Online Supplement

An Economical Fluorescent Method for Microplastic Detection in Soil Samples

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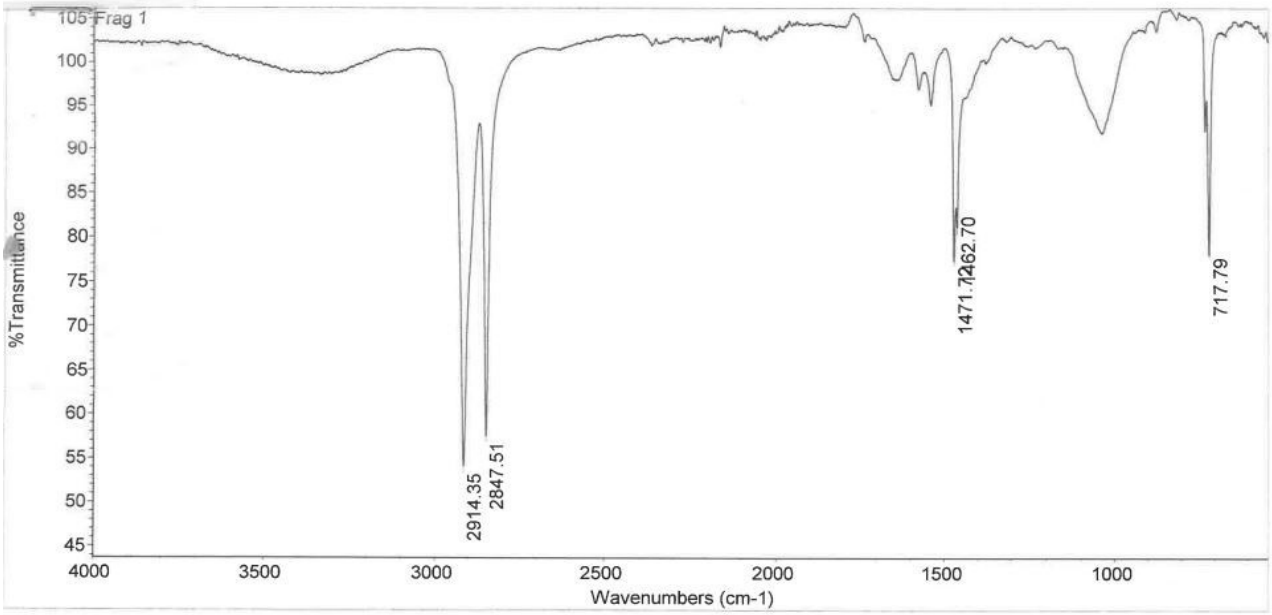
Table S1. Materials, sources, and costs* for the simplified fluorescent microplastic detection method (standard personal protection equipment included for layperson use)

Item	**Price ($)**
Nile Red dye solution, Acetone-Hexane based, 250 mL, 1 mg per mL (CrimeScene, Phoenix, AZ, USA).	47.00
Coarse Brown Playground Sand, 1Kg (Aldon, Innovating Science, Avon, NY, USA) *Not necessary if testing environmental samples	21.99
Blue light torch: 470nm (Blue Light Flashlight Hunting Torch 256 Yard (UltraFire, Shenzhen, China).	24.99
Lithium-ion 18650 rechargeable battery for torch with charger (Tokeyla, Guangzhou, China).	24.89
Orange filter, for images: 529 nm (58mm 21 Filter Orange) (Tiffen, Hauppauge, NY, USA).	29.95
Orange protection glasses for viewing (Uvex S0360X Ultraspec 2000, orange frame) (Honeywell; Fürth, Germany).	16.00
Milligram scale (AWS-100 Digital Pocket Scale, 100g X 0.01g Resolution) (American Weigh Scales; Allendale, Michigan, USA).	13.99
Graduated cylinder, glass, 10 mL (Karter Scientific; Lake Charles, LA, USA).	5.79
Set of 10 glass vials, 10mL (Jiuwu; Pukou, Nanjing, China).	7.99
1 Microliter syringes (BD Insulin syringes, 1 mL), a box of 90 (Franklin Lakes, NJ, USA).	9.90
Micro lab spoon/stainless steel spatula (Labware; Wilmington, Delaware, USA).	5.99
White coffee filter basket, 100-pack (Kroger; Dallas, TX, USA).	1.79
Standard household distilled water, 8 oz bottle (any producer)	~ 1.50
A set of 5 glass jars (typical household jam size, emptied and cleaned with soap and water).	Recycled
Standard laboratory safety glasses (Uvex Honeywell Ultra-spec Safety Glasses, Clear) (Charlotte, North Carolina, USA).	4.75

Standard white laboratory coat (Talvania Unisex) (Amazon; Seattle, Washington, USA).	23.95
Standard plastic gloves, latex-free (Amazon; Seattle, Washington, USA).	7.94
Total costs	**248.41**

Note: Prices as of 9/10/24 (subject to change); alternatives from other companies are available

a)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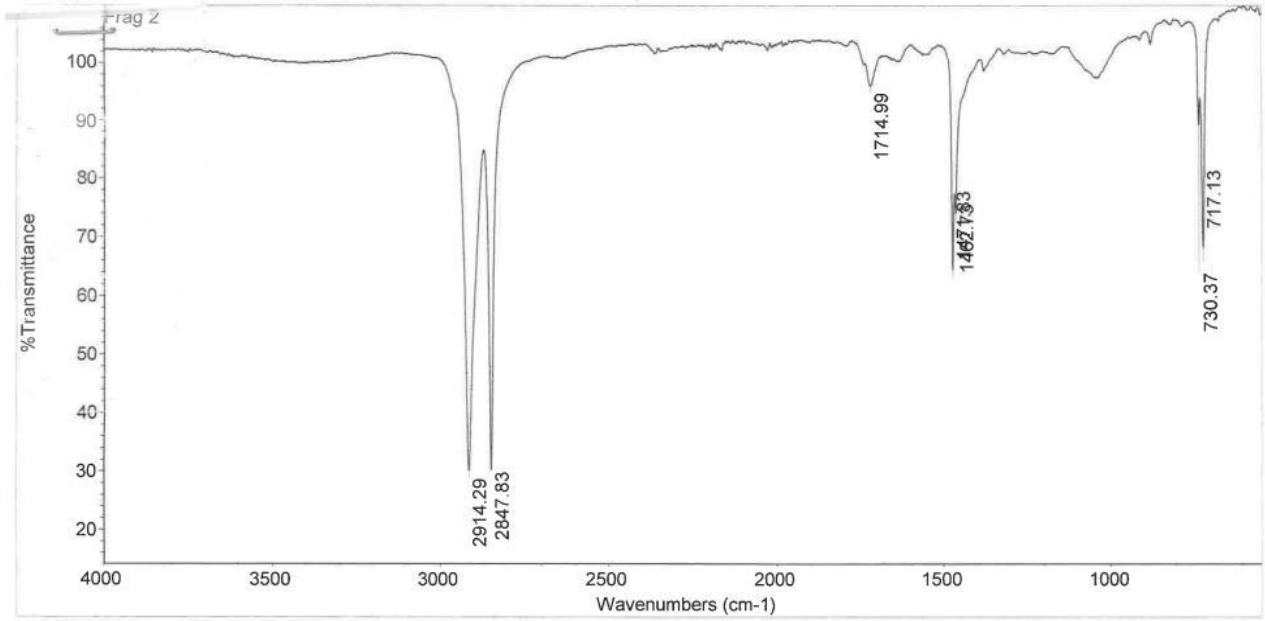
b)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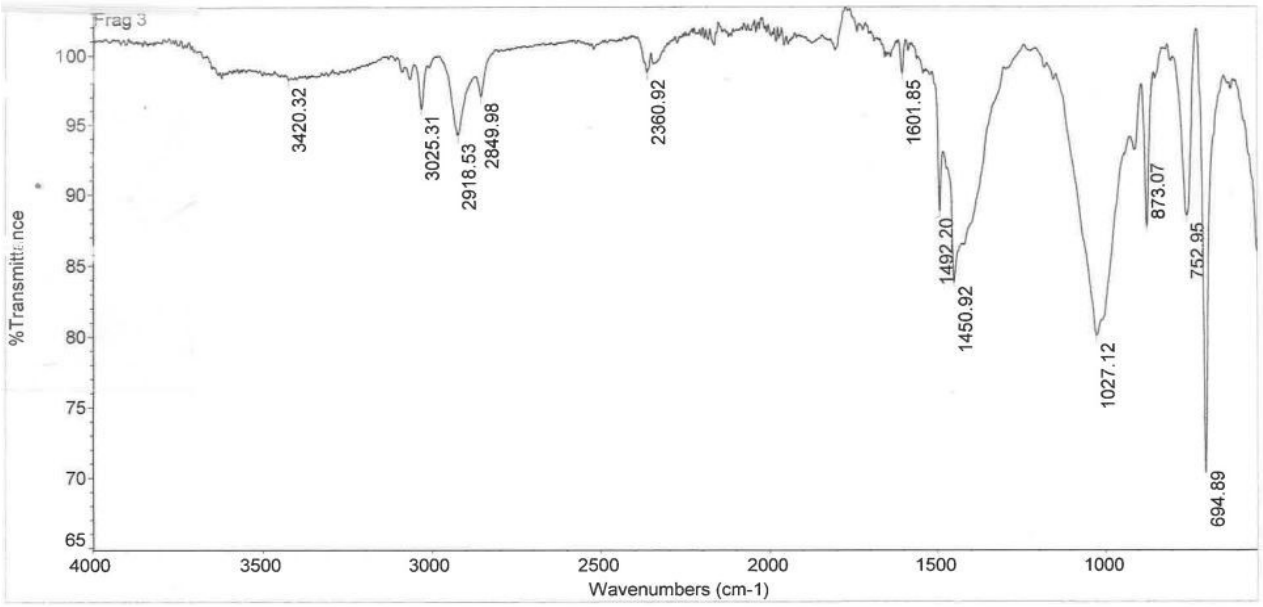
c)



d)

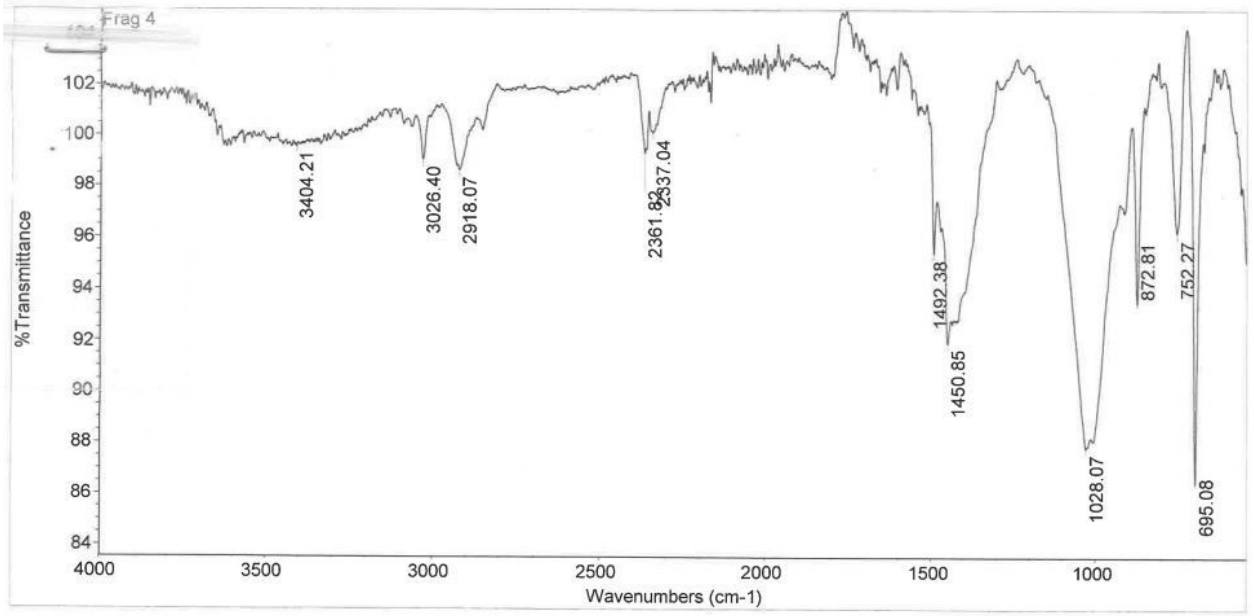


Figure S1: Spectra of four particles analyzed by Fourier-transform infrared spectrophotometry, a, b) polyethylene, and c, d) polystyrene.[1]

Student Protocol for Classroom Setting

Description: Microplastics are extremely small plastic particles (less than 5 mm in size) that result from broken-up plastic products and waste. They can be found across the globe and pose a threat to the health of plants, animals, and humans. Because of their size, which is largely invisible to the naked eye, it is hard to visualize them in our soil, water, and air. In this experiment, you will test soil samples that you have collected in your environment for the number of microplastics that contaminate them. You will use what is called a fluorescent method, in which you first treat your sample with a dye, then let it dry, and then examine it with a blue light torch while wearing orange glasses. This makes the microplastics that are otherwise undetected appear as glowing orange particles.

You can work in groups of two or three experimenters to assist each other when you complete each step, especially when shining a light on the sample, taking pictures, and counting the particles (always let two people count and then compare the numbers you come up with).

Materials you will use: Nile Red dye solution, blue light torch, orange filter for images, orange protection glasses for viewing, milligram scale, 10-ml graduated cylinder, 10 ml glass vials, 1 microliter syringes; micro lab spoon/stainless steel spatula, white coffee filter basket, glass jars, and distilled water. Consult Table S1 for a full list of materials.

Procedure:

*This is for one sample. Repeat the steps for more than one vial (3+ vials can be prepared at once, depending on the number of experimenters).

1. On a milligram scale, weigh ½ gram of sediment on a sheet of paper and transfer it into a 10 mL glass vial
2. Add 2.5 mL of distilled water to the vial using a graduated cylinder
3. Add 25 µL of Nile Red dye to the vial with a microliter syringe
4. Incubate the vial for 30 minutes, shake every 5 minutes for 1 minute
5. Dispose the sample onto a filter paper suspended over the opening of a glass jar and secured with a rubber band. Wash out any remaining analyte in the vial with additional water onto the filter paper
6. Let fluid content seep through filter paper and air dry (may take several hours)
7. Remove filter paper from the jar, spread it out on a table, and fix it with adhesive tape.
8. Illuminate the filter paper with a blue light torch while wearing orange glasses (take pictures by holding an orange filter in front of a cellphone lens).
9. For large particles, visually count fluorescent orange spots across the entire spread-out sample. For smaller particles, place a 16 x 16 cm grid with 4 cm square fields over the filter paper. Count all visible small particles across each of the 16 square fields.

Educator Instructions and Protocol for Classroom Setting

For instructors planning to implement this experiment as a group activity in a classroom setting. Instruct students to get into groups of 2+ students. This experiment may take more than an hour to complete with preparation and sample incubation. Shorter incubation times may also yield adequate results. For air-drying of samples, several hours of additional time needs to be budgeted, which may necessitate the deferral of visual inspection and analysis to the next school day. However, analysis of wet samples on filter paper can also be attempted. For visual inspection, students should be instructed in the independent rater method.

Safety instructions:

- Always ensure that students wear gloves, a lab coat, and safety glasses. All experimentation should be undertaken in a well-ventilated area.
- Nile Red dye is not considered dangerous if handled correctly. However, the acetone-hexane solvent is classified as a hazardous substance and has the potential to irritate skin and eyes with prolonged or concentrated contact. Consult safety data sheet for more information on how to handle and dispose of the substance. Use standard hazardous waste disposal procedures for analyzed samples and filter papers.

  Microliter syringes are not advised for students under high school age and can pose a major safety concern if not handled properly. Alternatives are low-cost glass micropipettes.

Materials and costs: Please consult *Table S1*.

Procedure:

*This is for one sample. Repeat the steps for more than one vial (3+ vials can be prepared at once, depending on the number of experimenters).

1. On a milligram scale, weigh ½ gram of sediment on a sheet of paper and transfer it into a 10 mL glass vial.
2. Add 2.5 mL of distilled water to the vial using a graduated cylinder
3. Add 25 µL of Nile Red dye to the vial with a microliter syringe
4. Incubate the vial for 30 minutes, shake every 5 minutes for 1 minute *(as a potentially more time-saving version, incubation times of 10 min with 2 x agitation for 1 min have also shown good results in detection of manually embedded particles)*
5. Dispose the sample onto a filter paper suspended over the opening of a glass jar and secured with a rubber band. Wash out any remaining analyte in the vial with additional water onto the filter paper
6. Let fluid content seep through filter paper *(note that time for passage through the filter paper depends on structure of the analyte)* and air-dry filter paper *(drying may take several hours, but note that drying may be skipped and step 8 already attempted with wet sample on filter paper)*
7. Remove filter paper from the jar, lay it out on a table, spread it out, and fix it with adhesive tape.
8. Illuminate the filter paper with a blue light torch while wearing orange glasses (take pictures by holding an orange filter in front of a cellphone lens).
9. For large particles, visually count fluorescent orange spots across the entire analyte spread. For smaller particles, place a 16 x 16 cm grid (fabricated from string or acquired as transparent

millimeter paper) with 4 cm square fields over the filter paper. Count all visible small particles across each of the 16 square fields.

Reference

1. Veerasingam S, Ranjani M, Venkatachalapathy R, Bagaev A, Mukhanov V, Litvinyuk D, Mugilarasan M, Gurumoorthi K, Guganathan L, Aboobacker VM, Vethamony P. Contributions of Fourier transform infrared spectroscopy in microplastic pollution research: A review. Crit Rev Environ Sci Technol. 2021;51(22):2681-743. doi:10.1080/10643389.2020.1807450.