An Economic Fluorescent Method for Microplastics Detection in Soil Samples

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Abstract

Microplastics from urban and industrial waste are threatening ecosystems worldwide. Quantification methods for soil samples have been proposed but typically require complex and expensive laboratory procedures, which are not accessible to the public. Therefore, we developed a simplified Nile red fluorescent dye method with low-budget materials that can be readily used as lone-standing demonstrations or implemented in environmental education modules. The method was validated on commercial coarse-grain sand spiked with microplastics (1-5 mm). Following incubation with Nile red dye, the analytes were visually inspected using blue light and orange filter glasses and counted by two independent masked assessors. Detection of particles was close to 100 percent. Four different types of environmental analytes were subsequently tested with this method: urban lake shore sediment, agricultural soil, gardening soil, and soil from a state park. Urban lake shore and garden soil samples showed the highest density of microplastic particles. Large numbers of smaller particles (<1 mm) were also identified and counted in these analytes, with very good reproducibility by the same assessor and replication of the rank order of analytes between two assessors. Visualizing microplastic pollution with this low-cost, scalable method can reach broad sections of educational settings and the broader public and thus raise awareness of the problem of microplastic pollution.

Keywords: Microplastic detection, soil, sediment, fluorescent dye method, low-cost

Introduction

Microplastics are extremely small plastic particles, ranging from 0.001 to 5 mm, which are found in the environment resulting from the breakdown of industrial waste and products (Hale et al., 2020). Pollution from microplastics is widespread and has attracted much scientific attention in recent years. Environmental analyses have discovered the small particles globally across varying environmental compartments and matrices, from the Mariana Trench to human placentas (e.g., Akdogan & Guven, 2020; Horton et al., 2017; Jamieson et al., 2019; Ragusa et al., 2021; Zhang & Liu, 2018). Owing to their chemical properties, microplastics release additives and absorb chemical pollutants, threatening ecosystems through bioaccumulation across the food chain (Horton et al., 2017; Miller et al., 2020). Microplastics can also access the living organism through the consumption of contaminated food. The average person ingests an estimated 50,000 microplastics per year. As microplastics break down into smaller particles through digestion, they can penetrate deeper layers of tissue, increasing the risk of gut and DNA dysfunction, inflammation, oxidative stress, and metabolic disorder (Das, 2023; Jin et al., 2018; Prata et al., 2020; Qiao et al., 2019; Wang et al., 2020).

In light of the escalating world plastic production and subsequent pollution, there is an urgent need to inform the greater public about the overlooked threat of these pollutants. While the study of marine microplastics has made progress in recent years, continental, including soil pollution with microplastics, has been lagging (Akdogan & Guven, 2019; Guo et al., 2020). Introduction of microplastics into the soil can originate from multiple sources, including agricultural and gardening activities (sewage, compost, mulch), garbage disposal and landfills, and atmospheric deposition (Guo et al., 2020; Yang et al., 2021). Once infiltrated, they are transported through the soil by ways of water infiltration, plant processes, animal activities, or perturbation by human agricultural, gardening, and building activities, and eventually enter the food chain (Dissanayake et al., 2022;

Guo et al., 2020). Studies testing polyacrylic fibers, polyamide beads, polyester fibers, and polyethylene fragments have demonstrated adverse effects on soil properties, including the increase in pH, the reduction in water holding capacity and bulk density, as well as the inhibition of microbial activity, all of which impact soil fertility (de Souza et al., 2018; 2019). However, soil integrity plays a vital role for living organisms by providing an array of ecosystem services, including nourishment for vegetation, habitat for animal species, and human agricultural, industrial, and medicinal activities.

Methods for detecting microplastics in soil have been proposed but typically involve laboratory equipment and procedures (Bläsing & Amelung, 2018; Coppock et al., 2017; Wohlschläger et al., 2024), which are cost- and labor-intensive and not publicly accessible. Therefore, it is essential to devise simpler methods for instructors in educational settings, low-budget scientists, and consumers to test soil samples for microplastic contamination. We, therefore, sought to devise a low-cost, scalable method for the detection of microplastic particles that could be readily used in educational and other daily life settings to raise awareness among educational settings and consumers about the ubiquity of this pollutant. For this purpose, we adapted a previously reported fluorescent method using Nile red dye (Erni-Cassola et al., 2017; Maes et al., 2017) and tested the performance on standard laboratory coarse-grain sand and a range of environmental soil and sediment samples contaminated with microplastics. We expected reliable performance of this method in detecting both systematically and naturally introduced particles in double-masked experiments, thus demonstrating the usefulness of this simplified method for daily life educational purposes. For the environmental samples, it was expected that those from the urban lake shore would be the most contaminated due to plastic trash carried by stormwater and runoff from roadways. In contrast, state park soil would be least contaminated given the distance from human settlements and activities.

Materials and Methods

Experimental design

The study included two phases. In the first validation phase, microplastic particles were embedded in standard coarse-grain sand in six trials, three of these unmasked and three masked. In the second phase, four different soil types were analyzed, with three samples each for spontaneously occurring microplastic contamination by two independent raters.

Instruments and materials

Instruments and materials were sourced with a focus on low-cost solutions that were freely available from standard stores, companies, and internet marketplaces. Table 1 provides a list of all materials used as part of the study for practical guidance. The Online Supplement (Table S1) provides approximate prices for all items as practical guidance (note that only estimates are provided that are current as of 9/15/2024, naturally, variation over time can be expected).

General procedure

The procedure was a simplification of fluorescent staining protocols that have been described before (Erin-Cassola et al., 2017; Maes et al., 2017). For an overview of the procedures, see the flowdiagram in Figure 1; for a full list of materials and producers, see *Supplement*. The desired analyte quantity, 1 gram for initial validation experiments with standard coarse-grain sand (0.5-1 mm, Innovating Science), ½ gram for environmental soil samples, was weighed on a milligram scale and transferred into a 10 mL glass vial, with 2.5 or 5 mL of water added. For initial validation experiments, small plastic particles (<5 mm) were scraped off a plastic bottle cap with a sharp knife and embedded with a laboratory spoon/stainless steel spatula in the coarse-grain sand. Using a disposable 1 mL syringe, 25 or 50 µL of Nile Red dye (acetone-hexane based, CrimeScene, Phoenix, AZ, USA) was then added. The sample was

then incubated for 30 minutes and shaken by hand for 1 minute every 5th min during that time. After that, the sample was disposed onto a filter paper (white household coffee filter, any company) that was suspended over the opening of a household glass jar and secured with a rubber band. Any remaining samples in the vials were washed out with additional water over the filter paper. After all water had passed through it, the filter paper was dried in room air for as long as necessary (typically multiple hours). The filter paper was then removed from the jar and laid out on a table, carefully stretched, and fixated with adhesive tape. The paper was then illuminated with a blue light torch (470 nm wavelength, UltraFire Blue Light Flashlight Hunting Torch 256 Yard; UltraFire, Shenzhen, China) and visually inspected by the experimenter while wearing orange glasses (Uvex S0360X Ultra-spec 2000, orange frame; Honeywell, Fürth, Germany). Images were taken by holding an orange filter lens (529 nm, Tiffen 58mm 21 Filter Orange., Hauppauge, NY, USA) in front of the lens of a standard cell phone. Large particles were counted across the complete content of the analyte spread out over filter paper. Small particles were counted with the help of a 16 x 16 cm grid of 4 cm square fields hand-fashioned from thin thread (0.3mm thickness). Fluorescent orange spots were visually identified and counted up across all 16 square fields. Standard lab coats and safety glasses are to be worn during all procedures. A random selection of four larger particles (1-5 mm) was also analyzed with a Fourier-transform infrared spectrophotometer to verify the identity of the particle material.

Table 1. Supply information on experimental items

Item
Nile Red dye solution, Acetone-Hexane based, 250 mL, 1 mg per mL (CrimeScene, Phoenix, AZ, USA).
Coarse Brown Playground Sand, 1Kg (Aldon, Innovating Science, Avon, NY, USA)
Blue light torch: 470nm (Blue Light Flashlight Hunting Torch 256 Yard (UltraFire, Shenzhen, China).
Lithium-ion 18650 rechargeable battery for torch, with charger (Tokeyla, Guangzhou, China).
Orange filter, for images: 529 nm (58mm 21 Filter Orange) (Tiffen, Hauppauge, NY, USA).

Orange protection glasses for viewing (Uvex S0360X Ultra-spec 2000, orange frame) (Honeywell; Fürth, Germany).
Milligram scale (AWS-100 Digital Pocket Scale, 100g X 0.01g Resolution) (American Weigh Scales; Allendale, Michigan, USA).
Graduated cylinder, glass, 10 mL (Karter Scientific; Lake Charles, LA, USA).
Set of 10 glass vials, 10mL (Jiuwu; Pukou, Nanjing, China).
1 Microliter syringes (BD Insulin syringes, 1 mL), a box of 90 (Franklin Lakes, NJ, USA).
Micro lab spoon/stainless steel spatula (Labware; Wilmington, Delaware, USA).
White coffee filter basket, 100-pack (Kroger; Dallas, TX, USA).
A set of 5 glass jars (typical household jam size, emptied and cleaned with soap and water).
Standard laboratory safety glasses (Uvex Honeywell Ultra-spec Safety Glasses, Clear) (Charlotte, North Carolina, USA).
Standard white laboratory coat (Talvania Unisex) (Amazon; Seattle, Washington, USA).
Standard plastic gloves, latex-free (Amazon; Seattle, Washington, USA).

Experimental design and analytes

Six samples of 1 mg of commercial coarse-grain sand were analyzed for initial validation. Three of these were spiked with 10 microplastic particles each by the experimenter and, following the staining protocol, visually inspected and counted by the experimenter as unmasked rater. An additional three samples were spiked with 4, 7, and 9 microplastic particles by the experimenter and visually inspected and counted by a masked assessor. One control trial was also performed with 1 mg of sand and no particles embedded. For the subsequent soil analysis, 4 small samples were sourced from different environments: (1) agricultural soil from a seasonally uncultivated field in West Texas; (2) roadside soil from a US state park sampled with the permission of a park ranger on duty, (3) commercial gardening soil (Miracle-Gro, Ohio, USA), and (4) dried sediment from an urban lake shore. Three 0.5 mg analytes were extracted from the environmental samples and analyzed. The analytes were examined visually by two independent assessors following the staining protocol. For small particles, one independent assessor performed the visual count twice. Large particles (> 0.5 mm) were counted without magnification, while smaller fluorescent spots (>0.1 mm) were counted using a 7 x magnifying glass. Since smaller fluorescent spots were also embedded in the coffee filter paper, a control filter was treated with 50 µL of Nile Red diluted

in 50 mL of water without analyte, and fluorescent spots were counted with the magnifying glass after drying. These control counts were then subtracted from the count of small particles.

Analytic strategy

Frequency counts were obtained and plotted in bar graphs for qualitative characterization of findings.

Results

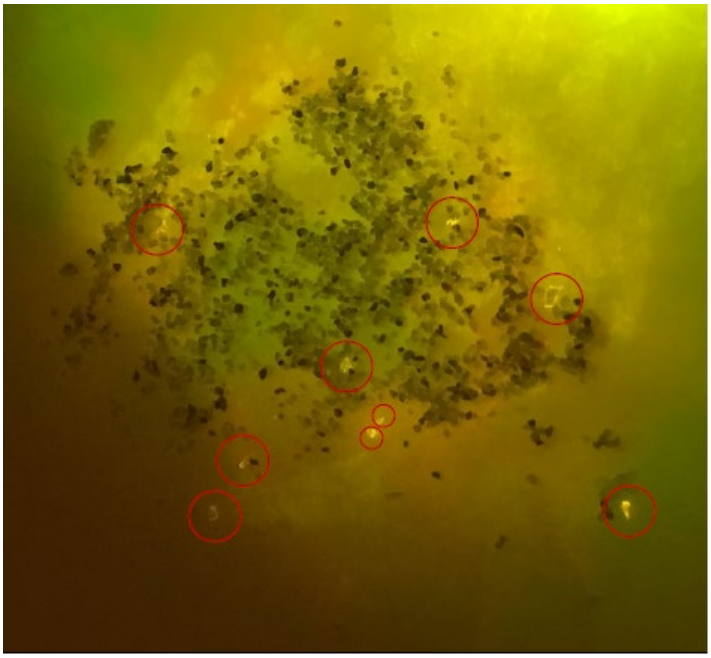
Validation experiment

In all three unmasked validation trials, the method successfully detected the ten microplastic particles embedded in the sediment (Table 1). The three masked validation experiments also demonstrated that all microplastics were identified and recovered except for one in one of the trials.

Table 2. Method validation for detection of microplastic particles in commercial coarse grain granulates

Experiment	Binding	Microplastics embedded	Microplastics detected	Microplastics recovered
1	unmasked	10	10	10
2	unmasked	10	10	10
3	unmasked	10	10	10
4	masked	7	6	6
5	masked	9	9	9
6	masked	4	4	4

Figure 2a shows trial 5, in which the masked assessor readily detected 9 particles. No particles were identified in the masked control trial (Figure 2b).



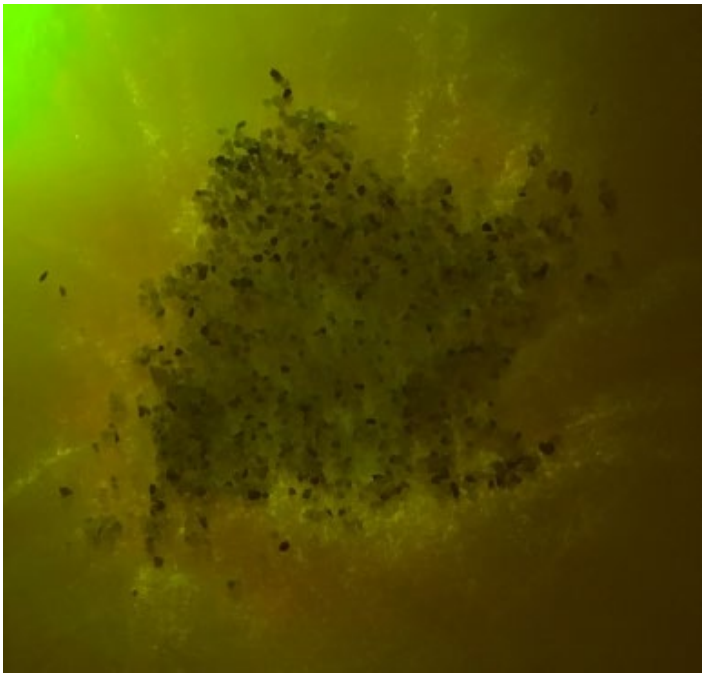


Figure 2. Example of a) an image of a validation trial with 9 identified microplastic pieces embedded in commercial coarse grain sand, and b) a control sample of sand without embedded microplastics

Environmental sample analysis

Microplastic frequencies varied substantially between sampling sites. Among all the samples, sediment from the urban lakeshore were the most contaminated with microplastics. The counts of small and large particles are shown in Figure 3. Small particles were widespread in both the urban lakeshore sediment and the other samples, adding up to a total of 46 particles across three trials. The agricultural soil also showed considerable contamination with large particles, followed closely by commercial gardening soil, which also had a particularly high number of small particles. The least contaminated samples were from the state park roadside soil. Fourier-transform infrared spectrophotometry identified two of the randomly selected particles as polyethylene and two as polystyrene according to commonly reported spectral peaks (Veerasingam et al., 2021) (see Figure S1 in Supplement for full spectra).

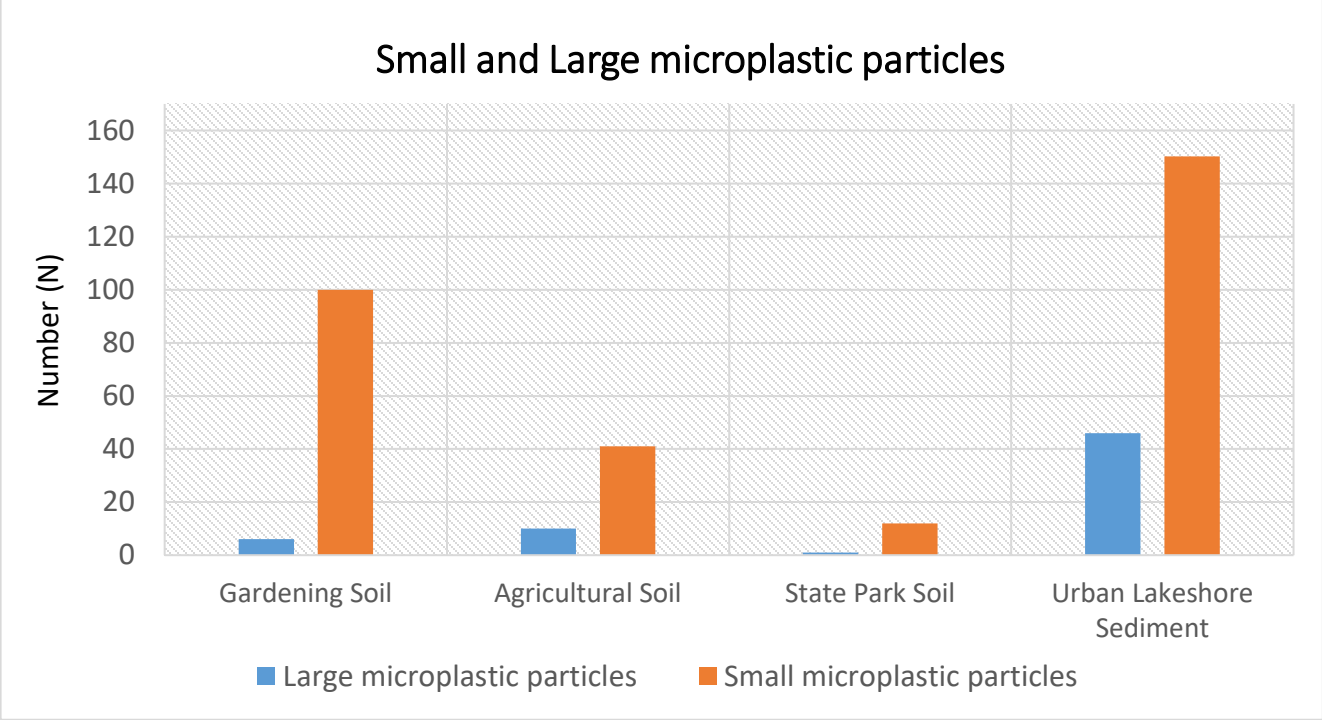
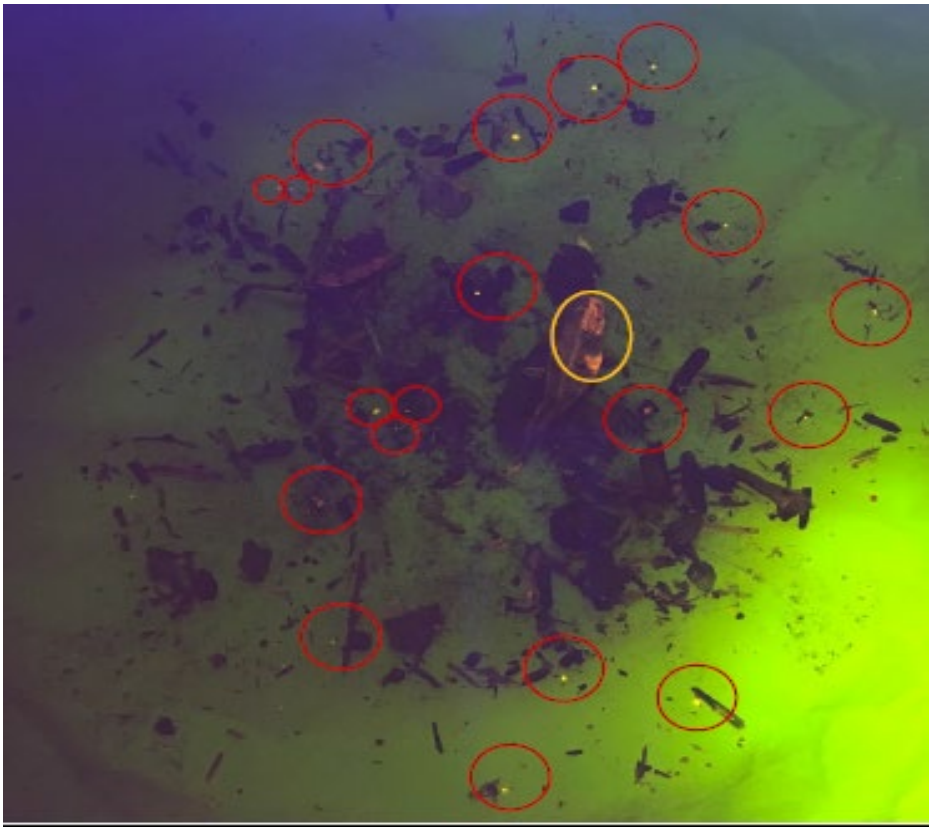
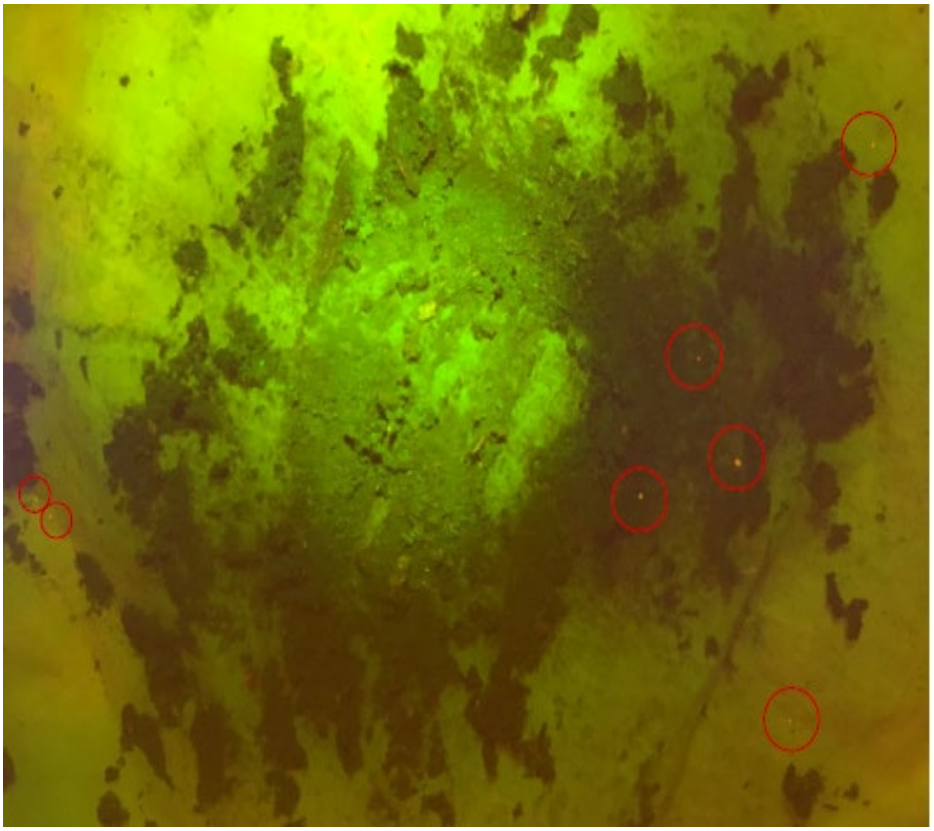


Figure 3. Total number of small and large microplastic particles across four environmental sampling sites with three analytical trials each (for small particles minus control filter only)

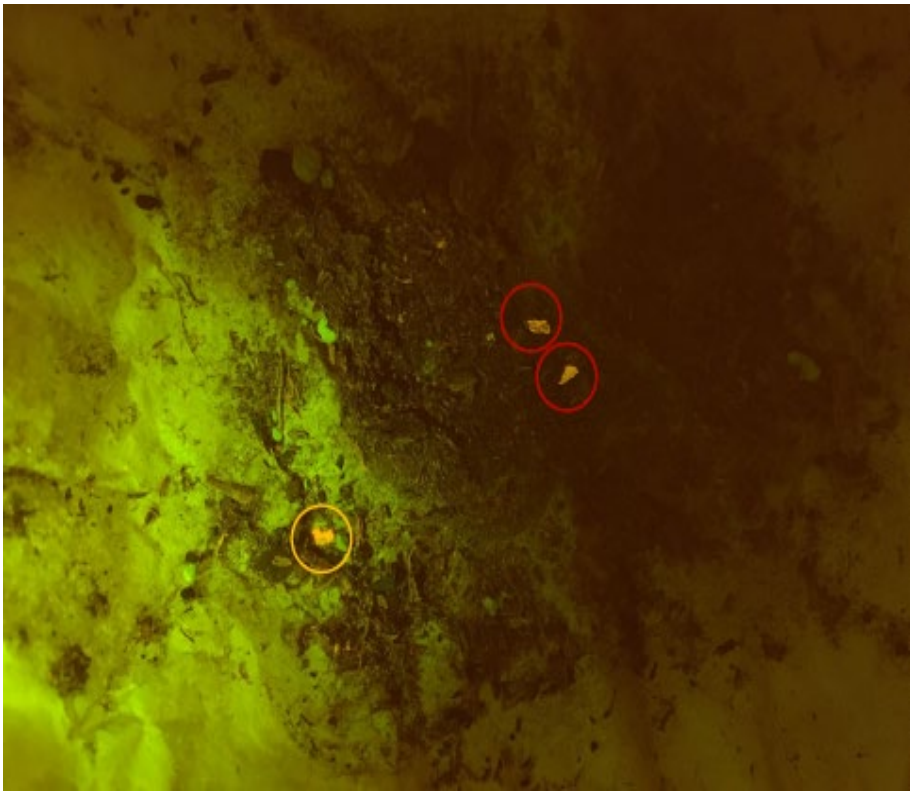
Figure 4 a-d shows examples of identified particles from the four sites.



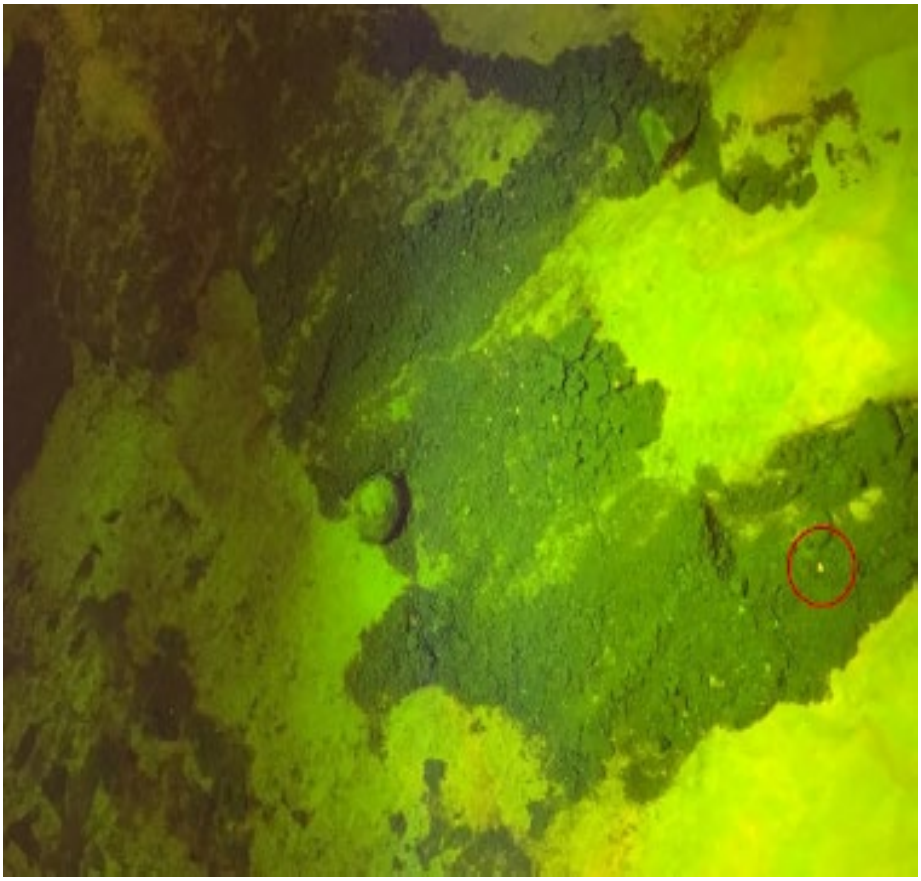
a)



b)



c)



d)

Figure 4. Examples of particles from the four sites: a) urban lakeshore sediment, b) agricultural soil, c) commercial garden soil, and d) state park soil

The two assessors were in agreement over the rank order of sampling sites for small particles, although they varied somewhat in the absolute number of particles counted (Figure 5). The repeated count of Assessor 1 yielded almost identical numbers.

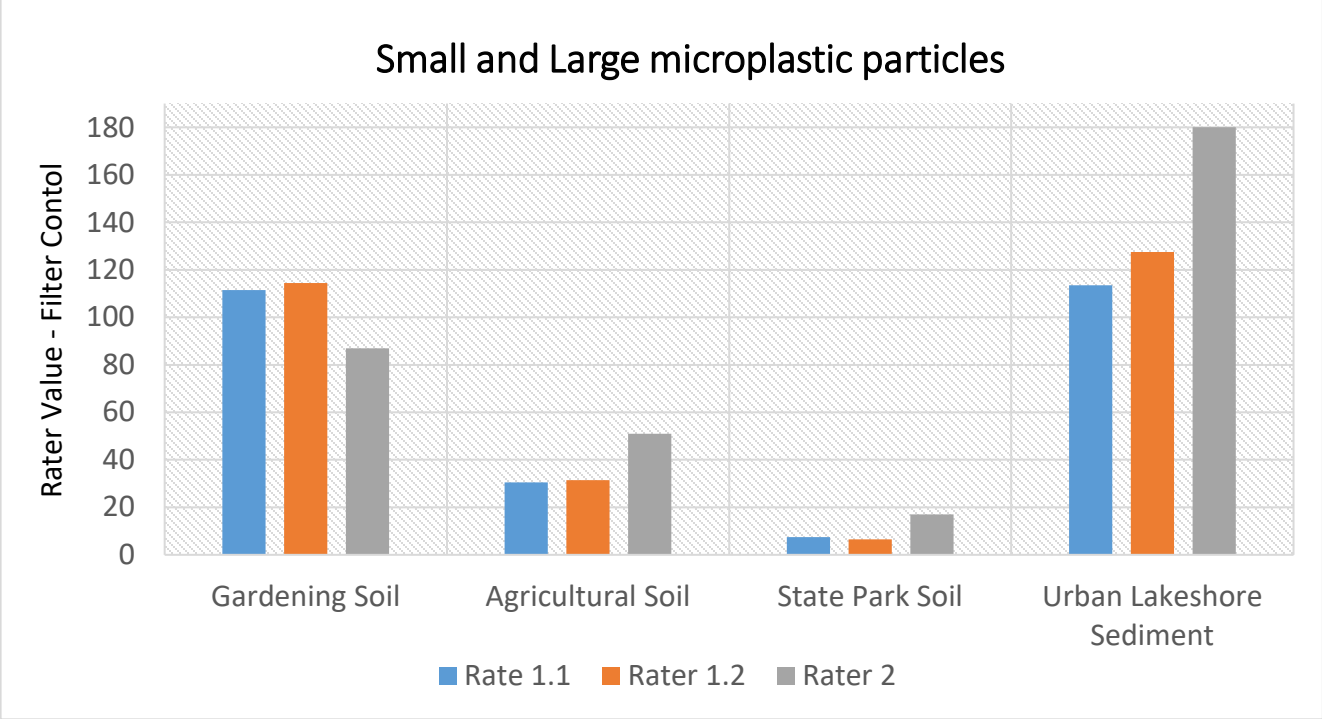


Figure 5: Number of small microplastic particles identified visually by two independent raters (one with two counts) in four different soil or sediment types (totals of three independent samples are shown)

Discussion

Our findings show that our simplified fluorescent method successfully detects microplastic particles in laboratory coarse-grain sand samples with high interrater reliability. It also readily detects microplastic pollution in samples sourced from the environment, meeting our expectations on

performance and confirming predicted pollution levels. The low costs of the method and the simplicity of its application make it ideally suited for an experimental module on microplastic detection in a curriculum of environmental education, starting as early as middle school students. Given the rising awareness of the critical importance of this long-neglected pollutant, simple and vivid demonstrations such as these are ideally suited for instructing students about the hazards of plastic waste and its widespread impact on the environment.

The almost perfect-match identification of experimentally embedded microplastic particles in standard coarse-grain sand provided the clearest demonstration of the high reproducibility of findings with this method. Given its predictability, it could be used as a first step of instructions in a microplastic teaching module, with multiple students participating in the preparation of standard samples by embedding self-produced plastic particles in the matrix, practicing the staining procedure, and then taking turns in masked and independent ratings of the level of particle pollution using orange glasses and the blue light torch. While larger microplastic particles will generate the most vivid demonstrations, our method also shows that smaller particles < 1 mm can be counted with the use of a simple 7 x magnifying glass. Although absolute levels of such smaller particles will likely vary between assessors due to varying personal criteria, our results show that the rank order of naturally occurring pollution levels in various environmental samples can be replicated, and replication within one assessor is high.

Our analysis of environmental soils and sediments confirmed the expectations regarding levels of microplastic pollution. Urban lake shore sediment showed the highest pollution levels, whereas state park soil showed the lowest level. Growing urbanization with unregulated plastic use and waste deposition can threaten freshwater species when microplastic particles are ingested. In freshwater fish, chemicals released from ingested microplastic particles alter immune and hormonal function, generate oxidative stress, and lead to organ and tissue damage (Parker et al., 2021). Commercial garden soil, and

to a certain extent, samples of agricultural soil, also showed substantial levels of pollution. Randomly selected particles from these sample types analyzed by Fourier Transform Infrared Spectrophotometer confirmed their nature as either polyethylene or polystyrene. The contamination of commercial and agricultural soils results mainly from two factors, one being fertilizers (ammonia, nitrate) that are polymer-coating for controlled release, and the other one the pervasive practice of mulching with polystyrene, resulting from long-held lay beliefs that this helps soil aeration and drainage. However, a variety of adverse effects of this practice have been shown more recently, including inhibition of water and nutrient uptake, compromise of plant growth, metabolic, hormonal, and antioxidant activity, cytotoxicity and genotoxicity, and disruption of soil enzyme activity and microbial communities (Iqbal et al., 2023; Jia et al., 2023; Lian et al., 2024; Liu et al., 2024).

Our analysis of environmental samples was not aimed at generating insights into such effects, nor was it planned to be a representative survey of specific environments or locations. Instead, its purpose was a demonstration of how the simplified technique can be applied to samples of soils and sediments in the environment. By analyzing collected samples from their home and neighborhood environments, students can thereby link the acquired laboratory technique with real-life applications and thus integrate the learned knowledge into a broader perspective of environmental preservation and threats to it.

Schools and universities have recently begun to incorporate learning modules on microplastic pollution (Rowe et al., 2018). In this effort, including illustrative hands-on activities and out-of-school learning sites can greatly support learning goals (Raab & Bogner, 2021). Our detection method is ideally suited to serve this purpose by providing a visually appealing demonstration. Evidence shows that experimental demonstrations of microplastic pollution are the one element of such learning modules that is most highly valued by students (Hogan & Urban-Rich, 2024). While some curricula are still missing such hands-on elements, others have incorporated more complicated experimental protocols of particle

separation or techniques that require expensive equipment set-ups such as fluorescent microscopy or spectroscopy methods (e.g., Collier et al., 2023; Forakis et al., 2024; Majcen et al., 2023; Scircle & Cizdziel, 2020). More invested methods have the advantage of teaching laboratory methods to advanced students who have an academic interest in relevant disciplines of the natural sciences.

On the other hand, our adaptation of the fluorescent dye method can target a wider section of the student population as it is comparably simple and cost-effective by not requiring expensive equipment, which makes it highly scalable. It can also address a wider audience as it captures students' attention effectively with appealing visuals. Despite its simplicity, our findings show that the method generates highly reproducible results. At this level, experiments can even be replicated by students in their home environment when packaged into small laboratory kits. Beyond that, such simple preparations may also be employed in public demonstrations at environmental fairs and by large-scale citizen science projects or consumers interested in examining potential pollution of their environment, thereby fostering a broader awareness of the problem of plastic pollution.

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