the corresponding site blanks was subtracted from the observed particle count, and the corrected count was divided by the dry mass of sediment processed to obtain particles per gram of dry sediment. Site-level means and standard deviations were then calculated from the individually corrected filters.

Sediment sample prep

Pretreatment with Hydrogen peroxide and size classing

Prior to chemical analysis and microscopy, sediment samples were preprocessed in the laboratory to separate potential plastic particles from the sedimentary matrix and

remove organic matter. Each 1 L sediment sample was pretreated with 30% hydrogen peroxide for approximately 7 days at room temperature (~22 °C). Treatment time varied slightly depending on the amount of organic matter as determined based on the termination of the bubbling reaction.

After initial digestion, sediments were rinsed and wet-sieved using a precleaned stainless steel sieve stack (5 mm, 833 µm, 500 µm, 250 µm, 63 µm). All material greater than 5 mm and smaller than 63 µm was discarded, and obvious organic matter, such as quagga mussel shells, were picked out and discarded. The sediment was then left to dry, covered in aluminum foil, and placed in an oven at 60 °C for ~24 hours, or until dry enough to dry-sieve. Material was size-classed into four size fractions (5 mm–833 µm, 833 –500 µm, 500- 250 µm, 250–63 µm), transferred to precleaned 600 mL glass beakers, and stored at room temperature covered in aluminum foil.

Density Separation using CaCl₂

For each site, roughly ten sediment subsamples weighing ~100g each (using aluminum weight boats) were transferred into a glass beaker, using a metal scoop. Subsamples from the largest size class (5 mm–833 µm) were processed first, then, depending on the amount of sediment in each size class, the remaining samples were taken from the second largest size class (833 µm–500 µm) to make up 10 total subsamples per site. Sediment from the two largest size classes were prioritized because material smaller than 500µm was difficult to manually subsample for

chemical analysis. All of the particles subsampled for Raman analysis across all sites were derived from the 5 mm–833 μm size class.

Sediment was processed following the Beaker method. Subsamples were treated with a density separation solution using CaCl_2 (density $\sim 1.4 \text{ g/cm}^3$) (Langknecht et al. 2023).

Briefly, the density separation consisted of adding 200 mL of CaCl_2 solution to the 100 g sediment subsample and stirring with a metal stir rod for 30 seconds. The sample was then covered with aluminum foil and left to settle for ~ 2 hours. After settling, the floating fraction was decanted into a separate precleaned glass beaker and additional salt solution was added. Stirring, and settling (~ 2 hours) was repeated.

After the second settling treatment, the floating fraction was again decanted into the second beaker. The contents of this beaker were allowed to settle for an additional ~ 1 hour before being decanted into a precleaned glass filtration system, using either a 25 mm stainless steel filter (20 μm pore size) if no further digestion was required, or a 45 mm stainless steel filter (20 μm pore size) for additional digestion of organic matter.

Secondary Digestion using Hydrogen Peroxide

Based on visual assessment following density separation, most filters underwent an additional digestion step using hydrogen peroxide to remove residual organic matter. For this procedure, the 45 mm stainless steel filters were transferred to pre-cleaned

glass petri dishes, after which 10 mL of hydrogen peroxide was added. The dishes were then covered with a glass crystallization dish and placed in an oven at 60 °C for 2 hours (Langknecht et al., 2023). Filters were then rinsed and re-filtered onto 25 mm diameter stainless steel filters (20 µm pore size).

Final filtration and storage

After processing, samples collected on 25 mm stainless steel filters were transferred to pre-cleaned glass slides and covered with pre-cleaned glass petri dishes for storage. Samples from the largest size class were stored until subsampling, while the remaining samples underwent Nile Red staining followed by fluorescence imaging. Covered samples were kept at room temperature in an enclosed bench-top cupboard until microscopy, chemical analysis, and fluorescence imaging were conducted.

Subsampling- Microscopy

Plastic particles were picked from all filters from the largest size class (5 mm-833 µm) across all sites as well as from the blank laboratory filters. Filters were observed under a Leica S8 APO Stereo Microscope and fine-tipped metal tweezers were used to manually pick out particles for chemical analysis. Particle picking guidelines followed recommendations from the Rochman Lab, focusing on particles that appeared anthropogenic in nature (Rochman et al. 2019). This included particles representing common MP morphologies: fiber, fiber bundles, fragments, foam, film, pellet, and sphere (Miller et al , 2021). While anthropogenic-seeming particles were

prioritized, other particles suspected to be inorganic in nature were also picked in order to reduce selection bias

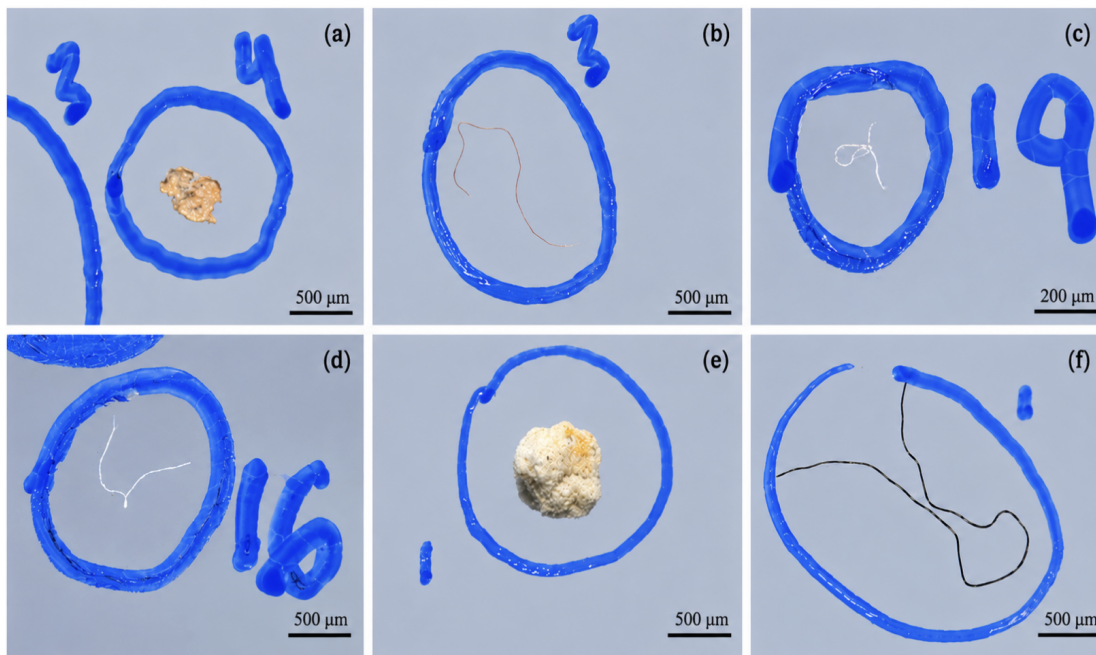


Figure 4: Particles subsampled from filters then staged onto a glass slide covered in double sided tape prior to chemical analysis. All particles are circled in sharpie and numbered. Classifications as follows: (a) tan fragment, (b) red fiber, (c) white fiber, (d) white fiber, (e) white foam (f) black fiber.

To prepare for chemical analysis using Raman spectroscopy, picked particles were placed onto double-sided tape, circled with a Sharpie, and labeled with a particle number (1-30). All particles were cataloged, and picking continued until 30 total particles were collected across all 10 subsamples of each site. Particles were selected by collecting up to ten of each unique morphology/color combination present on the filter (e.g., blue fibers), or until 30 total particles were collected for each of the five sites.

Analysis Methods

Chemical Analysis

Raman Spectroscopy

Raman Spectroscopy is one of the most popular methods for identifying and quantifying MP pollution. Raman spectroscopy uses visible or near infrared light to excite chemical bonds within a molecule and looks specifically for the Raman shifted photons that are scattered from the sample. Because chemical bonds in a molecule are unique and distinctive, the Raman spectrometer generates spectra which correspond to particular bonds. Interpreting/ analyzing the spectra and the bonds present for a given sample allows for chemical identification of the material in question (Forakis, J. PowerPoint presentation, June 3, 2025).

Raman spectroscopy was used to determine the polymer composition of MP samples. Raman spectra were collected using a Horiba LabRAM HR Evolution Raman spectrometer with a 50-milliwatt 532 nm excitation laser. Spectra were typically collected over the range of 400–3600 cm^{-1} . Initial acquisition parameters consisted of a 10 s integration time with three accumulations followed by an additional two scans at 20 s and three accumulations. An average of three spectra were captured per particle. Laser power was adjusted when required to optimize spectral intensity while minimizing the risk of thermal damage to the sample with most spectra collected with 0.1% laser power (using a neutral density (ND) filter).

Collected spectra were analyzed by identifying and recording prominent peaks and comparing both the peak positions and overall spectral profiles with reference spectra from the CMDR HPU Polykit 1.0 reference library provided on the accompanying flash drive.

QAQC Measures

Prior to Raman analysis of particles, the spectrometer was calibrated by analyzing several plastic standards from the Hawai'i Pacific University Center for Marine Debris Research Polykit 1.0. Specifically, polypropylene and polystyrene standards were analyzed with a 532 nm excitation laser, a 10 s integration time, and three accumulations (Figure 5). In addition, spectra of the double-sided tape were collected as background and referenced as needed to determine if during data collection the laser was missing the environmental particles and instead interacting with the background.

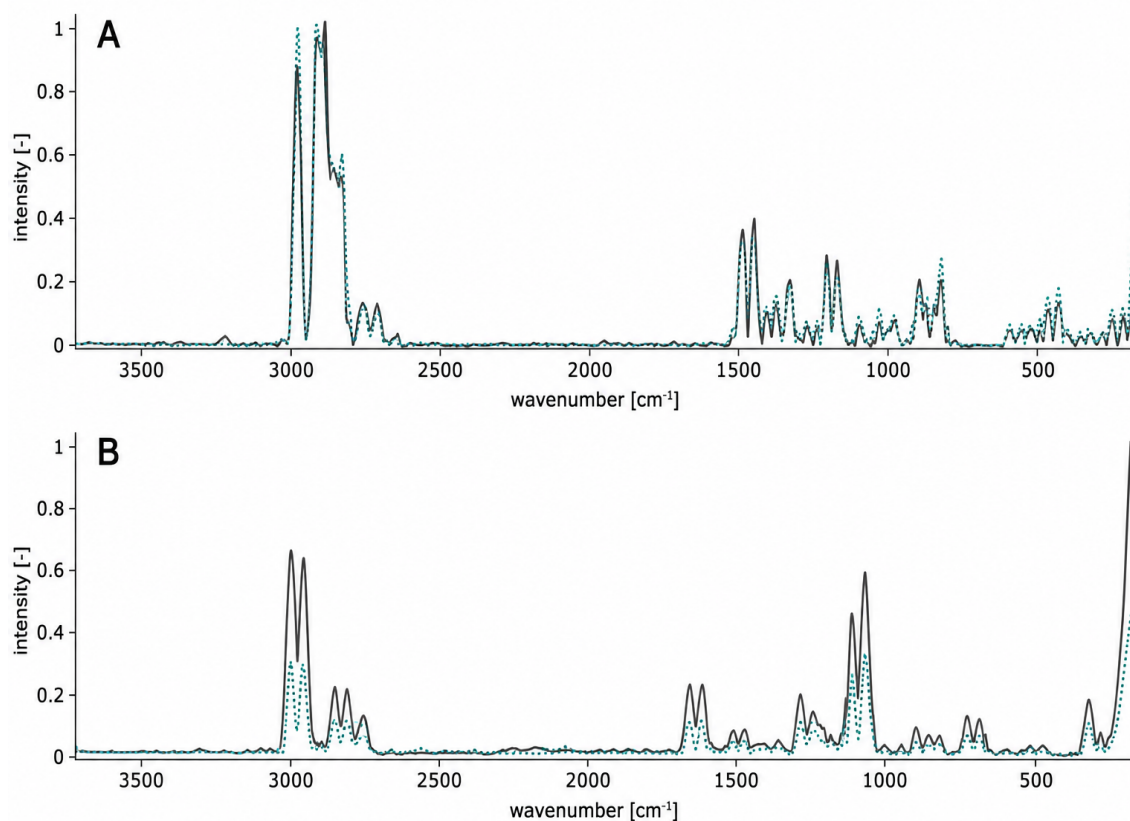


Figure 5: Raman spectra and corresponding OpenSpecy reference library matches for polymer standards from the Polykit 1.0. (A) Polypropylene and (B) polystyrene. Raman spectra were collected using a Horiba LabRAM HR Evolution spectrometer with a 532 nm excitation laser, a 10 s integration time, and three accumulations. The solid dark gray line represents the preprocessed sample spectrum, while the dotted turquoise line represents the best-matching reference spectrum from the OpenSpecy library. OpenSpecy analysis identified the polypropylene standard with a match value of 0.98 and the polystyrene standard with a match value of 0.92, indicating strong agreement between the measured and reference spectra.

Spectra Analysis

Raman spectra were analyzed by manual comparison with the CMDR HPU PolyKit 1.0 reference library to determine the polymer composition of individual particles.

Spectra were first imported as .txt files into LabSpec6 (Horiba Scientific), where the Analysis–Peak Matching function was used to identify prominent Raman peak

positions automatically. The resulting peak wavenumbers were recorded in a spreadsheet for subsequent comparison.

Spectra containing fewer than four reproducible peaks were classified as inconclusive and were not assigned a polymer identity. These spectra were typically dominated by fluorescence or exhibited low signal intensity relative to the baseline because of strong light absorption by dark or black materials. For the remaining spectra, a shortlist of candidate polymer matches was identified based on the observed peak wavenumbers in combination with particle morphology.

Candidate reference spectra from the CMDR HPU PolyKit 1.0 library were then loaded into LabSpec6 and visually compared with the measured spectra by overlaying the reference and sample spectra. Polymer identifications were assigned only when the characteristic peak positions and overall spectral profile showed good agreement between the measured and reference spectra. Confident matches were recorded as the final material identifications.

Quantification using Nile Red

Particle abundance per gram of lakebed sediment was estimated via fluorescence microscopy. Fluorescence microscopy is widely used for the detection of MP's, particularly in conjunction with lipophilic stains such as Nile Red (NR) (Maes et al, 2017, Ho et al. 2025). NR binds with polymers and emits at different wavelengths depending on the type of plastic it is attached to. The use of fluorescent dyes is not compatible with visible excitation Raman spectroscopy as dyed particles, when

excited by visible light fluoresce excessively, masking any Raman scattered signature of distinctive chemical bonds. For this reason, analysis using NR was performed on filters that had already been subsampled for Raman spectroscopy

Laboratory Method

The NR staining solution was prepared and applied to the filters prior to fluorescence microscopy. Briefly, filters were dyed using an in lab prepared NR staining solution with a dye concentration of 8 μM (2.5 $\mu\text{g/ml}$ NR) in 25% acetone, 75% H_2O (Wallner et al. 2026, in prep). After administering the staining solution directly onto the filters using a pipette, the filters were put in covered glass petri dishes and placed in the oven at 60°C for 20 min to dry. Dry filters were then rinsed and refiltered onto new precleaned 25mm stainless steel filters prior to fluorescence microscopy.

Fluorescence Microscopy

Fluorescence microscopy was performed using a Zeiss Axio Zoom.V16 microscope equipped with ZEN Pro imaging software (UCSC Life Sciences Microscopy Center). Images were acquired using a PlanApo Z 1.0 $\times$ objective lens at 20 $\times$ magnification. The resulting images had a pixel resolution of 3.23 $\mu\text{m} \times 3.23 \mu\text{m}$.

Samples were imaged using four fluorescence filter sets: DAPI (excitation 365 nm, emission 445/50 nm), GFP (excitation 470/40 nm, emission 525/50 nm), DsRed (excitation 545/25 nm, emission 605/70 nm), and Cy5 (excitation 640/30 nm,

emission 690/50 nm). Exposure times were adjusted manually for each filter channel to maximize fluorescence signal while minimizing image saturation.

Filters were secured in place using a stainless-steel washer containing a 20 mm diameter aperture. This ensured a standardized imaging area across all samples and treatments.

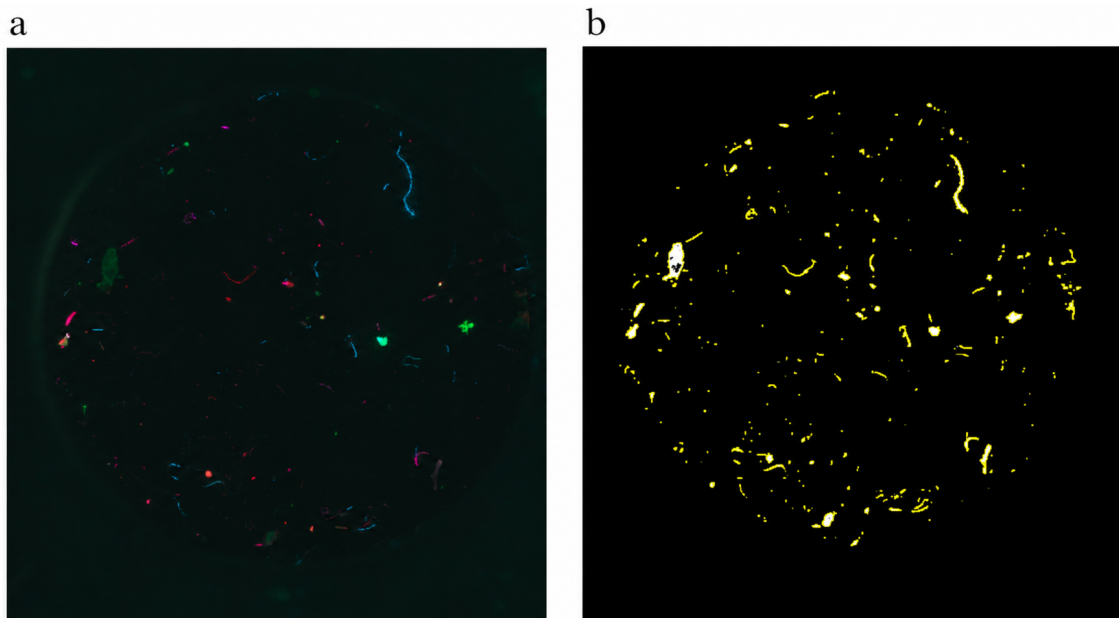


Figure 6: (a) Filter B.5 from Site ES2 (Zephyr Cove) imaged using a Zeiss Axio Zoom.V16 fluorescence microscope. The filter was imaged using superposition of the four channels: DAPI (excitation 365 nm, emission 445/50 nm), GFP (excitation 470/40 nm, emission 525/50 nm), DsRed (excitation 545/25 nm, emission 605/70 nm), and Cy5 (excitation 640/30 nm, emission 690/50 nm). (b) The same filter after image processing in ImageJ (Fiji), where a threshold-based mask was generated to identify and quantify all particles meeting the specified detection threshold.

After imaging multiple fields of view were combined using the image stitching function within ZEN Pro to generate composite images of the entire filter area.

Stitched images were subsequently analyzed using Fiji, a distribution of ImageJ that

includes a suite of pre-installed plugins for scientific image analysis (Schindelin et al., 2012).

ImageJ Analysis

Fiji was used for image preprocessing and automated particle counting to quantify the number of MP particles per filter for each of the ten filters from the 5 sites.

Images were uploaded into Fiji and a series of image processing steps were applied. These steps were optimized for the images and recorded as a macro to facilitate automated and standardized analysis for all filters.

These steps included converting the image to composite, stacking to RGB, and reducing the file size to 8-bit. A threshold mask was applied (Triangle) which looks for white particles on a black background. Then an erosion, followed by a dilation function were performed to clean up visual noise like light reflection on the stainless-steel mesh, and a filling holes function applied to ensure that fibers were counted as one object. After processing all particles between 200-5,000 pixels were assigned a number. All processing steps were recorded as a macro for reproducibility.

Statistics

Statistical analyses were conducted in R version 4.4.1 (R Core Team, 2024) using RStudio 2024.12.1 Build 563 (Posit Software, PBC, Boston, MA, USA). Data manipulation and summarization were performed using the dplyr package.

Generalized linear models were fitted using the MASS package, post hoc pairwise

comparisons were conducted using emmeans, and model diagnostics were evaluated using DHARMA. Figures were generated using the ggplot2 package.

Results

MP Particle Quantification

Particle abundance per gram of sediment

Following blank correction, particle abundances ranged from 1.01 to 4.96 particles g^{-1} dry sediment across the five sampling sites. The highest abundance was observed at East Incline (4.96 particles g^{-1}), followed by Zephyr Cove (3.73 particles g^{-1}) and South Sand Harbor (2.65 particles g^{-1}). Lower abundances were observed at Hidden Beach (1.17 particles g^{-1}) and Nevada Beach (1.01 particles g^{-1}).

Table 3: Summary of particle abundance for each sampling site. Values include the number of filters analyzed, mean dry sediment mass per filter, mean particle count per filter, blank-corrected particle count per filter, and the resulting blank-corrected particle abundance normalized to sediment mass (particles g^{-1} dry sediment). Blank correction was performed by subtracting the mean particle count observed in procedural sediment blanks from the mean particle count for each sampling site. The summary row shows the average blank-corrected particle abundance across the five sites, with ± 1.69 representing the standard deviation (SD).

Site	n filters	Mean sediment mass (g)	Blank-corrected abundance (particles g^{-1} dry sediment)
ES1	12	86.58	1.01
ES2	10	100.11	3.73
ES4	9	78.61	2.65
ES6	10	95.86	4.96
ES7	10	97.13	1.17
Overall mean $\pm$ SD	51	91.66	2.70 ± 1.69

Blanks

Particle counts from environmental samples were compared with those from laboratory blank filters to assess the contribution of laboratory contamination. The

relatively high and variable particle counts observed in the laboratory blanks introduced uncertainty into the quantified microplastic abundances. Overall, environmental samples contained higher particle counts (mean = 433 particles, SD = 319, $n = 51$) than laboratory blanks (mean = 197 particles, SD = 130, $n = 19$). Blank correction was performed using the laboratory blanks associated with each processing batch. The estimated blank contribution varied among sites, accounting for approximately 69% of the measured particles at Nevada Beach, 67% at Hidden Beach, 39% at Zephyr Cove, 36% at South Sand Harbor, and 28% at East Incline. Across all sites, laboratory blanks accounted for an average of 46.9% of the measured particle counts. These results indicate that laboratory contamination represented a substantial proportion of the measured particle counts, particularly at Nevada Beach and Hidden Beach, and therefore the reported microplastic abundances and differences among sites should be interpreted with caution.

Statistical analysis using Welch's two-sample t -test indicated that particle counts in samples were significantly greater than those observed in blanks ($t = 4.38$, $p < 0.001$). This result was further supported by a non-parametric Mann–Whitney U test ($U = 772$, $p < 0.001$). These findings suggest that, although background contamination was present in procedural blanks, the particle abundances measured in environmental samples (all sites aggregated) were statistically higher than laboratory blank levels.

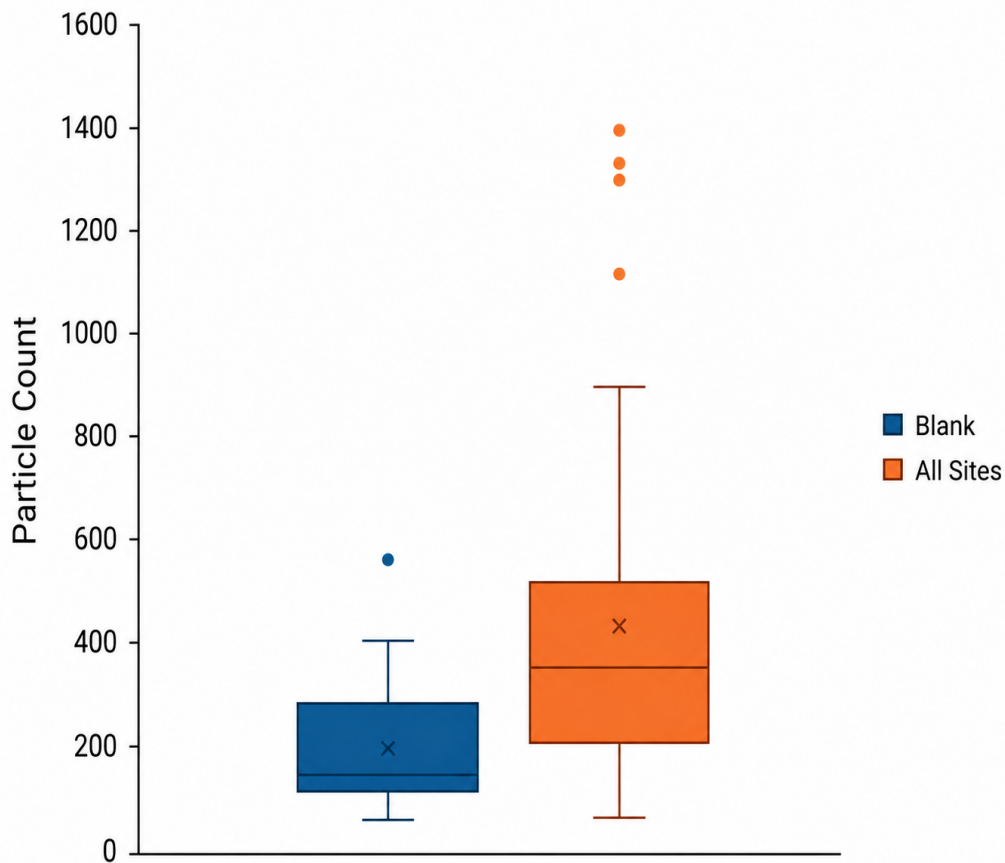


Figure 7: Mean particle counts measured in procedural blanks ($n = 19$) and environmental samples ($n = 51$). Error bars represent ± 1 standard deviation. Environmental samples contained significantly higher particle counts than blanks (Welch's t -test, $p < 0.001$).

Particle abundance across sites

Particle counts varied among sampling sites, with mean abundances ranging from 245.6 particles at Nevada Beach to 660.5 particles at East Incline. A one-way analysis of variance (ANOVA) indicated significant differences in particle counts among sites ($F_{4,44} = 3.51$, $p = 0.014$). Post-hoc Tukey's HSD testing revealed that only ES6 exhibited significantly greater particle counts than Nevada Beach ($p = 0.024$). No other pairwise comparisons were significant.

Due to variable particle counts and not normally distributed data, a non-parametric Kruskal–Wallis test was also performed. This analysis did not detect a significant overall difference among sites ($H = 8.97, p = 0.062$). This is likely because although higher mean particle counts were observed at ES2 and ES6, the large variability among particle counts per filter within these sites weakened statistical support for spatial differences in particle abundance between sites.

Table 4: Summary statistics for particle counts at each sampling site. The number of filters analyzed using fluorescence imaging and Fiji image analysis is reported as n . Mean particle count and standard deviation (SD) are presented for each site.

Site	n	Mean Particle Count	SD
ES1	10	245.6	106.8
ES2	10	566.8	385.7
ES4	9	326	187.5
ES6	10	660.5	456.8
ES7	10	346.8	163.2

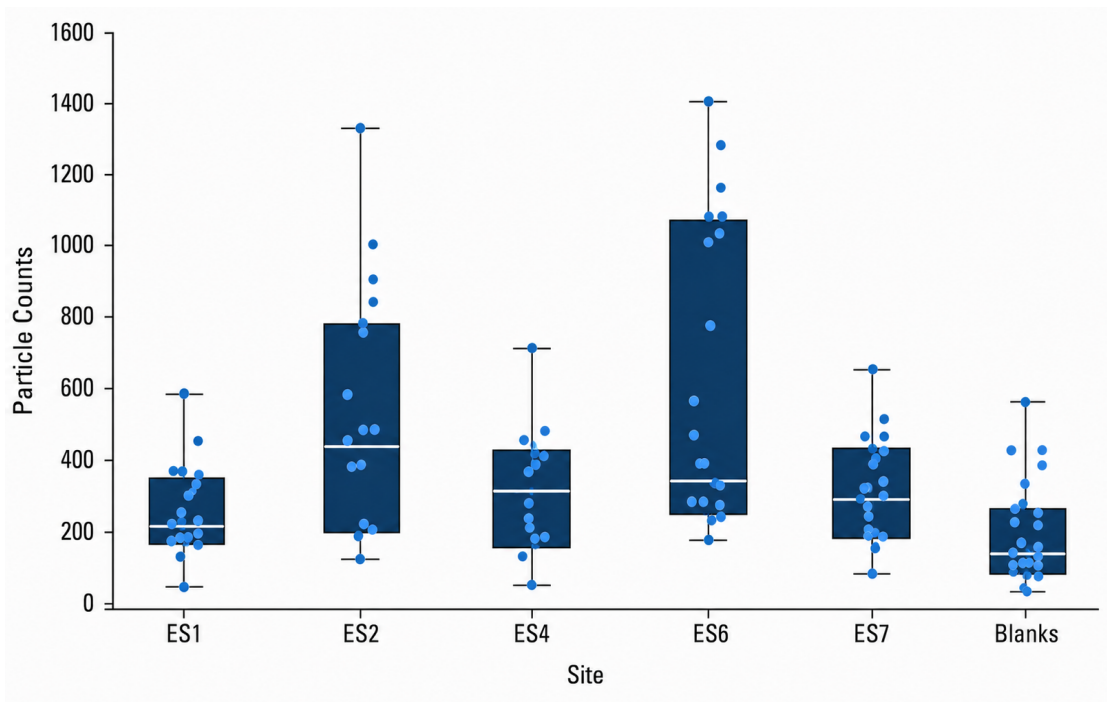


Figure 8: Distribution of particle counts across all sites not blank corrected. Boxplots show the median (white line), interquartile range (box), and minimum and maximum values (whiskers). Individual points represent count values for each filter. East Incline (ES6) demonstrated the highest mean particle abundance as well as the largest variation.

Particle size across sites

Particle abundance differed significantly among sampling sites and among particle size classes. Furthermore, a significant interaction between site and size class indicated that the distribution of particle sizes varied among sampling locations.

Particle size data was collected via initial sediment size classing not through individual particle measurement.

Preliminary two-way ANOVA identified significant effects of site ($F = 4.49$, $p = 0.0056$), size class ($F = 3.60$, $p = 0.0244$), and their interaction ($F = 5.22$, $p < 0.001$).

However, because particle count data was not normal (skewed right) a generalized

linear model with a negative binomial distribution was used to test differences in particle abundance among sites and size classes.

The negative binomial model confirmed that particle abundance varied among sites and size classes, with the greatest abundances generally observed in the smallest size class (250–63 μm), particularly at ES6. Conversely, larger particle size classes were more abundant at ES1 and ES2. These results demonstrate that both sampling location and particle size significantly influenced observed particle abundances.

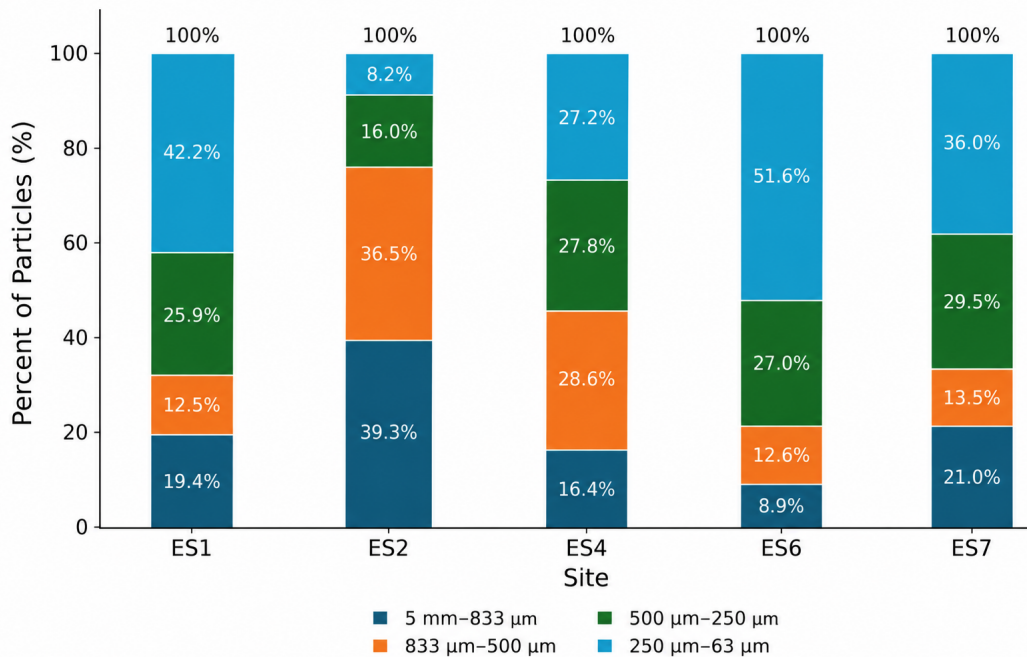


Figure 9: Percent composition of microplastic particle size classes at sampling sites ES1, ES2, ES4, ES6, and ES7. Stacked bars show the proportion of particles in each size class (5 mm–833 μm , 833 μm –500 μm , 500 μm –250 μm , and 250 μm –63 μm), normalized to 100% of the particles collected at each site. Values within each bar indicate the percentage of total particles represented by each size class.

Based on the quantification of particles from the blank filters, these findings suggest that particle counts within the 500–250 μm and 250–63 μm size classes were likely overestimated across filters from all sites. This potential background contamination should be considered when interpreting the particle size distributions presented in Figure 9.

To further characterize the blank particles, particle size analysis was performed in Fiji using fluorescent images captured with the Axio Zoom microscope. The Feret diameter was calculated for each detected particle, where the Feret diameter represents the maximum caliper diameter, or the longest distance between any two points along a particle's boundary. An average Feret diameter was then calculated for each blank filter. These average measurements (Table X) provide an estimate of the size distribution of particles present in the blank filters. The average Feret diameters ranged from 217.3 to 436.9 μm , with most blank filters (68.4%) having average Feret diameters within the 500–250 μm size class and the remaining 31.6% within the 250–63 μm size class. This indicates that most particles contributing to the blank counts were within these two size classes, supporting the likelihood that particle counts in these size ranges were inflated by background contamination.

Table 5: all blank filters with the average ferret diameter for each filter. This average was calculated by taking the ferret diameter of each counted particle then averaging it.

ID	Average Feret diameter (μm)
SedBlank2	319.1
SedBlank4	239.4
SedBlank5	254.9
SedBlank6	226.7
SedBlank7	217.3
SedBlank8	436.9
SedBlank9	291.1
SedBlank10	285.5
SedBlank11	319.8
SedBlank12	323.3
SedBlank13	257.9
SedBlank14	279.7
SedBlank15	241.4
SedBlank16	226
SedBlank18	337.4
SedBlank19	237
SedBlank20	288.8
SedBlank21	256.8
SedBlank22	414.2

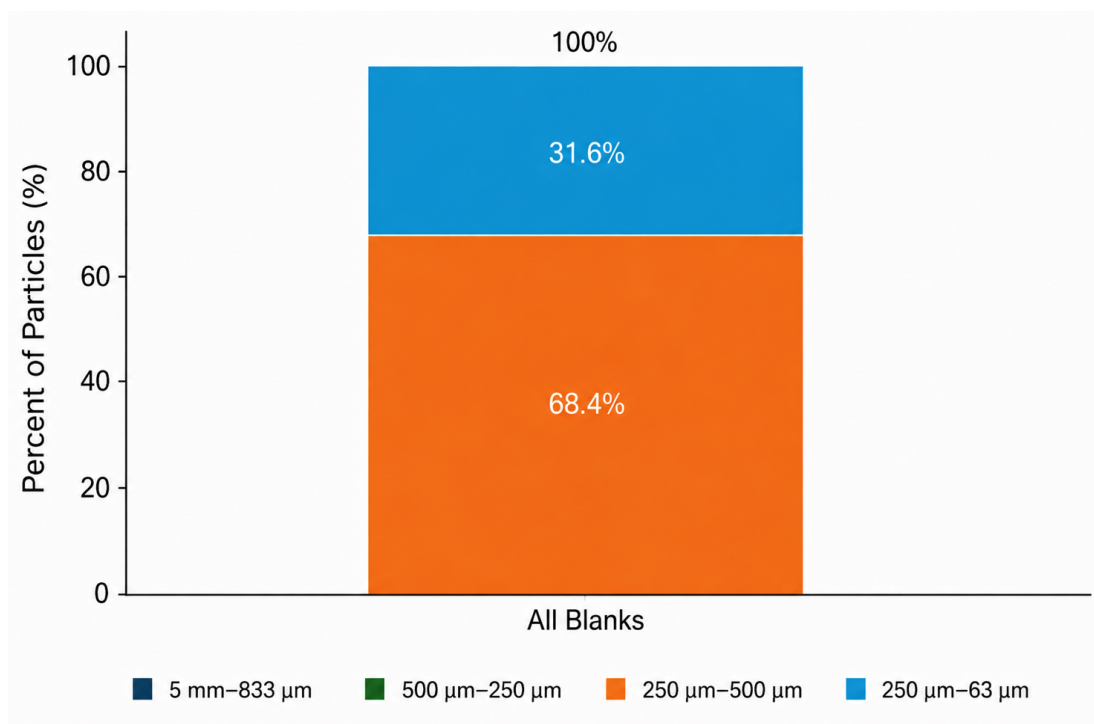


Figure 10: Distribution of the average Feret diameter measurements for all blank filters. Most blank filters (68.4%) had average Feret diameters within the 500–250 μm size class, while the remaining 31.6% had average Feret diameters within the 250–63 μm size class.

Morphology and color

Morphology

Morphology composition differed significantly between sampling sites and laboratory blanks based on a chi-square test of independence with Monte Carlo simulation, $\chi^2(30) = 111.31$, $p < 0.001$. The Monte Carlo simulation was used to accommodate the data contingency table for these data which had more data than a 2x2 table format. The association between site and morphology was moderate in strength (Cramér's $V = 0.353$). Because several morphology categories had low expected counts, rare categories (cylindrical pellet and foam) were collapsed into an “Other” category and the analysis was repeated; the overall result remained significant, $\chi^2(25) = 111.31$, $p < 0.001$.

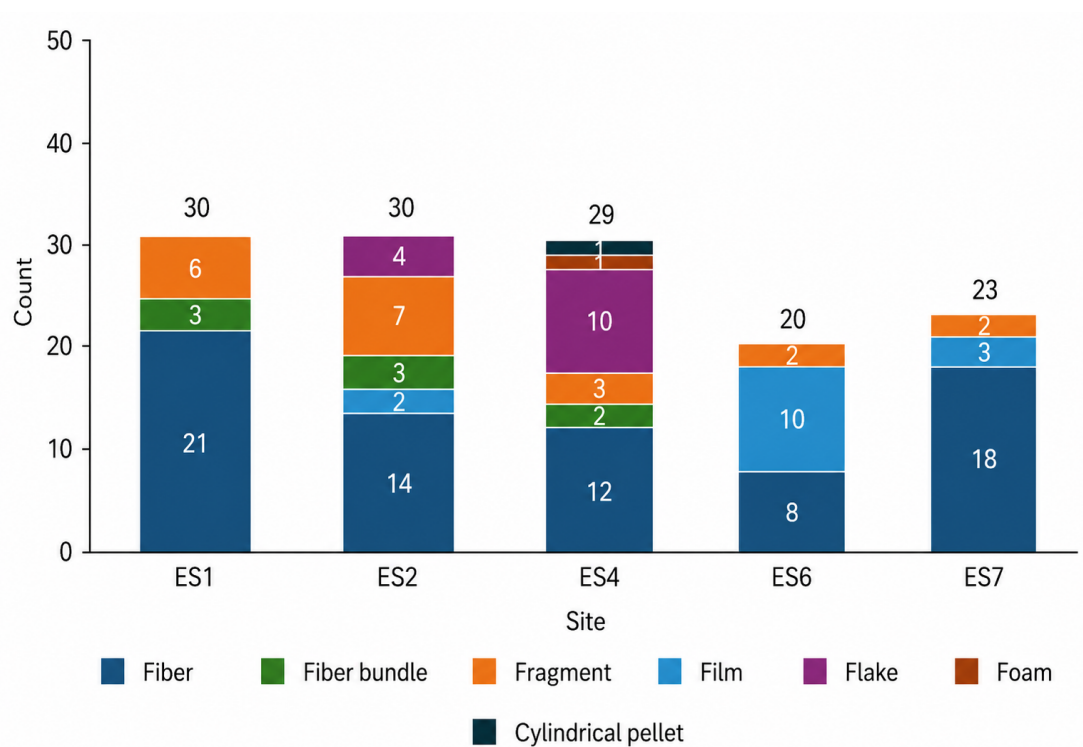


Figure 11: Distribution of particle morphologies identified at each sampling site. Stacked bars represent the number of particles assigned to each morphology category (fiber, fiber bundle, fragment, film, flake, foam, and cylindrical pellet). Values within each bar indicate the number of particles in each morphology category, and the value above each bar indicates the total number of particles identified for that group.

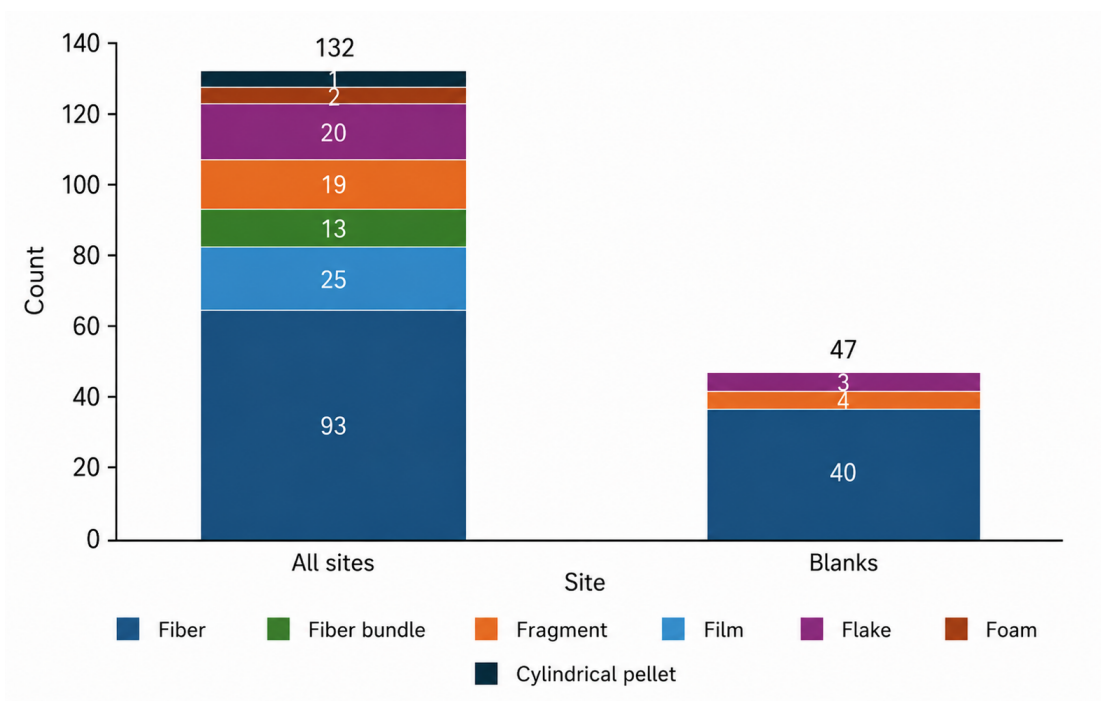


Figure 12: Morphologies for all sites aggregated compared to all blanks. Stacked bars represent the number of particles assigned to each morphology category (fiber, fiber bundle, fragment, film, flake, foam, and cylindrical pellet). Values within each bar indicate the number of particles in each morphology category, and the value above each bar indicates the total number of particles identified for that group.

Color

A chi-square test of independence indicated that particle color distributions differed significantly between sampling sites compared to laboratory blanks ($\chi^2 = 97.55$, $df = 40$, $p < 0.001$; Cramér's $V = 0.33$). Because several color categories had counts less than five (gray, green, tan), the assumptions of the chi-square test were violated: therefore, these results should be interpreted with caution.

Pairwise Fisher-style Monte Carlo comparisons with false discovery rate correction indicated that ES4 differed significantly from ES1, ES2, ES6, ES7, and the laboratory blanks. No other pairwise comparisons were significant after correction. These differences were largely driven by the high count (n=9) of brown particles observed at ES4 compared with the other sites and blanks.

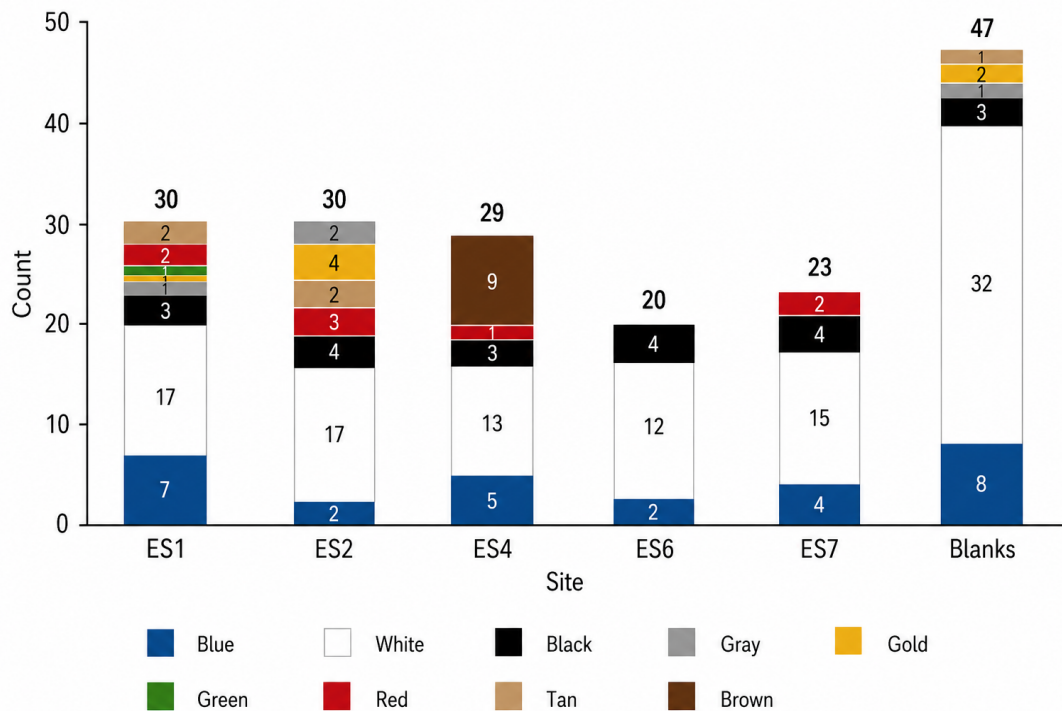


Figure 13: Distribution of particle colors identified at each sampling site and in the aggregated laboratory blanks. Bar segments represent the number of particles assigned to each color category, and values within each segment indicate particle counts. Numbers above each bar denote the total number of particles analyzed for each site. White particles were the dominant color at all sampling sites and in the laboratory blanks, followed by blue and black particles. Brown particles were observed exclusively at ES4, while green and gray particles occurred only infrequently.

Morphology and Color combined

Of the 179 total particles that were subsampled certain color/morphology combinations dominated. Across all environmental sampling sites, white fibers were the most common color–morphology combination, accounting for 30.3% (40 of 132 particles) of all identified particles (Table 5). Blue fibers and gold flakes were the next most abundant combinations, each representing approximately 11% of the total particles (11.4% and 10.6%, respectively), followed by white films (10.6%), black fibers (7.6%), and white fiber bundles (5.3%). In comparison, laboratory blanks were dominated by white fibers, which comprised 57.4% (27 of 47 particles) of all particles recovered, followed by blue fibers (19.1%) and gold flakes (6.4%). White films and white fiber bundles were not observed in the laboratory blanks. Overall, fibrous particles, particularly white and blue fibers, were proportionally more abundant in laboratory blanks than in environmental samples, whereas white films and gold flakes were comparatively more representative of the environmental samples.

Table 6: Top color–morphology combinations identified across all environmental sampling sites and laboratory blanks. Environmental samples (ES1, ES2, ES4, ES6, and ES7) and laboratory blanks are presented as aggregated datasets. Counts represent the total number of particles assigned to each color–morphology combination, and percentage columns indicate the proportion of particles represented by each combination within the aggregated environmental samples (n = 132) and aggregated laboratory blanks (n = 47).

Color–Morphology	All Sites (n = 132)	% of Sites	Blanks (n = 47)	% of Blanks
White Fiber	40	30.3	27	57.4
Blue Fiber	15	11.4	9	19.1
Gold Flake	14	10.6	3	6.4
White Film	14	10.6	0	0
Black Fiber	10	7.6	2	4.3
White Fiber Bundle	7	5.3	0	0
Other combinations	32	24.2	6	12.8

Raman Spectroscopy

In total 179 particles were analyzed including 46 laboratory blanks and 118 environmental particles using Raman spectroscopy. Of these 179 samples, 10 unique spectra categories were assigned. These were: Inconclusive, Inconclusive Organic, Blue Disperse Dye on PET Fiber, Dye-Dominated, PP, PET, PS, PE, Carbonaceous Black Material, Sulfate Mineral.

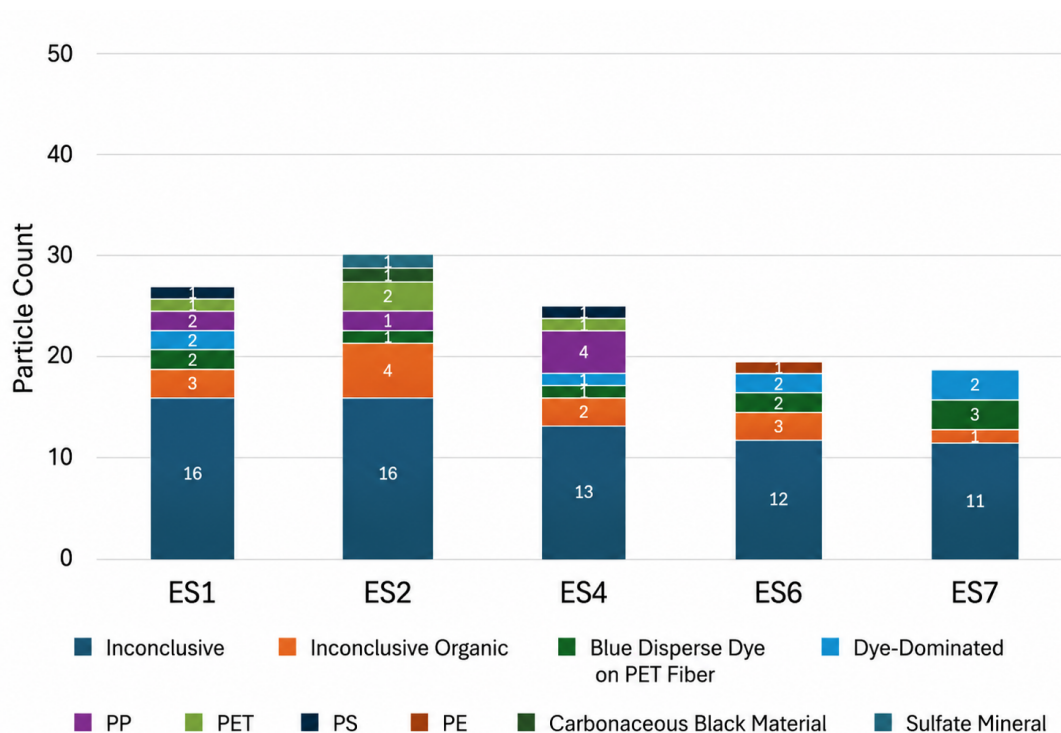


Figure 14: Distribution of Raman spectroscopy material identifications by sampling site for environmental particles analyzed from sites ES1, ES2, ES4, ES6, and ES7. Stacked bars represent the number of particles assigned to each material category at each site, including Inconclusive, Inconclusive Organic, Blue Disperse Dye on PET Fiber, Dye-Dominated, PP, PET, PS, PE, Carbonaceous Black Material, and Sulfate Mineral.

Based on spectral analysis, most particles (69.3%) were classified as Inconclusive, indicating that no reliable material identification could be assigned from the acquired Raman spectra. An additional 10.6% of particles were classified as Inconclusive Organic, as weak but discernible C–H stretching bands were present, indicating an

organic composition; however, the spectra were insufficient for confident material identification. Blue Disperse Dye on PET Fiber was the most common positively identified material (6.1%). A further 5.0% of particles were classified as Dye-Dominated, indicating that Raman spectra were dominated by dye-related peaks, preventing confident identification of the underlying polymer. The remaining particles were identified as PP, PET, PS, PE, carbonaceous black material, or sulfate mineral.

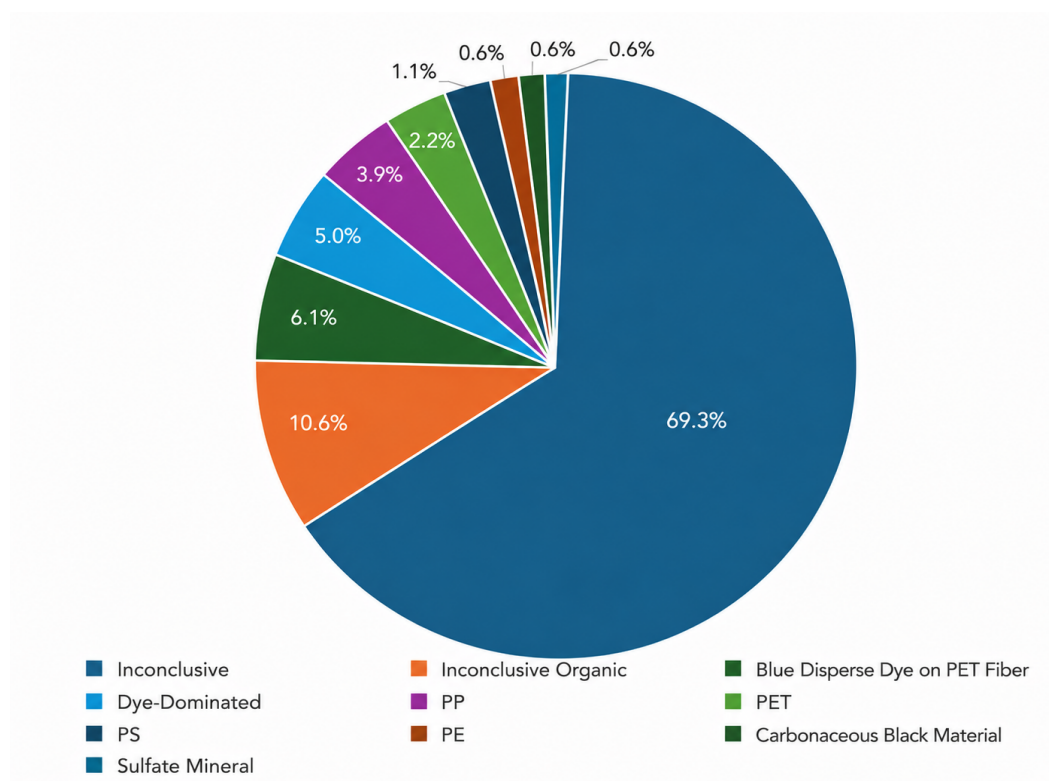


Figure 15: Polymer identification results for all particles analyzed, including both environmental samples (ES1, ES2, ES4, ES6, and ES7) and laboratory blanks (n = 179). Slice labels indicate the percentage of particles assigned to each material category. Most particles were classified as Inconclusive (69.3%), followed by Inconclusive Organic (10.6%), Blue Disperse Dye on PET Fiber (6.1%), Dye-Dominated (5.0%), PP (3.9%), PET (2.2%), PS (1.1%), and PE, Carbonaceous Black Material, and Sulfate Mineral (0.6% each).

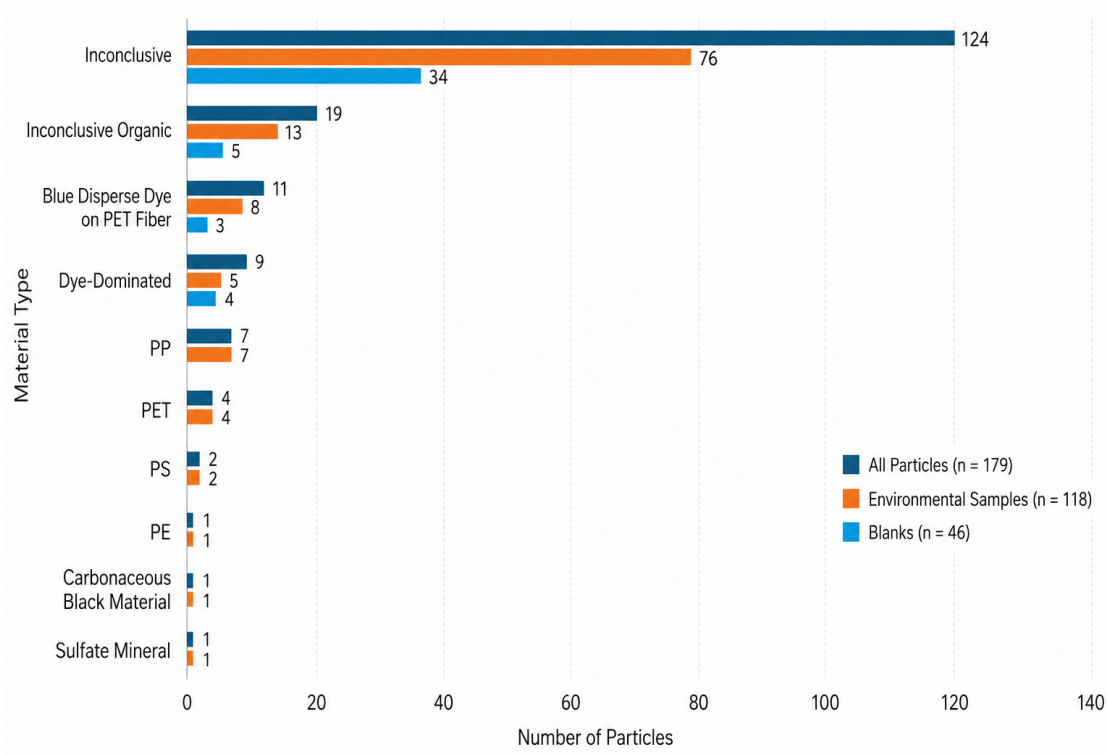


Figure 16: Comparison of Raman spectroscopy material identifications for all analyzed particles (n = 179), environmental samples (n = 118), and laboratory blank samples (n = 46). Material identifications are grouped by unique Raman spectral matches and shown side-by-side for the complete dataset, environmental samples, and blanks.

Material Assignments

Inconclusive

69.3% of all particles analyzed were categorized as inconclusive including 76 environmental particles and 34 blank particles from a total of 179 particles analyzed. These spectra that did not contain enough distinguishing peaks to be confidently assigned a material ID. Most typically these spectra exhibited a pronounced sloping, curving or wavy baseline that was several orders of magnitude greater than the Raman signal, obscuring characteristic Raman peaks (Figure 16 panel C). These features are indicative of fluorescence interference (Q. Williams, personal

communication, March 2026); elastic scattering of light from imperfections or complex surfaces may also play a role in these backgrounds. Fluorescence occurs when molecules within the sample absorb the incident laser energy and subsequently emit light over a broad range of wavelengths. This issue was particularly common among fibers (white and blue) and white particles making confident material identification difficult.

Particles that were black in color often produced “scratchy” low y axis spectra with no discernible peaks above the baseline (Figure 16 panel A). This is likely because of the strong optical absorption of black materials, which absorb a significant proportion of the incident laser energy (Q. Williams, personal communication, March 2026).

Spectra produced from cylindrical particles or some fibers resulted in spectra where the signal intensity increased rapidly and reached the upper limit of the detector, resulting in a clipped or saturated spectrum (Figure 16 panel B). The most likely explanation is that these particles generated a strong optical signal, either through intense fluorescence or strong multi-angular scattering the incident laser (Q. Williams, personal communication, March 2026).

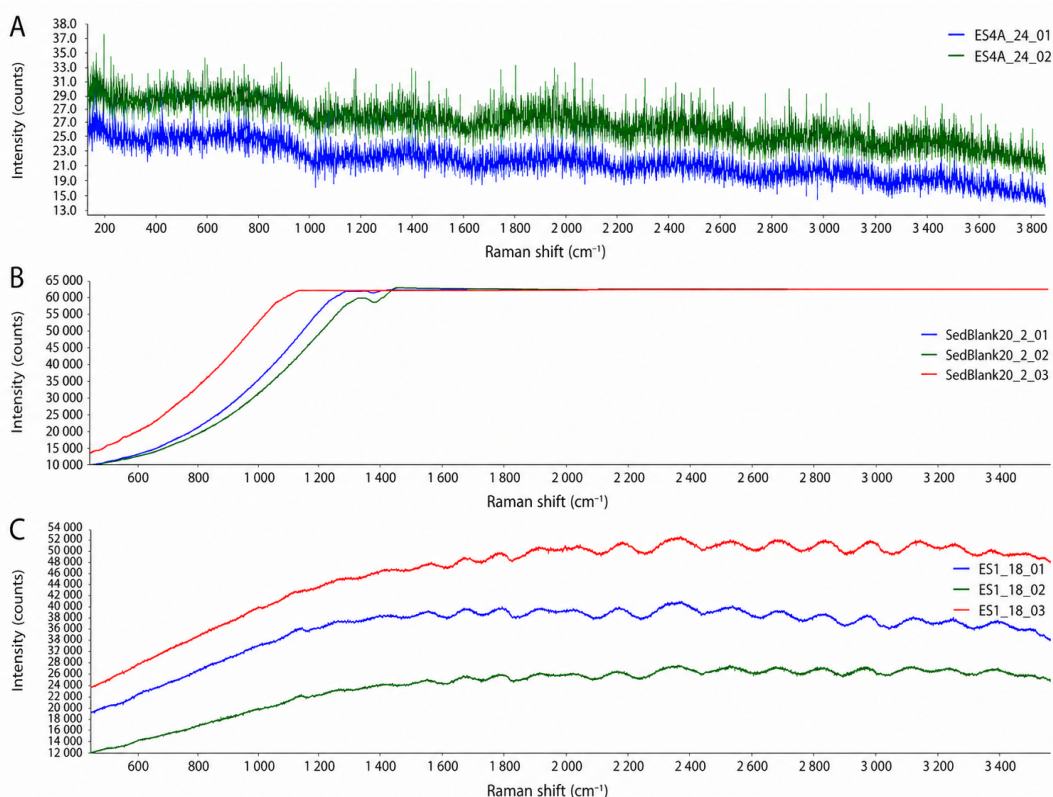


Figure 17: Three representative Raman spectra classified as inconclusive due to spectral interference and poor signal quality due to possible diffuse scattering and/or fluorescence. (A) Raman spectra from particle 24 collected at Site ES4, exhibiting very low signal intensity and a noisy, irregular ("scratchy") spectral profile. (B) Three spectra from particle 2 collected on laboratory blank filter 20. The spectra rapidly increase in intensity before saturating at the detector's upper intensity limit, producing clipped signals. This saturation pattern was commonly observed for tubular-shaped particles. (C) Raman spectra from particle 18 collected at Site ES1. Beginning at approximately 1500 cm⁻¹, the spectra display a repetitive sinusoidal pattern characteristic of excessive light from either fluorescence or diffuse scattering interacting with the optics. Fluorescence was the most frequently observed source of spectral interference across all analyzed samples.

Inconclusive Organic

Spectra classified as Inconclusive Organic exhibited weak but discernible bands within the C–H stretching region (2800–3100 cm⁻¹), indicating the presence of organic material. However, the spectra lacked sufficient diagnostic peaks in the

fingerprint region ($500\text{--}1800\text{ cm}^{-1}$) to permit confident identification of a specific polymer or compound.

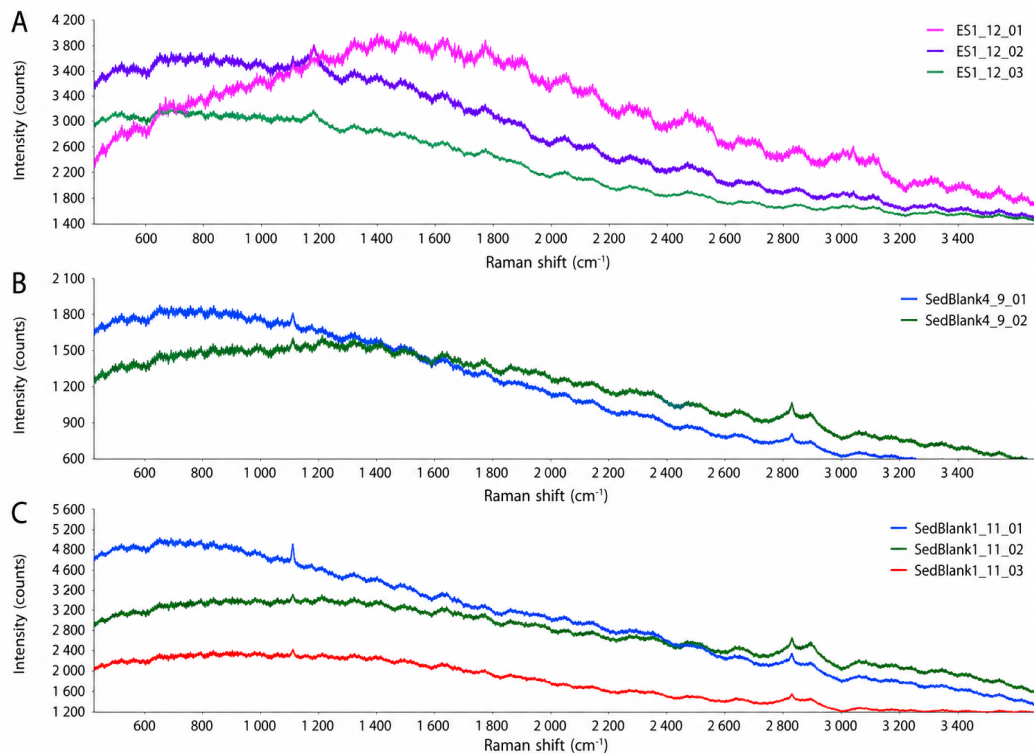


Figure 18: Three representative spectra classified as inconclusive organic. (A) Spectra collected from particle ES1 (B) Spectra collected from laboratory blank particle . (C) Spectra collected from laboratory blank particle . All spectra exhibit a broad fluorescent background and only weak but discernible C–H stretching bands in the high-wavenumber region (approximately $2800\text{--}3100\text{ cm}^{-1}$). All spectra show another weak peak at $\sim 1112\text{ cm}^{-1}$, however more information is needed for a confident material ID.

Blue Disperse dye on PET Fiber

The Raman spectrum exhibits several bands that are consistent with a polyester material, most notably the peak at approximately 1612 cm^{-1} , which is characteristic of the aromatic ring stretching vibration of polyethylene terephthalate (PET) (Oßmann, 2022). Additional bands observed at approximately 1142 , 1173 , and 1200 cm^{-1} are also consistent with PET and have been reported within regions commonly

influenced by textile dyes (Figure 18). Weak features at approximately 820 and 887 cm^{-1} further support a possible PET assignment, corresponding to aromatic ring deformation modes.

Several prominent bands, including those at approximately 1341, 1420, and 1576 cm^{-1} , are more consistent with the presence of a blue colorant than with the polymer backbone itself, suggesting that the Raman spectrum is significantly influenced by the fiber dye (Śmigiel-Kamińska et al., 2019). Most likely is a Disperse Blue dye (60/79/165) which is an anthraquinone-based or related aromatic disperse dye typically used on polyester textiles and often dominate Raman spectra.

Overall, the spectral features are most consistent with a blue PET (polyester) fiber, although the spectrum is substantially influenced by dye-related bands. The polymer identification is therefore considered probable rather than definitive.

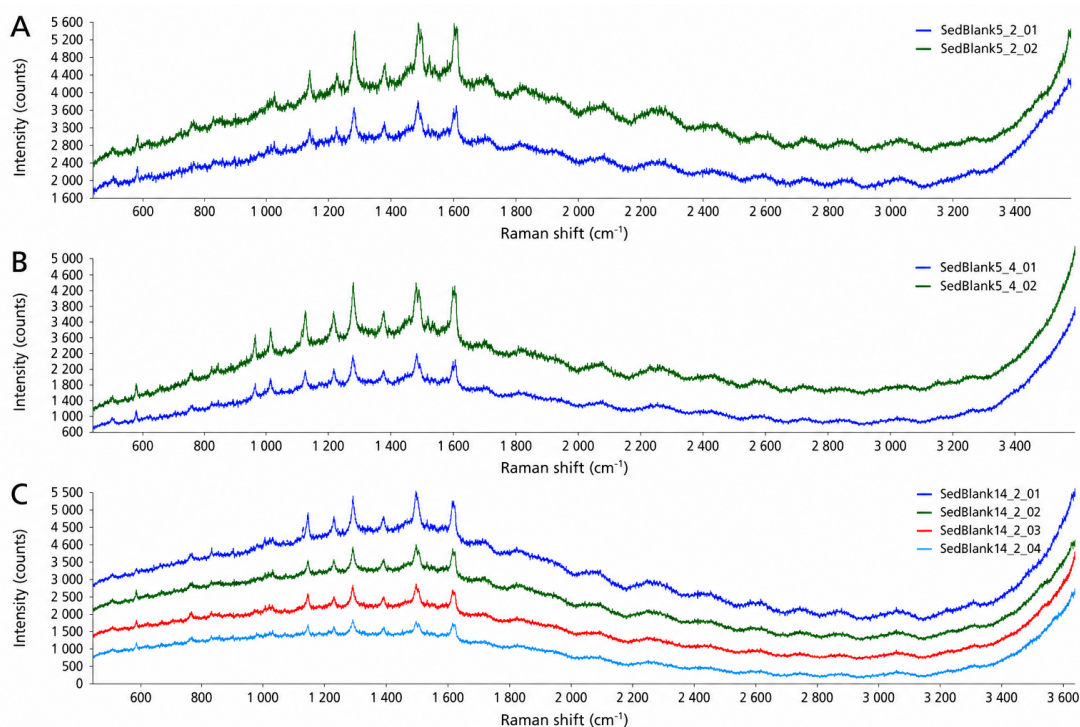


Figure 19: Three representative spectra classified as Blue Disperse dye on PET fiber. (A) Spectra collected from laboratory blank particle SedBlank5_2. (B) Spectra collected from laboratory blank particle SedBlank5_4. (C) Spectra collected from laboratory blank particle SedBlank14_2. All panels show reproducible peaks at ~ 980 , 1040 , 1112 , 1200 , 1290 , 1370 , 1490 , 1600 cm^{-1} ; weak C–H stretching bands at approximately 2800 – 3100 cm^{-1} .

Dye-Dominated

Several blue fiber samples produced spectra with reproducible Raman bands occurring at approximately 520 – 550 cm^{-1} , 1340 – 1380 cm^{-1} , 1450 cm^{-1} , and 1550 – 1610 cm^{-1} . This spectral profile is not characteristic of a clean polymer spectrum, instead the Raman signal appears to be dominated by contributions from the blue colorant associated with the fiber. The strong band between approximately 1550 and 1610 cm^{-1} is consistent with aromatic C=C and C=N stretching vibrations commonly observed in conjugated blue dyes, while the bands between approximately 1340 and 1450 cm^{-1} are compatible with C–N and C–C vibrational modes typical of aromatic

dye molecules (Śmigiel-Kamińska et al., 2019). Characteristic fingerprint bands for common synthetic polymers, including polyethylene terephthalate (PET), polypropylene (PP), polyethylene (PE), and nylon, are not clear.

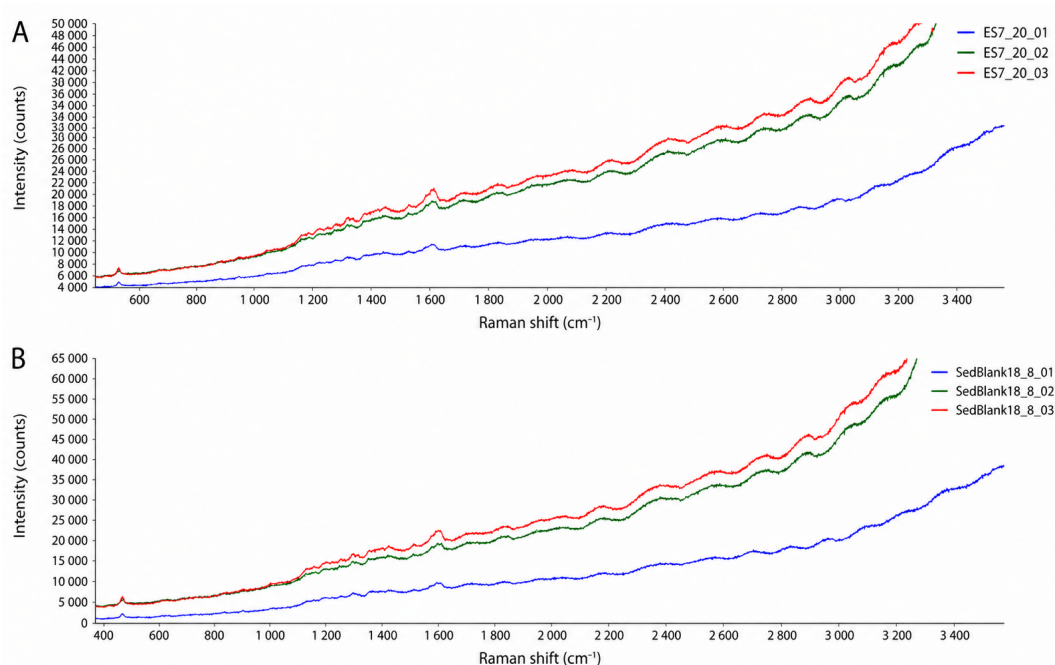


Figure 20: (A) Spectra collected from particle ES7_20. (B) Spectra collected from laboratory blank particle SedBlank18_8. All spectra exhibit a strong fluorescence background with weak, broad Raman features.

Polymer ID

The Raman bands used for polypropylene (PP) identification were approximately 809, 841, 973, 1167, 1330, 1450, 2838, 2880, 2905, and 2950 cm⁻¹ (Oßmann, 2022). While the presence of C–H stretching bands confirms that a material is organic, the presence of peaks in the fingerprint region (200–1800 cm⁻¹), in combination with the CH stretch region led to PP identification (Figure 20 panel A) .

Polystyrene (PS) was identified by its characteristic aromatic Raman fingerprint, including prominent bands at approximately 620, 1001, 1030, and 1602 cm^{-1} , with additional C–H stretching bands near 2910 and 3050 cm^{-1} (Figure 19 panel B). The strong phenyl ring breathing mode at $\sim 1001 \text{ cm}^{-1}$, together with the aromatic C=C stretching band near 1602 cm^{-1} , provided the most diagnostic evidence for PS identification (Oßmann, 2022). Spectra exhibiting this characteristic combination of peaks were classified as polystyrene with high confidence.

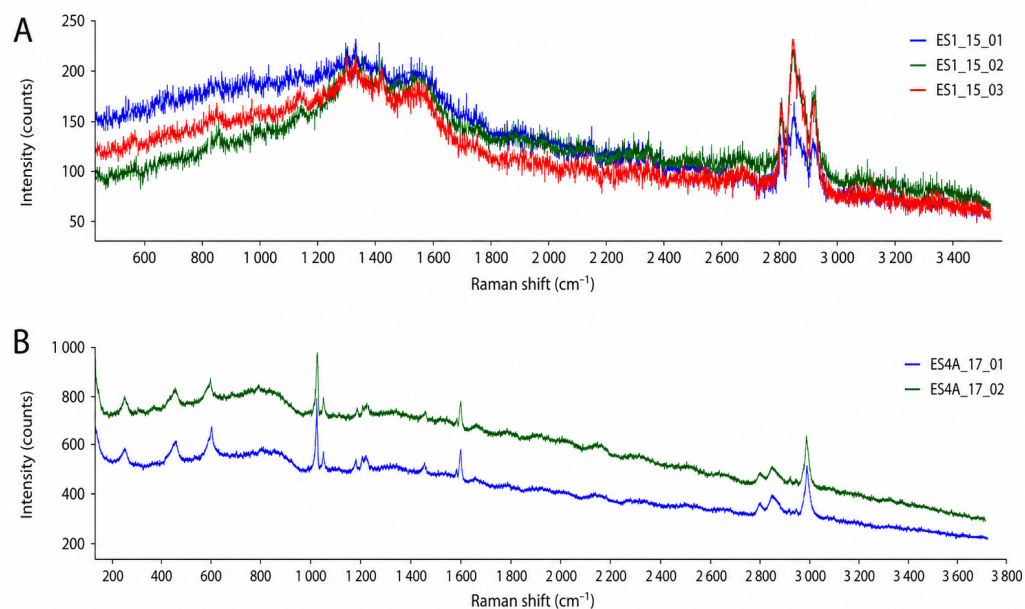


Figure 21: Representative Raman spectra of two commonly identified polymer types. (A) polypropylene (PP) from Nevada Beach (ES1), and (B) polystyrene (PS) from South Sand Harbor (ES4).

Rare assignments

One example of a rare but definitive spectra match was Carbonaceous black material (carbon black) which was identified by the presence of the characteristic Raman D and G bands, located at approximately 1350 and 1580 cm^{-1} , respectively (Q. Williams, personal communication, May 2026) (Figure 21). This material was only identified once indicating that the other black particles analyzed were not likely carbonaceous in nature.

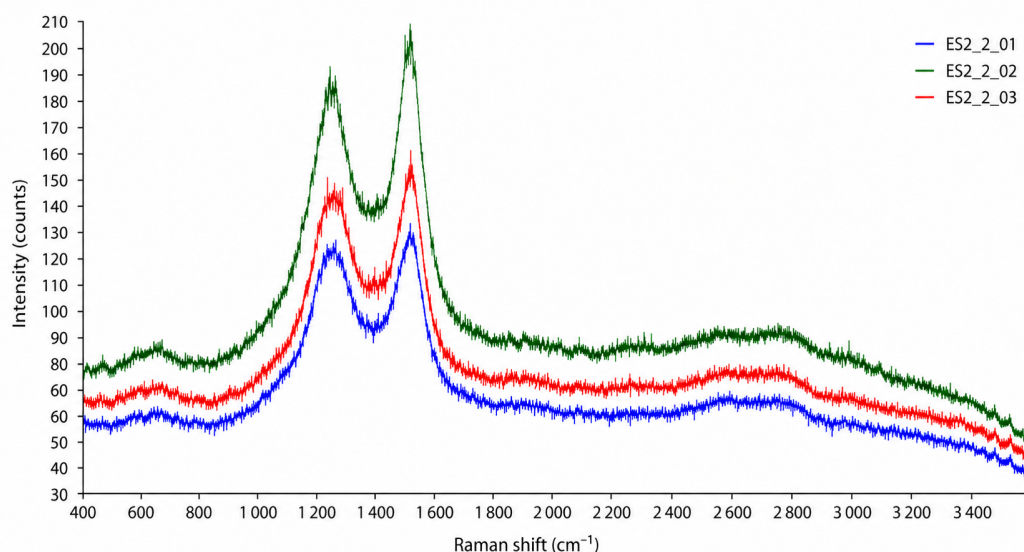


Figure 22: Raman spectra collected from particle ES2_2. The spectra exhibit the characteristic D and G bands of carbonaceous black material (carbon black), located at approximately 1350 cm^{-1} and 1580 cm^{-1} , respectively.

Discussion

The findings of this study are subject to uncertainty due to the relatively high and variable particle counts observed in laboratory blank filters (blanks contributing up to 44% of particles) compared with the environmental samples. Despite these

limitations, the results provide preliminary evidence of microplastic contamination in sediments along the east shore of Lake Tahoe. Further research is needed to more confidently quantify microplastic abundances and better characterize their spatial distribution.

The highest MP particle abundance was observed at East Incline (ES6; 4.96 particles g^{-1}), followed by Zephyr Cove (ES2; 3.73 particles g^{-1}) and South Sand Harbor (ES4; 2.65 particles g^{-1}). Lower abundances were observed at Hidden Beach (ES7; 1.17 particles g^{-1}) and Nevada Beach (ES1; 1.01 particles g^{-1}). These values are conservative because they do not account for the laboratory method recovery rate of 78.44% however they may be impacted by highly variable blanks.

The finding that East Incline exhibited the highest particle abundance is consistent with the documented hydrodynamics of Lake Tahoe. Hydrodynamic modeling has consistently demonstrated a counterclockwise gyre in the northern basin that transports suspended particles eastward across the north shore before circulating south along the eastern shoreline (University of California, Davis Tahoe Environmental Research Center [TERC], 2026). The finding that East Incline contained the highest microplastic abundance is therefore consistent with the lake's circulation patterns. However, the results of this study do not provide a clear explanation for why Zephyr Cove and South Sand Harbor exhibited the next highest abundances, or why lower abundances were observed at Hidden Beach and Nevada Beach. Furthermore, no clear north-to-south spatial trend was evident. From north to

south, the study sites were East Incline, Hidden Beach, South Sand Harbor, Zephyr Cove, and Nevada Beach, yet microplastic abundances did not follow this geographic sequence.

Of the 179 subsampled particles for which color and morphology were recorded, white fibers were the most prevalent. Considering the findings of the 2025 study of clothes dryer emissions from local Tahoe Basin households (Arienzo et al., 2025), the high proportion of fibers was not surprising, however many fibers were present in laboratory blanks indicating that this MP morphology is likely widespread in both indoor and outdoor environments. Arienzo et al. (2025) found that captured textile-derived microfibers in the Tahoe Basin were predominantly cellulosic, followed by polyethylene terephthalate (PET), other synthetic polymers, and polyolefins. In the present study, PET fibers with dye signals were positively identified, which is consistent with the findings of Arienzo et al. (2025). During examination of Raman spectra, cellulosic fiber reference spectra were compared with many fiber particles. In numerous cases, the spectra appeared to be potential matches; however, peak-matching confidence was too low to support definitive identification. This result is consistent with the tendency of cellulose-based materials to produce strong fluorescence under visible laser excitation, which can interfere with Raman analysis (Poisson & Zhang, 2024). Therefore, despite a positive ID based on a spectral match, the large number of inconclusive Raman results from fiber specimens agrees with the apparent abundance of cellulose fibers, whether dyed or undyed, that has been

documented in this region (Arienzo, 2025). In the present study these fibers were conservatively classified as inconclusive rather than identified as cellulosic.

One unexpected finding was the predominance of white and blue fibers in the laboratory blanks. This highlights the ubiquitous nature of airborne fibers, which are common contaminants in both indoor and outdoor environments (Dris et al., 2017). The increasing recognition of fibers as a significant environmental pollutant in recent literature underscores the importance of rigorous contamination control during MP analysis.

The present study found a greater microplastic abundance per gram of sediment than the only previous study examining Lake Tahoe lakebed sediments (Gjeltema et al., 2023). Gjeltema et al. identified three suspected plastic particles in 1.53 g of sediment; after blank correction, one confirmed polypropylene particle remained, corresponding to an estimated abundance of 0.65 particles g⁻¹. In comparison, the present study measured an average abundance of 2.70 ± 1.69 particles g⁻¹ dry sediment, approximately 4.2 times greater than previously reported. This difference may partly reflect differences in sampling design, methods used, and indoor MP contamination. The present study analyzed sediment collected from three homogenized grabs at each of five sampling sites, totaling approximately 5.73kg of sediment, whereas Gjeltema et al. analyzed a single 1.53 g sample. Differences in sampling location may also have contributed. In this study, samples were collected from heavily visited recreational beaches along Lake Tahoe's east shore, whereas

Gjeltema et al. sampled approximately 8 km offshore near Tahoe City Marina at a depth of approximately 470 m.

Direct comparison of polymer composition between the present study and the lakebed litter survey conducted by Davidson et al. is limited by the high proportion of particles that remained chemically unidentified. The large proportion of inconclusive and inconclusive organic spectra, together with the relatively small number of confidently identified polymers, precludes meaningful comparison of polymer composition among sites despite sampling the same transects (Nevada Beach, Zephyr Cove, East Incline, South Sand Harbor, Hidden Beach) examined by Davidson et al. However, comparison of the proportion of unidentified particles is possible if the “unknown” polymer category reported by Davidson et al. is considered broadly comparable to the “inconclusive” and “inconclusive organic” categories used in the present study.

Davidson et al. reported that unknown polymers comprised 37%, 27%, and 29% of litter collected from the Nevada Beach ($n = 43$), Zephyr Cove ($n = 75$), and East Incline ($n = 137$) transects, respectively. In contrast, 73.3%, 83.3%, and 85.0% of particles from the corresponding transects in the present study (ES1, ES2, and ES6) were classified as inconclusive or inconclusive organic. Although the proportion of unidentified particles was substantially greater in the present study, it is noteworthy that the unknown polymer category also represented the single largest material category reported by Davidson et al. at each of the three shared sampling locations.

This suggests that unidentified or difficult-to-characterize particles comprise a substantial component of Lake Tahoe lakebed debris. The much higher proportion observed here likely reflects methodological differences, including the smaller particle sizes analyzed, greater weathering or surface coatings on particles, fluorescence or dye-dominated Raman spectra, and the conservative spectral identification criteria applied in the present study.

Limitations

Preprocessing limitations

Several methodological limitations should be considered when interpreting the results of this study. The study focused on four larger size classes within the MP size range (5 mm–833 μm , 833–500 μm , 500–250 μm , and 250–63 μm) and excluded the smallest size class $< 63 \mu\text{m}$. Furthermore, only particles from the two largest size classes (5 mm–833 μm and 833–500 μm) underwent chemical characterization because these particles were large enough to be manipulated using micro tweezers. Consequently, the chemically identified particles may not be representative of the smaller MP size fractions, which could differ in both abundance and polymer composition.

Particle loss during laboratory processing represents another potential source of bias. Samples underwent multiple filtration steps, including filtration following density separation, chemical digestion, and Nile Red staining. Recovery experiments indicated that each additional filtration step increased the likelihood of particle loss,

suggesting that the final particle counts may underestimate the true abundance of MP's present in the original samples. The extent of this loss could not be quantified for individual processing stages but should be considered when interpreting particle abundance data.

Particle selection for chemical analysis also introduced a degree of sampling bias. Manual isolation of particles under the stereomicroscope was labor-intensive, requiring visual prioritization of particles that appeared anthropogenic based on characteristics such as color, shape, and texture. While this approach improved analytical efficiency, it may have resulted in the underrepresentation of less conspicuous MPs, including white, tan, brown, and transparent particles, which are also commonly reported in environmental samples (Kozolski et al., in preparation). Consequently, the subset of particles selected for Raman analysis may not fully represent the diversity of MPs present in the samples.

Analysis limitations

Laboratory blanks had relatively high and variable particle counts (mean = 197 particles per filter, SD = 130). Blank particles therefore represented a substantial proportion of the uncorrected environmental particle counts, introducing considerable uncertainty into the corrected abundances. Therefore absolute concentrations and differences among sites should be interpreted cautiously, particularly where site means were similar.

A major limitation of this study was the quality of the Raman spectra generated by the Raman spectrometer and LabSpec 6 software. Many spectra exhibited a pronounced sloping or curved fluorescence baseline that was several orders of magnitude greater than the Raman signal, obscuring characteristic Raman peaks. This issue is well-known for microplastics, and especially those containing dyes (e.g., Liu et al., 2023). In this study, such fluorescence was found to be particularly common among white, blue, and black fibers, which comprised 63.21% of the subsampled particles. In addition, many spectra contained only a small number of reproducible peaks, and these peaks were frequently broad rather than sharp, making confident material identification difficult.

The poor quality of many spectra also limited the effectiveness of both manual and automated spectral identification methods. Manual interpretation by peak matching and visual comparison with reference spectra is inherently time-consuming and subjective and is therefore not well suited for high-throughput analysis when spectra are of poor quality.

Automated spectral matching was initially performed using OpenSpecy. Spectra were exported from LabSpec 6 as .txt files and uploaded to the OpenSpecy web application for preprocessing and library matching. Spectra were preprocessed using the recommended OpenSpecy workflow (cite OpenSpecy documentation), which included min–max normalization, Savitzky–Golay smoothing and first-derivative transformation (third-degree polynomial, 90 cm^{-1} window), followed by linear

interpolation to standardize wavenumber spacing prior to comparison with the reference library (Cowger et al., 2021).

Despite following the recommended preprocessing workflow, the resulting library matches were inconsistent with the expected materials. Many spectra were assigned to obscure mineral species rather than common synthetic polymers or textile fibers, and these matches were not reproducible across similar particles. This outcome likely reflects the quality of the original spectra rather than deficiencies in the OpenSpecy library or matching algorithm. When fluorescence backgrounds dominate the Raman signal and characteristic peaks are weak or poorly resolved, preprocessing algorithms may amplify spectral artefacts or noise, leading to unreliable library matches. Consequently, automated spectral identification was not considered sufficiently reliable for this dataset, and material identification instead relied primarily on manual interpretation through comparison with the CMDR HPU Polykit 1.0 reference library.

Conclusion

MP's were detected in sediments at all five east shore sampling sites, with blank-corrected concentrations ranging from 1.01 to 4.96 particles g^{-1} dry sediment. The highest abundance was observed at East Incline, followed by Zephyr Cove and South Sand Harbor. The elevated particle abundance at East Incline is consistent with documented hydrodynamic circulation patterns that transport and concentrate

suspended material along the northeastern shoreline, suggesting that lake circulation may influence the spatial distribution of MP's.

Fibers were the dominant particle morphology, with white fibers being the most prevalent. The identification of PET fibers with dye signals is consistent with recent studies identifying textile-derived microfibers as an important source of environmental contamination in the Lake Tahoe Basin. Inconclusive analyses were most associated with fibers, which frequently were colored (contained dyes), and could have had a substantial cellulose population, as would be expected from dryer outflows (e.g., containing cotton and rayon) in the Tahoe Basin. While much of the chemical identification remained inconclusive because of weathering, fluorescence, and dye-dominated Raman spectra, the conservative identification approach used in this study minimized the risk of false polymer identification.

Overall, this study provides the first spatial assessment of MPs in Lake Tahoe's east shore sediments and establishes an important baseline for future monitoring. The higher particle abundance observed compared with previous lakebed sediment studies suggests that additional research is needed to better understand the sources, transport, and long-term accumulation of MPs within the Lake Tahoe Basin.

CHAPTER 3: COMPARING THE USE OF DCVJ VS. NILE RED FOR SOLVATOCHROMISM AND EMISSION INTENSITY USING PLASTIC STANDARDS AND ENVIRONMENTAL SAMPLES

Introduction

Microplastic (MP) pollution is ubiquitous in all of earth's compartments. Therefore, developing methods for MP detection in environmental matrices is important.

Fluorescence microscopy is a relatively simple method for detecting MP's that relies on using specific wavelengths of light to excite plastic particles. Some plastic polymers have intrinsic fluorescence however the use of lipophilic stains is a popular method to enhance the fluorescence of plastic particles (Maes et al., 2017). Nile Red (NR) is a commonly used lipophilic fluorescent dye in biological and microplastic research. It stains microplastics by interacting with and adsorbing onto polymer surfaces primarily through van der Waals forces, with additional dipole interactions occurring in more polar polymer types (Maes et al., 2017). Because of the hydrophobic property of many MP's, caused by the multiple C-H bonds in their polymer chains, NR has been known to stain MP (Shim et al., 2016, Maes et al., 2017). Outside of MP studies, NR has been used to study cells and microorganisms, with applications such as determining lipid content in animal cells (Chen et al 2009, Rumen et al 2015) . Other dyes have also been explored as potential stains in MP studies. Specifically, the fluorescent molecular rotor, 9-(dicyanovinyl)-julolidine (DCVJ), has recently been shown to stain polystyrene spheres (Gagne 2019; Moraz

and Brieder 2021). DCVJ is a fluorescent molecular rotor, which is a molecule whose brightness, after excitation by a light source, changes depending on how freely the molecule can rotate. When excited with light, the molecule rotates to dissipate energy, resulting in weak fluorescence, whereas if molecular mobility is restricted it exhibits strong fluorescence (Haidekker & Theodorakis, 2007). There is limited information on using DCVJ to stain plastics, making this study important. Previous research suggests that DCVJ can stain some plastic polymers, mainly at the nanoscale, but its effectiveness is not well understood.

Motivation for this research

The study by Maes et al. (2017) suggested that, due to its solvatochromic properties, NR may be useful for determining polymer type or polymer family groups based on the emitted wavelength of light from stained particles. Part of the goal of this research was to further investigate this theory by testing it on plastic standards from the Polykit 1.0, and on environmental samples. Additionally, while Maes et al. (2017) evaluated NR alongside several other dyes, no study has directly compared NR and DCVJ, despite recent literature showing promising results for DCVJ at the nanoscale (Gagne 2019; Moraz and Breider 2021). Therefore, a direct comparison of the two dyes using polymer standards from the Polykit 1.0 and environmental samples would be useful.

The analysis of environmental samples in addition to the plastic standards serves two purposes. First, it allows an assessment of how effectively each dye stains unknown environmental pollutants. Second, because the polymer composition of the Hawaiian Microplastic (HMP) samples is unknown, comparing their emission wavelengths to those of the stained standards may help infer their likely polymer composition based on the dyes' solvatochromic responses.

Literature review NR

The first peer-reviewed use of NR for microplastic applications was by Shim et al (2016). This study found that NR was useful for detecting micro-sized polyethylene, polypropylene, polystyrene, polycarbonate, polyurethane, and poly(ethylene-vinyl acetate) particles from a control sand matrix (Shim et al 2016). This study developed the first methodology for NR preparation and staining which included testing eight solvents before landing on five mg/L NR solution in *n*-hexane (Shim et al 2016). Maes et al. (2017), evaluated several other dyes, including Oil Red EGN, Eosin B, Rose Bengal, Hostasol Yellow 3G, and Nile Red, for their effectiveness in staining and fluorescing MP. This study modified the Shim et al (2016) staining method. Maes et al (2017) found that NR is solvatochromic, meaning its fluorescence emission spectrum red shifts as the polarity of the surrounding solvent increases. As a result, the study suggested that NR could potentially be used not only for microplastic detection, but also for plastic categorization based on the surface polarity characteristics of identified particles.

Prasad et al. (2024) tested seven common polymers stained with Nile Red and recorded fluorescence spectra using a Cary Eclipse fluorescence spectrometer under three excitation wavelengths. They found that excitation at 525 nm produced poor peak differentiation, whereas 465 nm and 405 nm excitation generated more distinct characteristic peaks that enabled improved discrimination among polymer types. However, it turns out that the peaks seen with 405 nm are a scattering artifact caused by the monochromator in the fluorimeter. The study supports the use of Nile Red fluorescence for distinguishing polymers; however, the authors noted that fluorescence analysis alone is less reliable than when combined with complementary infrared (IR) techniques.

Since 2017, an increasing number of studies have used Nile Red (NR) staining to quantify MP's in a variety of environmental matrices, including water and wastewater (Bagaev et al 2017., Hendrickson et al. 2018, Kaile et al. 2020), marine and freshwater sediments (Tamminga et al. 2017, Catarino et al. 2018), and biological materials (Cole et al. 2014, Karlsson et al. 2017, Dehaut et al. 2016, Cowger et al. 2021). Collectively, these studies demonstrate that NR is a useful and cost-effective prescreening tool for environmental samples. However, they also highlight several limitations associated with NR-based microplastic analysis, including false positives caused by the staining of natural organic matter,

background interference in complex soil matrices, and workflow constraints such as incompatibility with Raman spectroscopy following NR staining.

Literature Review DCVJ

Compared to Nile Red, there is less literature regarding the use of DCVJ for MP applications. While peer reviewed studies using DCVJ for plastic identification are limited, more studies that use DCVJ focus on the nano plastic (NP) scale rather than the micro scale. Recent peer reviewed literature regarding DCVJ has focused on testing the fluorescent capabilities of this molecule in different solvents as DCJV is a molecular rotor whose fluorescent capability is determined by how freely the molecule can move in its environment.

One of the first peer-reviewed studies to apply DCVJ for plastic detection was conducted by Gagné et al. (2019). In this study, 50 and 100 nm polystyrene nanobeads were analyzed, and DCVJ was found to be effective for detecting nanoplastics both in clean water and in biological matrices derived from mussel tissue. The addition of nanoplastics to the DCVJ probe solution resulted in increased fluorescence intensity, measured using a microplate fluorimeter, as well as a bathochromic shift in the emission maximum from approximately 500 to 650 nm (Gagné et al., 2019).

These findings were later supported by Moraz and Brieder (2021), who employed similar experimental protocols and observed that polystyrene nanobeads stained with

DCVJ exhibited a secondary fluorescence peak near 620 nm. Furthermore, the intensity of this peak increased with increasing concentrations of polystyrene nanobeads, suggesting that DCVJ fluorescence may provide a concentration-dependent response for nanoplastic detection.

Experimental Design

A three-tier analytical approach was developed to compare the performance of Nile Red (NR) and 4-(dicyanovinyl)julolidine (DCVJ) as fluorescent dyes for microplastic detection and characterization. The experimental design incorporated both polymer standards and environmental microplastic samples to evaluate dye performance under controlled and environmentally relevant conditions.

The first tier consisted of fluorescence imaging following dye staining, using a handheld blue light source and a Nikon camera to provide a qualitative assessment of staining effectiveness and fluorescence color. The second tier involved quantitative image analysis using ImageJ, where RGB values were extracted to compare fluorescence intensity and emission characteristics between dye treatments. The final tier employed fluorometric analysis to obtain high-resolution fluorescence emission spectra and determine emission maxima.

Plastic Standards

Materials

Nile Red in powdered form was purchased from MP Biochemicals (via VWR Ltd) and 9-(dicyanovinyl)-julolidine (DCVJ) was obtained from Cayman Chemical (Ann Arbor). The Polymer kit 1.0 was obtained from the Hawai'i Pacific University and contained polyethylene (PE) of differing densities (LDPE, MDPE, HDPE), polypropylene (PP), polyethylene terephthalate (PET), ethylene-vinyl acetate (EVA), acrylon-butadiene-styrene (ABS), polystyrene (PS), polyamide (Nylon, PA), polyvinyl chloride (PVC) pellets (~3 to 5 mm diameter) and polyester fabric (PEST). Other plastics tested included eight micro-macroplastic samples collected from Waimānalo beach, O'ahu, Hawaii in June 2025. These are referred to as Hawaiian Microplastics (HMP)

Staining Procedure

To compare the fluorescent capabilities of NR and DCVJ plastic standards from the Polykit 1.0 were subject to three treatments. These included staining with a NR solution, a DCVJ solution and a control treatment where no stain was applied. The polymers analyzed included ultra-low-density polyethylene (ULDPE), low-density polyethylene (LDPE), linear low-density polyethylene (LLDPE), medium-density polyethylene (MDPE), high-density polyethylene (HDPE), polypropylene (PP), polyethylene terephthalate (PET and PEST), ethylene-vinyl acetate (EVA), acrylonitrile butadiene styrene (ABS), expanded polystyrene (EPS), polystyrene (PS), polyamide (PA), and polyvinyl chloride (PVC).

To create staining solutions, first stock solutions of NR and DCVJ were made up at 1 mg/ml in isopropyl alcohol, and sub stocks made at 0.1 mg/ml. A dye concentration of 8 μ M (2 μ g/ml DCVJ or 2.5 μ g/ml NR) in 25% acetone and 75% H₂O was used to stain pellets from the Polykit 1.0. Individual pellets were arranged in a 96 well plate and covered with 100 μ l of the dye solution using a pipette. Control samples were run with solvent only to check for any intrinsic fluorescence from the pellets. The 96 well plate was placed on a shaking hotplate at 35 °C. After 1 hour, the pellets were rinsed in Milli-Q H₂O and allowed to dry. The samples were stored in the dark prior to analysis. The pellets were transferred to a black metal tray for photography or a modified cuvette for spectrophotometric analysis (Figure 23). In both cases pellets were held in place using double-sided tape which showed no significant fluorescence.

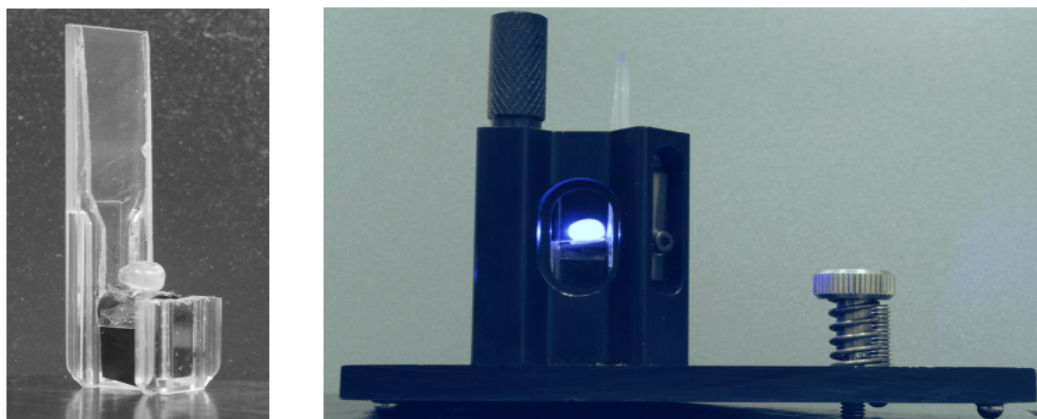


Figure 23: Modified cuvette designed for fluorescence analysis of solid polymer pellets using a Varian Eclipse spectrofluorimeter. Three side walls were removed and an internal mounting platform fitted with double-sided adhesive tape was added to secure pellets within the optical path during measurement. Photo credit: Clive Bagshaw.

The eight HMP samples collected from Waimānalo beach were subsampled into ~3mm x 3mm fragments and treated with staining solutions of NR and DCVJ as well as a control treatment in the same fashion as described above. These unknown samples, labeled HMP 1-8, were white or translucent, except HMP6 which was pale blue and HMP8 which was a green thread. All HMP samples showed melting with the hot needle test using a soldering iron tip at 350° C (Beckingham 2023). In addition to these environmental samples, fragments cut from a polystyrene Petri dish (aPS) , a white HDPE food container (HDPE), nylon fishing line and a cigarette filter (CA) were examined, along with a selection of MP's from the Polymer kit as standards.

Photography

Stained pellets were arranged on a gridded black metal tray and illuminated with a blue (450 nm LED) flashlight (Xinfeibei) without a focusing lens, held at 45° and 15 cm away. Photographs were taken with Nikon D3000 10.2 MP dSLR camera with a 55 mm lens set at f/16 aperture and exposure times 0.25 to 1 s. This camera has 3,872 x 2,592 pixels with an area of 6.1 µm square. A Zeiss 515 nm long-pass filter was attached to the camera lens using filter stepping rings. Files were saved in .jpg and .NEF formats then analyzed using ImageJ.

Stained environmental samples were similarly mounted to a gridded black metal tray using double sided tape before photography following the same method as above.

ImageJ Analysis

Red Green Blue (RGB) channel values for the stained and imaged pellets were recorded using ImageJ. The .jpeg image of the stained pellets (Figure 23) was uploaded into ImageJ and RGB values of the stained pellets were determined by selecting a Region of Interest followed by analysis. Measurement settings were configured to record mean pixel intensity values with results reported to two decimal places. Each RGB image was converted into a three-channel stack using the “RGB Stack” function, which separated the image into individual red, green, and blue channels. The mean intensity of each channel was then measured sequentially by selecting a Region of Interest from the corresponding slice for the red, green, and blue channels. The resulting mean RGB intensity values were subsequently used for color and chromaticity analysis. Red chromaticity was figured for each sample from the RGB analysis. This calculation gives information about how red a sample appeared from the .jpeg image. When the blue component is minimized by the use of a 515nm long pass filter during photography, a value approaching 1 indicates that the sample is red, a value approaching 0.5 indicates yellow and a value approaching zero indicates green. This value was calculated by dividing the Red channel value by the sum of the red, green and blue channels (where blue $\sim$ 0) :

$$\text{Red Chromaticity} = \frac{R}{(R+G+B)}$$

Relative total intensity was also calculated from the RGB analysis by adding the individual RGB values for each sample and then dividing this number by 3×255 , where 255 represents the maximum value of each channel. Note an 8-bit number has $2^8 = 256$ values, but these are defined on a scale of 0 to 255.

Fluorescence Spectroscopy

Fluorescence emission spectra for dyed pellets were recorded using a Varian Eclipse spectrofluorometer with excitation set at 450 nm and the emission scanned from 470 to 800 nm with 5 nm slit widths and photomultiplier voltage from 600 to 900v.

Polymer pellets were mounted in a modified acrylic cuvette with 3 sides cut-away and the sample mounted on double-sided tape at a height of 15 mm (Figure 22). The pellet was centered in the 450 nm excitation beam using the height adjustment screw to achieve maximum visual scattering.

Raman Spectroscopy

For the HMP samples, each sample was cut into subsamples to generate replicate treatments. One set of subsamples was stained with two different fluorescent dyes (NR and DCVJ) following the previously stated staining solution preparation, while a corresponding set remained unstained for Raman spectroscopic analysis to determine polymer chemical composition.

The unstained samples were analyzed using a Horiba Labram HR Evolution spectrometer using the 532 nm diode excitation laser at 50 mW input power. The spot size at the sample was about 2 μm . Spectra were recorded from 200 to 3800 cm^{-1} .

¹, with 15 s acquisition times and 3 accumulations for each sample. A diffraction grating with a grating density of 1200 lines/mm was used for all measurements with a spectral resolution of $\sim 1 \text{ cm}^{-1}$. Spectra were exported as .txt files and then analyzed in OpenSpecy to determine polymer composition.

Results

Photography analysis

Photograph of the Polymer Standards

Pellets from the Polymer kit 1.0, which were stained with NR and DCVJ, and the control set were excited with a 450 nm LED flashlight then imaged. The control pellets provided a baseline for intrinsic fluorescence of the polykit pellets under 450 nm light. Based on the image, both samples of PET showed strong intrinsic fluorescence, while weaker fluorescence was seen in the PEST and PA samples. Figure 23 demonstrates the ability of NR to distinguish different classes of polymer based on the color of their fluorescence emission, as reported previously (Maes 2017, Prasad 2024). For example, the polyethylene polymers (ULDPE, LDPE, LLDPE, MDPE, and HDPE) exhibited fluorescence in the yellow region when stained with NR. In contrast, DCVJ produced little to no observable fluorescence for these hydrophobic polymer classes. Those polymers that were stained by DCVJ appeared predominantly green, particularly PA and PVC.

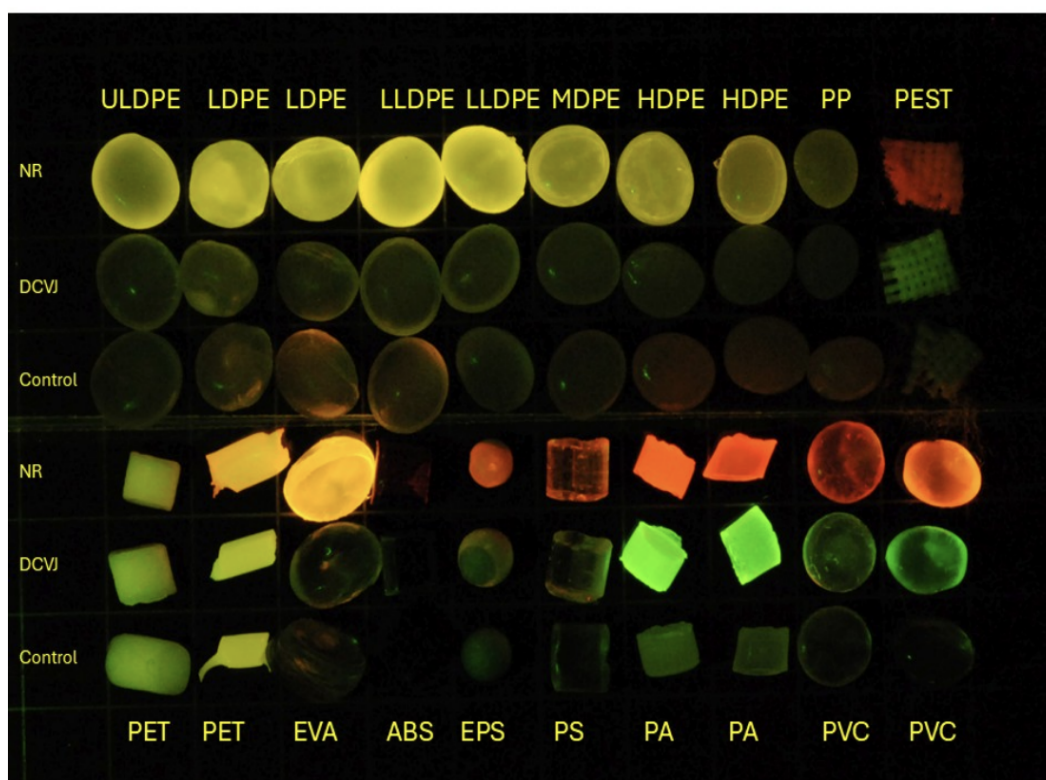


Figure 24: Fluorescence responses of reference polymer standards from the Polykit 1.0 under three treatment conditions: NR, DCVJ, and unstained control. Polymers included are polyethylene variants (ULDPE, LDPE, LLDPE, MDPE, HDPE), polypropylene (PP), polyester (PEST), polyethylene terephthalate (PET), ethylene-vinyl acetate (EVA), acrylonitrile butadiene styrene (ABS), expanded polystyrene (EPS), polystyrene (PS), polyamide (PA), and polyvinyl chloride (PVC). Fluorescence intensity and color variation illustrate differences in dye affinity and staining response among polymer types. Photo credit: Clive Bagshaw, M. Wallner

Photograph of Hawaiian Microplastics

The HMP samples of unknown chemical composition that were collected from Waimanalo beach, Oahu, HI, were similarly stained and imaged (Figure 25). Based on the image, the HMP beach samples, when stained with Nile Red, generally showed strong fluorescence signals and most had a distinct orange hue compared

with the yellow color of standard polyethylene samples. In contrast, DCVJ staining of HMPs showed dull to strong yellow-green fluorescence.

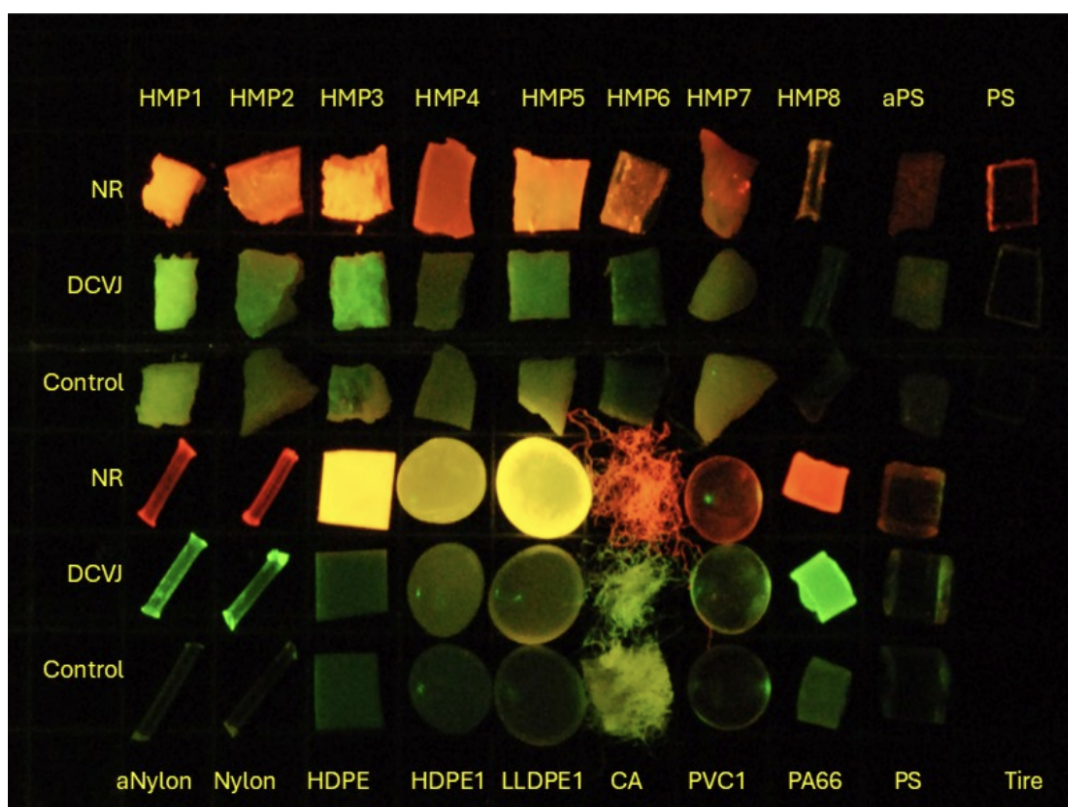


Figure 25: Fluorescence responses of HMP (Waimanalo Beach, Oahu, HI) and reference polymer standards under three treatment conditions: NR, DCVJ, and unstained control. Polymer standards included are Nylon, Polyethylene (HDPE, LLDPE), cellulose acetate, PVC, Polyamide (PA66), polystyrene and crumb rubber (Tire). The unknown environmental samples are HMP1-8. Photo credit: Clive Bagshaw, Amelia Labbe

ImageJ Results

RGB Analysis of Polymer standards

Further investigation of RGB values using ImageJ provided a way to quantify these observed differences in fluorescence emission wavelength and intensity from the .jpeg images. Regarding intensity, based on the RGB analysis of Figure 24 the

sample that showed the most intense fluorescence was the EVA dyed with NR. This sample demonstrated an intensity value of 0.55 and became the normalization for the relative intensity calculation in Table 6. The polymers in the PE class when dyed with NR showed Red chromaticity values approaching 0.5 which is consistent with the yellow color which is seen from the .jpeg image.

Based on these results, relative intensity was significantly lower in DCVJ-treated polymers compared to NR-treated polymers with the mean relative intensity decreasing from 54.4 in the NR treatment to 22.8 in the DCVJ treatment (paired t-test, $p = 0.00068$). This difference is even more marked given that the camera is more sensitive to green than red light.

Table 7: Red chromaticity and relative fluorescence intensity of dyed polymer samples under three conditions (NR, DCVJ, and control). Red chromaticity quantifies the apparent emission color, where values approaching 1 indicate red emission, values near 0.5 appear yellow, and values approaching 0 indicate green emission. Relative fluorescence intensity values were normalized to EVA dyed with NR, which exhibited the highest fluorescence intensity and was assigned a value of 100. Values in red indicate that the polymer demonstrated relative intensity of values lower than 20 which are comparable to the background signal from unstained samples meaning that chromacity value is not well defined.

Polymer	NR		DCVJ		Control	
	Red chromaticity	Relative Intensity	Red chromaticity	Relative Intensity	Red chromaticity	Relative Intensity
ULDPE	0.48	45.83	0.36	11.66	0.38	10.40
LDPE	0.51	82.64	0.42	17.47	0.50	19.74
LLDPE	0.51	77.46	0.41	14.97	0.43	13.07
MDPE	0.51	59.98	0.39	12.87	0.40	9.72
HDPE	0.52	52.93	0.39	11.79	0.55	14.18
PP	0.47	20.39	0.36	8.43	0.49	12.46
PEST	0.80	32.27	0.28	24.14	0.43	11.84
PET	0.51	78.64	0.45	73.42	0.45	68.12
EVA	0.58	100.00	0.42	13.92	0.45	11.68
ABS	0.62	8.69	0.33	2.53	0.34	2.14
EPS	0.73	60.72	0.34	18.60	0.20	13.83
PS	0.69	24.63	0.38	18.48	0.36	4.88
PA	0.76	75.89	0.27	72.66	0.32	21.21
PVC	0.78	41.90	0.22	17.92	0.36	5.20

Fluorescence Spectroscopy Analysis

Spectra of the dyed and control pellets from the Polymer kit 1.0 were recorded by mounting the dry pellets in the excitation beam of a Varian Eclipse spectrofluorometer. Fluorescence emission peaks were determined for Nile Red stained samples between 534 nm (PE) and 615 nm (PA and PVC) (Figure 26). This corresponds with the colors seen by eye (Figure 24) . In contrast, fluorescence emission from DCVJ stained pellets, where visible, was generally in the region of 500 nm and not always clearly resolved from the background light scatter. Exceptions were those from PA and PVC which showed very intense peaks at 497 nm and 495 nm respectively (Figure 26). The reported emission spectra are uncorrected for the instrument sensitivity which gives a stronger signal in the green compared with the red region. While the correction factors for the Varian fluorimeter were not available across the complete range of interest, those for an SLM

fluorimeter having the same R928 photomultiplier are: 500 nm, 1.00; 550 nm, 1.57; 600 nm, 2.64; 650 nm, 5.17; 700nm, 11.9.

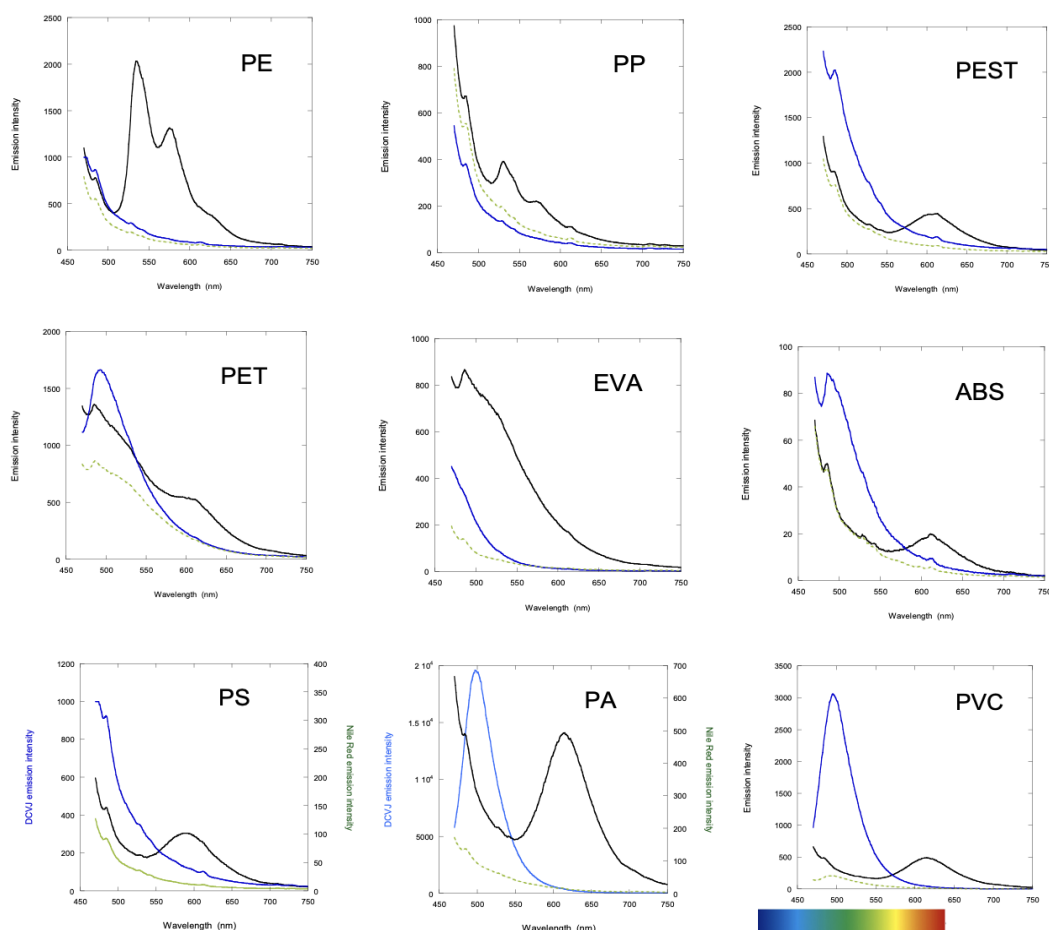


Figure 26: Fluorescence emission spectra of selected polymers measured using a Varian Eclipse spectrofluorometer following staining with NR and (DCVJ). Black lines represent NR-stained polymers, blue lines represent DCVJ-stained polymers, and green dashed lines represent unstained controls. The x-axis shows emission wavelength (nm), corresponding approximately to the visible colors indicated by the color bar, and the y-axis shows fluorescence emission intensity. Emission profiles are shown for polyethylene (PE), polypropylene (PP), polyester (PEST), polyethylene terephthalate (PET), ethylene-vinyl acetate (EVA), acrylonitrile butadiene styrene (ABS), polystyrene (PS), polyamide (PA), and polyvinyl chloride (PVC).

Raman Spectroscopy Results of Hawaiian Microplastics

Raman spectra were collected for the eight HMP samples and subsequently analyzed using OpenSpecy. The analysis identified two samples (HMP3 and HMP5) as polypropylene, while the remaining six samples were identified as polyethylene (Table 7, Figure 26). All samples produced match values exceeding the accepted threshold of 0.70, indicating reliable polymer identification. Furthermore, all samples except HMP1 exhibited match values greater than 0.90, providing strong confidence in the spectral assignments.

Table 8: Spectral matches for HMP samples 1-7. All exhibit strong match values. Signal to noise ratio (SNR) is variable among samples.

Sample	Material	Match Value	SNR
HMP1	poly(ethylene)	0.85	8.61121
HMP2	poly(ethylene)	0.98	130.843
HMP3	poly(propylene)	0.91	8.95247
HMP4	poly(ethylene)	0.99	171.582
HMP5	poly(propylene)	0.93	10.7923
HMP6	poly(ethylene)	0.98	106.338
HMP7	poly(ethylene)	0.9	10.872

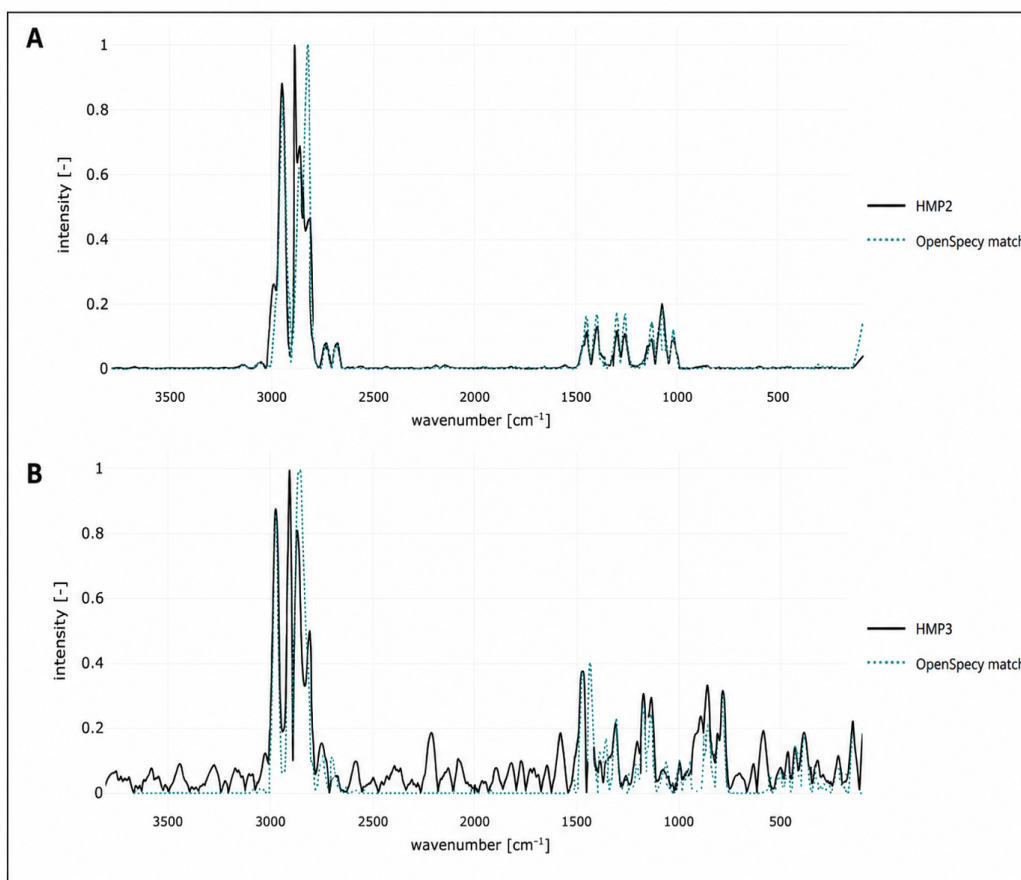


Figure 27: OpenSpecy spectral match results for representative polymer particles. (A) Representative spectrum identified as polyethylene (PE) (HMP2). (B) Representative spectrum identified as polypropylene (PP) (HMP3). Spectra were generated in OpenSpecy to assess material identification through spectral matching. The solid black line represents the preprocessed sample spectrum, while the dashed turquoise line represents the corresponding OpenSpecy reference library match. The close alignment between the sample and reference spectra supports the polymer identifications.

Discussion

The three-tier analytical approach employed in this study provided a comprehensive framework for comparing Nile Red (NR) and DCVJ as fluorescent dyes for MP identification. The first tier involved staining both polymer standards and environmental samples with NR and DCVJ, followed by fluorescence imaging using readily accessible equipment (a handheld blue light source and a Nikon camera). Visual assessment of the resulting images provided an initial qualitative evaluation of staining effectiveness (brightness) and apparent fluorescence color. The second tier involved quantitative image analysis using ImageJ, where RGB values were extracted to assess differences in fluorescence intensity and emitted wavelength between dye treatments. The final tier consisted of fluorometric analysis using XXX, which provided higher spectral resolution and allowed the precise determination of fluorescence emission maxima and wavelength resolution of the peaks. Together, these complementary methods enabled both qualitative and quantitative comparisons of dye performance.

The use of polymer standards from the Polykit 1.0 enabled a direct comparison between the RGB image analysis and fluorometric measurements for the two dye treatments. Results from both approaches demonstrated that the fluorescence emission characteristics of the dyes varied among polymer types, providing evidence for solvatochromic behavior. This effect was particularly evident for NR, which exhibited more pronounced shifts in emission wavelength among polymer classes. For example, certain polymer groups consistently emitted fluorescence within

distinct wavelength ranges, suggesting that the polymer matrix influences the fluorescence response of the dye. These findings support previous suggestions that solvatochromic dyes may have potential for distinguishing among polymer types or polymer families.

The inclusion of Hawaiian beach microplastic (HMP) samples provided an opportunity to evaluate whether the solvatochromic patterns observed in polymer standards could be applied to environmental MP's of unknown composition. While this approach was useful for testing the solvatochromic hypothesis under realistic environmental conditions, the results ultimately revealed more complexity than was observed for the standards alone. Raman spectroscopic analysis identified HMP3 and HMP5 as polypropylene, while the remaining samples were identified as polyethylene. Based on the fluorescence behavior observed in the polymer standards, polypropylene particles would be expected to emit fluorescence within the 525–550 nm range (yellow by eye). However, HMP3 and HMP5 exhibited fluorescence that appeared closer to 600 nm (orange-red by eye) (Figure XX). Similarly, the polyethylene samples (HMP1, HMP2, HMP4, HMP6, HMP7, and HMP8) displayed fluorescence colors that were more orange or red than expected based on the responses of the polyethylene standards.

These discrepancies suggest that fluorescence emission characteristics observed in environmental MP's cannot be explained solely by polymer identity. Environmental weathering, surface oxidation, biofouling, and the adsorption of organic

contaminants may alter the local chemical environment surrounding dye molecules, thereby affecting their fluorescence response.

Consequently, while the results obtained from the polymer standards demonstrate that both NR and DCVJ exhibit solvatochromic behavior, the environmental samples indicate that polymer composition alone may not be the only parameter influencing fluorescence emission wavelength, and that real-world sample emission is more complex. This finding highlights an important limitation of using solvatochromic fluorescence as a standalone method for polymer identification and underscores the continued need for spectroscopic confirmation when analyzing environmental MP's.

To determine whether surface conditions were influencing polymer identification by Raman spectroscopy, undyed HMP samples (HMP1–HMP8) were prewashed with acetone to remove any organic material present on the particle surface and then reanalyzed. In addition, each undyed HMP sample was cut, and Raman spectra were collected from the interior of the particle to assess whether polymer composition differed between the exterior and interior regions, or whether oxidation effects were influencing spectral identification.

In both cases, the resulting spectra matched the initial Raman identifications, with samples consistently identified as either polypropylene or polyethylene. These findings suggest that biofouling, surface oxidation, and heterogeneous polymer

composition between the exterior and interior of the particles did not influence the polymer identifications obtained by Raman spectroscopy.

Beyond the scope of the experiments presented in this chapter, additional work conducted by collaborators (Clive R. Bagshaw, Justin Diaz and James Jacob) investigated the performance of NR and DCVJ at the nanoscale using fluorescently stained polystyrene nanoparticles. Fluorescence imaging of the stained particles suggested that DCVJ may offer advantages over NR for nanoplastic applications, particularly when analyzing polystyrene particles.

In these experiments, the NR treatment exhibited a higher level of apparent false-positive signal relative to the control treatment, which consisted of dye in clean water without polystyrene particles. The fluorescence observed in the NR control is believed to result from dye aggregation, where NR molecules precipitated from solution and formed fluorescent aggregates that could potentially be misidentified as nanoplastic particles. In contrast, the DCVJ control exhibited substantially lower background fluorescence and fewer apparent aggregates, suggesting reduced susceptibility to this source of analytical interference.

These findings indicate that, despite the lower fluorescence intensity exhibited by DCVJ relative to NR in both the microplastic and nanoplastic experiments, DCVJ may offer greater specificity for nanoscale applications due to its lower background signal and reduced propensity for aggregation. Minimizing false-positive detections

is particularly important in nanoplastic research, where particles approach the limits of optical resolution and accurate identification becomes increasingly challenging.

Additional research is needed to evaluate the performance of both dyes across a broader range of polymer types and environmental conditions at the nanoscale. Such work would help determine whether the advantages observed for DCVJ are specific to polystyrene or represent a more general improvement for nanoplastic detection and characterization.

Conclusion

This study demonstrated that both Nile Red (NR) and DCVJ are effective fluorescent dyes for microplastic detection, with each exhibiting distinct strengths. While NR produced stronger fluorescence and more pronounced solvatochromic responses in polymer standards, these patterns were not consistently observed in environmental microplastics, indicating that fluorescence emission is influenced by factors beyond polymer composition, such as environmental weathering and surface chemistry. Consequently, fluorescence staining alone is insufficient for reliable polymer identification, and spectroscopic confirmation remains essential. Although DCVJ exhibited lower fluorescence intensity, its reduced background signal and lower propensity for dye aggregation suggest it may offer advantages for nanoplastic applications.

References

- Amato-Lourenço, L. F., Carvalho-Oliveira, R., Júnior, G. R., dos Santos Galvão, L., Ando, R. A., & Mauad, T. (2021). Presence of airborne microplastics in human lung tissue. *Journal of Hazardous Materials*, 416, 126124. <https://doi.org/10.1016/j.jhazmat.2021.126124>
- Andrady, A. (2022). *Plastics and the Ocean*. 2022 John Wiley & Sons, Inc. <https://doi.org/10.1002/9781119768432>
- Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, 62(8), 1596–1605. <https://doi.org/10.1016/j.marpolbul.2011.05.030>
- Andrady, A. L., & Rajapakse, N. (2019). Additives and Chemicals in Plastics. In H. Takada & H. K. Karapanagioti (Eds.), *Hazardous Chemicals Associated with Plastics in the Marine Environment* (pp. 1–17). Springer International Publishing. https://doi.org/10.1007/698_2016_124
- Arienzo, M. M., Collins, M., Justice Frey, E., Movius, M., Patten, L., DePauw, A., & Kozloski, R. (2025). A participatory science approach to quantify microfiber emissions from clothes dryers. *Environmental Toxicology and Chemistry*, vgaf222. <https://doi.org/10.1093/etjnl/vgaf222>
- Bagaev, A., Mizyuk, A., Khatmullina, L., Isachenko, I., & Chubarenko, I. (2017). Anthropogenic fibres in the Baltic Sea water column: Field data, laboratory and numerical testing of their motion. *Science of the Total Environment*, 599–600, 560–571. <https://doi.org/10.1016/j.scitotenv.2017.04.185>
- Beckingham, B., Apintiloaiei, A., Moore, C., & Brandes, J. (2023). Hot or not:

- systematic review and laboratory evaluation of the hot needle test for Microplastic identification. *Microplastics and Nanoplastics*, 3(1), 8.
<https://doi.org/10.1186/s43591-023-00056-4>
- California State Water Resources Control Board, Division of Drinking Water. (2022). Water. California State Water Resources Control Board
- Carpenter, E. J., & Smith, K. L. (1972). Plastics on the Sargasso Sea Surface. *Science*, 175(4027), 1240–1241.
<https://doi.org/10.1126/science.175.4027.1240>
- Cashman, M. A., Langknecht, T., El Khatib, D., Burgess, R. M., Boving, T. B., Robinson, S., & Ho, K. T. (2022). Quantification of microplastics in sediments from Narragansett Bay, Rhode Island USA using a novel isolation and extraction method. *Marine Pollution Bulletin*, 174, 113254.
<https://doi.org/10.1016/j.marpolbul.2021.113254>
- Catarino, A. I., Macchia, V., Sanderson, W. G., Thompson, R. C., & Henry, T. B. (2018). Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. *Environmental Pollution*, 237, 675–684.
<https://doi.org/10.1016/j.envpol.2018.02.069>
- Chaïb, I., Doyen, P., Merveillie, P., Dehaut, A., & Duflos, G. (2025). Microplastic contaminations in a set of beverages sold in France. *Journal of Food Composition and Analysis*, 144, 107719.
<https://doi.org/10.1016/j.jfca.2025.107719>

- Chen, R., Shi, Y., Fang, N., Shao, C., Huang, H., Pan, R., Xu, Y., Wang, M., Liu, X., Xu, K., Zhu, R., & Wang, M. (2024). Bronchoalveolar lavage fluid analysis in patients with checkpoint inhibitor pneumonitis. *Cancer Immunology, Immunotherapy* : CII, 73(11), 235.
<https://doi.org/10.1007/s00262-024-03834-y>
- Chen, W., Zhang, C., Song, L., Sommerfeld, M. & Hu, Q. A high throughput Nile red method for quantitative measurement of neutral lipids in microalgae. *J. Microbiol. Methods* 77, 41–7 (2009).
- City of South Lake Tahoe. (2018). Polystyrene and plastic food packaging regulations (City Code Chapter 4.175). <https://www.cityofslt.gov>
- City of South Lake Tahoe. (2022). Single-use plastic water bottle ordinance.
<https://www.cityofslt.gov>
- City of South Lake Tahoe. (n.d.). Plastic waste reduction.
<https://www.cityofslt.gov/2267/Plastic-Waste>
- Clean Up the Lake—Diving to Clean Up Lakes and Oceans. (n.d.). Clean Up The Lake. Retrieved June 28, 2026, from <https://cleanupthelake.org/>
- Cole, M., Webb, H., Lindeque, P. K., Fileman, E. S., Halsband, C., & Galloway, T. S. (2014). Isolation of microplastics in biota-rich seawater samples and marine organisms. *Scientific Reports*, 4, 4528. <https://doi.org/10.1038/srep04528>
- Conley, K., Clum, A., Deepe, J., Lane, H., & Beckingham, B. (2019). Wastewater treatment plants as a source of microplastics to an urban estuary: Removal

- efficiencies and loading per capita over one year. *Water Research X*, 3, 100030. <https://doi.org/10.1016/j.wroa.2019.100030>
- Cowger, W., Gray, A., Guiling, J., Fong, P. P., & Waldschläger, K. (2021). Critical review of processing and classification techniques for images and spectra in microplastic research. *Applied Spectroscopy*, 75(9), 989–1010. <https://doi.org/10.1177/00037028211012637>
- Davidson, J., & Arienzo, M. (2024). (PDF) Polymer Characterization of Submerged Plastic Litter from Lake Tahoe, United States. ResearchGate. <https://doi.org/10.1177/00037028231201174>
- Dehaut, A., Cassone, A.-L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., & Paul-Pont, I. (2016). Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environmental Pollution*, 215, 223–233. <https://doi.org/10.1016/j.envpol.2016.05.018>
- Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V., & Tassin, B. (2017). A first overview of textile fibers, including microplastics, in indoor and outdoor environments. *Environmental Pollution*, 221, 453–458. <https://doi.org/10.1016/j.envpol.2016.12.013>
- Forakis, J. (2025, June 3). Microplastic analysis background [PowerPoint presentation]. Presented by M. Calderon, R. Nakamoto, C. Megill, K. Shaw, J. Axworthy, C. Wardinski, S. Williams.
- Gagné, F. (2019). On the use of fluorescence to detect the presence of microplastics

- in environmental samples: A review. *Applied Sciences*, 9(22), 4814.
<https://doi.org/10.3390/app9224814>
- Geyer, R. (2020). Chapter 2—Production, use, and fate of synthetic polymers. In T. M. Letcher (Ed.), *Plastic Waste and Recycling* (pp. 13–32). Academic Press.
<https://doi.org/10.1016/B978-0-12-817880-5.00002-5>
- Gjeltema, J., Senft, K., Lang, J., Sesma, S., & Schladow, G. (2023). To sink or swim: A snapshot evaluation of the fate and types of microplastics in Lake Tahoe. Report prepared for the Nevada Division of Environmental Protection, University of California, Davis, Tahoe Environmental Research Center.
- Goldman, C. R. (1989). Lake Tahoe: Preserving a Fragile Ecosystem. *Environment: Science and Policy for Sustainable Development*, 31(7), 6–31.
<https://doi.org/10.1080/00139157.1989.9928958>
- Goßmann, I., Halbach, M., & Scholz-Böttcher, B. M. (2021). Car and truck tire wear particles in complex environmental samples – A quantitative comparison with “traditional” microplastic polymer mass loads. *Science of The Total Environment*, 773, 145667. <https://doi.org/10.1016/j.scitotenv.2021.145667>
- Haidekker, M. A., & Theodorakis, E. A. (2007). Molecular rotors—fluorescent biosensors for viscosity and flow. *Organic & Biomolecular Chemistry*, 5(11), 1669–1678.
- Harrold, Z., West, C., Easler, S., Urrita, H., Arienzo, M., Senft, K., & Davis, U. (n.d.). Results of the 2020 Lake Tahoe SCUBA- Enabled Litter Cleanup Dives.

- Hendrickson, E., Minor, E. C., & Schreiner, K. (2018). Microplastic abundance and composition in western Lake Superior as determined via microscopy, Pyr-GC/MS, and FTIR. *Marine Pollution Bulletin*, 150, 110762.
<https://doi.org/10.1016/j.marpolbul.2019.110762>
- Ho, D., & Masura, J. (2025). Dyeing to Know: Harmonizing Nile Red Staining Protocols for Microplastic Identification. *Colorants*, 4(2), 20.
<https://doi.org/10.3390/colorants4020020>
- Huang, S., Huang, X., Bi, R., Guo, Q., Yu, X., Zeng, Q., Huang, Z., Liu, T., Wu, H., Chen, Y., Xu, J., Wu, Y., & Guo, P. (2022). Detection and Analysis of Microplastics in Human Sputum. *Environmental Science & Technology*, 56(4), 2476–2486. <https://doi.org/10.1021/acs.est.1c03859>
- Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R., & Law, K. L. (2015). Plastic waste inputs from land into the ocean. *Science*, 347(6223), 768–771.
<https://doi.org/10.1126/science.1260352>
- Kaile, N., El Dana, D. R., Chokwe, T. B., & Musee, N. (2020). First identification and quantification of microplastics in wastewater from wastewater treatment plants in South Africa. *Environmental Monitoring and Assessment*, 192, 477.
<https://doi.org/10.1007/s10661-020-08412-8>
- Karlsson, T. M., Vethaak, A. D., Almroth, B. C., Ariese, F., van Velzen, M., Hassellöv, M., & Leslie, H. A. (2017). Screening for microplastics in sediment, water, marine invertebrates and fish: Method development and

- microplastic accumulation. *Marine Pollution Bulletin*, 122(1–2), 403–408.
<https://doi.org/10.1016/j.marpolbul.2017.06.081>
- Lake Tahoe Destination Stewardship Council. (2023). Lake Tahoe destination stewardship plan. Lake Tahoe Destination Stewardship Council.
<https://stewardshiptahoe.org/>
- Langknecht, T., Lao, W., Wong, C. S., Kotar, S., El Khatib, D., Robinson, S., Burgess, R. M., & Ho, K. T. (2023). Comparison of two procedures for microplastics analysis in sediments based on an interlaboratory exercise. *Chemosphere*, 313, 137479.
<https://doi.org/10.1016/j.chemosphere.2022.137479>
- Law, K. L., Starr, N., Siegler, T. R., Jambeck, J. R., Mallos, N. J., & Leonard, G. H. (2020). The United States' contribution of plastic waste to land and ocean. *Science Advances*, 6(44), eabd0288. <https://doi.org/10.1126/sciadv.abd0288>
- Leslie, H. A., van Velzen, M. J. M., Brandsma, S. H., Vethaak, A. D., Garcia-Vallejo, J. J., & Lamoree, M. H. (2022). Discovery and quantification of plastic particle pollution in human blood. *Environment International*, 163, 107199.
<https://doi.org/10.1016/j.envint.2022.107199>
- Liu, Y., Hu, J., Lin, L., Yang, B., Huang, M., Chang, M., ... & Li, C. (2023). Overcoming the fluorescent interference during Raman spectroscopy detection of microplastics. *Science of The Total Environment*, 897, 165333.
- Lukasik, H. L. (2024). Microplastics occurrence and distribution in two western arid

- river systems: The Truckee River and the Lower Colorado River (Master's thesis, University of Nevada, Reno). ScholarWolf.
<https://scholarwolf.unr.edu/items/9dfad219-0a3f-4aeb-a692-b39b11f2b218>
- Lynch, J. M., Knauer, K., & Shaw, K. R. (n.d.). Plastic Additives in the Ocean. Retrieved October 1, 2025, from
<https://onlinelibrary.wiley.com/doi/10.1002/9781119768432.ch2>
- Maes, T., Jessop, R., Wellner, N., Haupt, K., & Mayes, A. G. (2017). A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. *Scientific Reports*, 7(1), 44501.
<https://doi.org/10.1038/srep44501>
- Megill, C., Shaw, K., Knauer, K., Seeley, M., & Lynch, J. (2024). Plastic additives in the ocean: Use of a comprehensive dataset for meta-analysis and method development. *Chemosphere*, 358, 142172.
<https://doi.org/10.1016/j.chemosphere.2024.142172>
- Miller, E., Sedlak, M., Lin, D., Box, C., Holleman, C., Rochman, C. M., & Sutton, R. (2021). Recommended best practices for collecting, analyzing, and reporting microplastics in environmental media: Lessons learned from comprehensive monitoring of San Francisco Bay. *Journal of Hazardous Materials*, 409, 124770. <https://doi.org/10.1016/j.jhazmat.2020.124770>
- Moraz, A., & Breider, F. (2021). Detection and Quantification of Nonlabeled Polystyrene Nanoparticles Using a Fluorescent Molecular Rotor. *Analytical Chemistry*, 93(45), 14976–14984.

<https://doi.org/10.1021/acs.analchem.1c02055>

Nava, V., Chandra, S., Aherne, J., Alfonso, M. B., Antão-Geraldes, A. M.,
Attermeyer, K., Bao, R., Bartrons, M., Berger, S. A., Biernaczyk, M.,
Bissen, R., Brookes, J. D., Brown, D., Cañedo-Argüelles, M., Canle, M.,
Capelli, C., Carballeira, R., Cereijo, J. L., Chawchai, S., ... Leoni, B. (2023).
Plastic debris in lakes and reservoirs. *Nature*, 619(7969), 317–322.

<https://doi.org/10.1038/s41586-023-06168-4>

Negrete Velasco, A. de J., Rard, L., Blois, W., Lebrun, D., Lebrun, F., Pothe, F., &
Stoll, S. (2020). Microplastic and Fibre Contamination in a Remote Mountain
Lake in Switzerland. *Water*, 12(9), Article 9.

<https://doi.org/10.3390/w12092410>

Our Mission | Protect & Preserve. (n.d.). Keep Tahoe Blue. Retrieved June 28, 2026,
from <https://www.keeptahoeblue.org/mission/>

Oßmann, B. E. (2022). Microplastics Characterization by Raman Microscopy. In
Handbook of Microplastics in the Environment (pp. 113–139). Springer,
Cham. https://doi.org/10.1007/978-3-030-39041-9_36

O'Brien, S., Rauert, C., Ribeiro, F., Okoffo, E. D., Burrows, S. D., O'Brien, J. W.,
Wang, X., Wright, S. L., & Thomas, K. V. (2023). There's something in the
air: A review of sources, prevalence and behaviour of microplastics in the
atmosphere. *The Science of the Total Environment*, 874, 162193.

<https://doi.org/10.1016/j.scitotenv.2023.162193>

Pastorino, P., Anselmi, S., Esposito, G., Bertoli, M., Pizzul, E., Barceló, D., Elia, A.

- C., Dondo, A., Prearo, M., & Renzi, M. (2023). Microplastics in biotic and abiotic compartments of high-mountain lakes from Alps. *Ecological Indicators*, 150, 110215. <https://doi.org/10.1016/j.ecolind.2023.110215>
- Poisson, J., & Zhang, K. (2024). Unique optical properties of cellulosic materials. *Accounts of Materials Research*, 5(8), 920–932. <https://doi.org/10.1021/accountsmr.4c00013>
- Prasad, S., Bennett, A., & Triantafyllou, M. (2024). Characterization of Nile Red-stained microplastics through fluorescence spectroscopy. *Journal of Marine Science and Engineering*, 12(8), 1403. <https://doi.org/10.3390/jmse12081403>
- Qin, X., Cao, M., Peng, T., Shan, H., Lian, W., Yu, Y., Shui, G., & Li, R. (2024). Features, Potential Invasion Pathways, and Reproductive Health Risks of Microplastics Detected in Human Uterus. *Environmental Science & Technology*, 58(24), 10482–10493. <https://doi.org/10.1021/acs.est.4c01541>
- Rochman, C. M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., De Frond, H., Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S. B., Wu, T., Santoro, S., Werbowski, L. M., ... Hung, C. (2019). Rethinking microplastics as a diverse contaminant suite. *Environmental Toxicology and Chemistry*, 38(4), 703–711. <https://doi.org/10.1002/etc.4371>
- Rosso, B., Scoto, F., Hallanger, I. G., Larose, C., Gallet, J. C., Spolaor, A., Bravo, B., Barbante, C., Gambaro, A., & Corami, F. (2024). Characteristics and

quantification of small microplastics (<100 µm) in seasonal svalbard snow on glaciers and lands. *Journal of Hazardous Materials*, 467, 133723.

<https://doi.org/10.1016/j.jhazmat.2024.133723>

Rumin, J. et al. The use of fluorescent Nile red and BODIPY for lipid measurement In microalgae. *Biotechnol. Biofuels* 8, 42 (2015).

Shaw, K. R., Sandquist, R., Fairclough, C., Black, J., Fitzgerald, A., Shaw, J. T., Gallagher, S., & Lynch, J. (2024). Separation of microplastics from deep-sea sediment using an affordable, simple to use, and easily accessible density separation device. *Microplastics and Nanoplastics*, 4(1), 16.

<https://doi.org/10.1186/s43591-024-00093-7>

Shaw, K, CMDR Marine Debris Circularity Academy Microplastic Quantification Short Course, June 2025.

Shim, W. J., Song, Y. K., Hong, S. H., & Jang, M. (2016). Identification and quantification of microplastics using Nile Red staining. *Marine Pollution Bulletin*, 113(1–2), 469–476.

<https://doi.org/10.1016/j.marpolbul.2016.10.049>

Śmigiel-Kamińska, D., Pośpiech, J., Makowska, J., Stepnowski, P., Wąs-Gubała, J., & Kumirska, J. (2019). The Identification of Polyester Fibers Dyed with Disperse Dyes for Forensic Purposes. *Molecules*, 24(3), 613.

<https://doi.org/10.3390/molecules24030613>

State of California. (n.d.). Single-use carryout bags.

<https://calrecycle.ca.gov/plastics/carryoutbags/>

Tamminga, M., Hengstmann, E., & Fischer, E. K. (2017). Nile Red staining as a subsidiary method for microplastic quantification: A comparison of three solvents and factors influencing application reliability. *SDRP Journal of Earth Sciences & Environmental Studies*, 2(2), 1–9.

Thornton Hampton, L. (2023, June 2). Microplastics method standardization [PowerPoint slides]. Southern California Coastal Water Research Project (SCCWRP).

https://ftp.sccwrp.org/pub/download/PRESENTATIONS/Commission_Meetings/23_06.02/Microplastics.pdf

Tian, Z., Gonzalez, M., Rideout, C. A., Zhao, H. N., Hu, X., Wetzel, J., Mudrock, E., James, C. A., McIntyre, J. K., & Kolodziej, E. P. (2022). 6PPD-Quinone: Revised Toxicity Assessment and Quantification with a Commercial Standard. *Environmental Science & Technology Letters*, 9(2), 140–146.
<https://doi.org/10.1021/acs.estlett.1c00910>

Town of Truckee. (2024). Ordinance No. 2024-01: An ordinance prohibiting the sale and commercial distribution of water in single-use plastic bottles and paper cartons under one gallon in volume.
<https://mccmeetingspublic.blob.core.usgovcloudapi.net/truckee-ca-meet-1e71c452c3c143689e98e939404c304f/ITEM-Attachment-001-9f1183498a5b434eabfc886f57cb58f7.pdf>

U.S. Environmental Protection Agency. (2011). Lake Tahoe total maximum daily

load (TMDL).

<https://archive.epa.gov/region09/water/archive/watershed/tahoe/pdf/final-lake>

-tahoe-tmdl.pdf

Umurhan, Y., Songsart-Power, M., Limbu, T. B., & Phan, T. (2025). Applications of Raman spectroscopy for microplastic detection and characterization: A comprehensive spectral reference. *Environmental Science and Pollution Research International*, 32(50), 28630–28677.
<https://doi.org/10.1007/s11356-025-37224-3>

United States Department of Transportation (2022). Mitigating Traffic Congestion - The Role of Demand-Side Strategies: Lake Tahoe Basin - CA. United States Department of Transportation - Federal Highway Administration. Available at:https://ops.fhwa.dot.gov/publications/mitig_traf_cong/lake_tahoe_case.htm#:~:text=About%2056%2C000%20live%20in%20the,catering%20primarily%20to%20Basin%20visitors.

University of California, Davis Tahoe Environmental Research Center. (2026). Lake Tahoe's physical dynamics. Tahoe Environmental Research Center

Wang, S., Chen, H., Zhou, X., Tian, Y., Lin, C., Wang, W., Zhou, K., Zhang, Y., & Lin, H. (2020). Microplastic abundance, distribution and composition in the mid-west Pacific Ocean. *Environmental Pollution*, 264, 114125.
<https://doi.org/10.1016/j.envpol.2020.114125>

Wiesinger, H., Wang, Z., & Hellweg, S. (2021). Deep Dive into Plastic Monomers, Additives, and Processing Aids. *Environmental Science & Technology*,

55(13), 9339–9351. <https://doi.org/10.1021/acs.est.1c00976>

Yu, L., Zhang, J., Liu, Y., Chen, L., Tao, S., & Liu, W. (2021). Distribution characteristics of microplastics in agricultural soils from the largest vegetable production base in China. *Science of The Total Environment*, 756, 143860. <https://doi.org/10.1016/j.scitotenv.2020.143860>

Yu, Y., & Flury, M. (2021). Current understanding of subsurface transport of micro- and nanoplastics in soil. *Vadose Zone Journal*, 20(2), e20108. <https://doi.org/10.1002/vzj2.20108>

Zhu, L., Kang, Y., Ma, M., Wu, Z., Zhang, L., Hu, R., Xu, Q., Zhu, J., Gu, X., & An, L. (2024). Tissue accumulation of microplastics and potential health risks in human. *The Science of the Total Environment*, 915, 170004. <https://doi.org/10.1016/j.scitotenv.2024.170004>