Enhancing Microplastic Transport Research in Agricultural Soils through Fluorescent Particles: A Simplified Method for Detection and Quantification

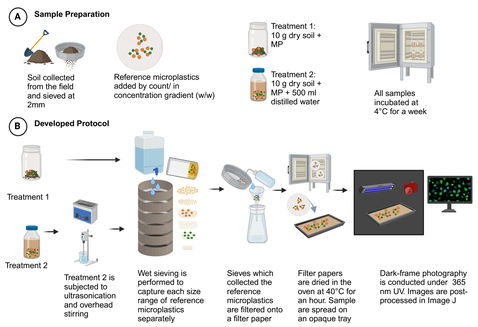
Saunak Sinha Ray*, Tomas Dostal, David Zumr
Czech Technical University in Prague, Department of Landscape and Water Conservation, Prague 16629, Czech Republic.
*Corresponding author: (saunak.sinha.ray@fsv.cvut.cz)

INTRODUCTION

- Fluorescent microplastics are widely used in studying microplastics' (MP) fate, transport, and deposition, but their identification process is still time-consuming and equipment-dependent.
- This work highlights a rapid, reliable protocol for identifying and counting microplastics within soil/ sediment samples without extraction, showcasing a non-destructive processing with precise recovery.
- In this study, we assessed fluorescent PE and Polylactic acid (PLA) MP particles, focusing on (1) the identification of MP particles without extraction, (2) quantification of count and size of particles, and (3) validation of the protocol recovery.

METHODS

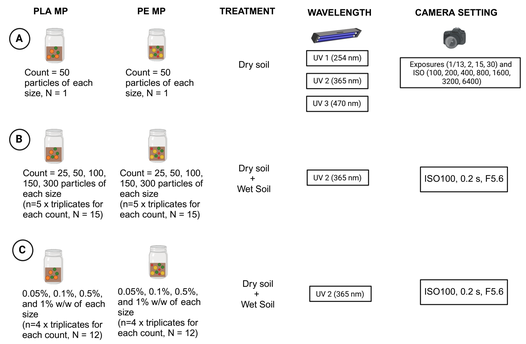
- Fluorescent PE microspheres in 125-150 µm (green) and 425-500 µm (red) colors were sourced from Cospheric (Santa Barbara, USA), with densities of 1 and 1.09 g/cm^3.
- Fluorescent PLA MP particles in 125-150 µm (orange) and 250-300 µm (green), with a density of 1.24 g/cm^3, were obtained by cryo-milling and dry sieving commercially available 1.75 mm fluorescent PLA filament.
- The MP detection protocol involved two treatments: Treatment 1 simulated induced MP in topsoil by mixing 10 g of dry soil with known amounts of PE and/or PLA particles in a glass jar, adjusting the moisture to 20%, while Treatment 2 replicated eroded sediment by adding 500 ml of distilled water to a similar mixture, both incubated at 4°C for a week to incorporate microplastics into the soil matrices.



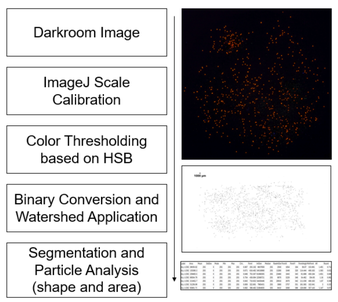
We primarily used wet sieving to separate fluorescent microplastics from samples in both treatments and aligned sieve diameters with fluorescent PE and PLA MP sizes, using extra sieves to exclude larger soil and organic matter particles, capturing induced microplastics and soil particles in the same size range and reducing initial sample volumes significantly.

METHODS

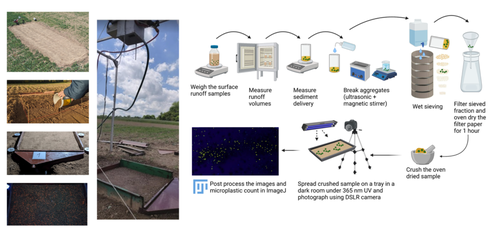
Framework of the tests conducted to develop the protocol - initial test with reference microplastics to identify optimal wavelength and camera settings (A), and validation of reference microplastics by count (B), and by w/w (C)



For the first validation, we calculated true positives (TP), false positives (FP), and false negatives (FN) based on the actual class identified in each photographed sample. In the second validation, particle area relative to mass was analyzed, and regression analysis was performed for two equations: (i) weight (PLA and PE) versus observed total particle count, and (ii) weight (PLA and PE) versus total particle count observed in post-processed images using Image J.



Schematic of automatic detection of the fluorescent MP in an image.



Application of the protocol to an agricultural plot-scale case study - Investigation was conducted on five plots (1m x 1m) subjected to rainfall simulations at an intensity of 60 mm h^{-1}, involved the addition of 7.1 g m^{-2} of fine (size 125-150 µm) and coarse (size 425-500 µm) fluorescent polyethylene to the topsoil (1 cm).

RESULTS - METHODOLOGY

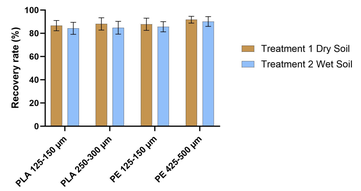
Polymer Type	Size (µm)	Precision (mean ± STD)		Recall (mean ± STD)		F-Score (mean ± STD)	
		Treatment 1	Treatment 2	Treatment 1	Treatment 2	Treatment 1	Treatment 2
PLA	125 – 150	0.94 ± 0.12	0.95 ± 0.07	0.85 ± 0.04	0.83 ± 0.04	0.89 ± 0.07	0.89 ± 0.05
	250 – 300	0.95 ± 0.05	0.92 ± 0.1	0.85 ± 0.04	0.84 ± 0.04	0.90 ± 0.04	0.88 ± 0.06
PE	125 – 150	0.96 ± 0.04	0.94 ± 0.08	0.85 ± 0.03	0.85 ± 0.04	0.90 ± 0.03	0.89 ± 0.05
	425 – 500	0.96 ± 0.06	0.97 ± 0.03	0.91 ± 0.04	0.90 ± 0.05	0.93 ± 0.04	0.93 ± 0.03

Table 1 The precision, recall, and F-score values for the reference polymers in different treatments from Experiment 1

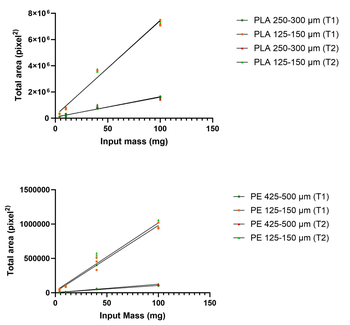
Microplastic Added (count)	Recovery (%, count) (mean ± STD)			
	Treatment 1			
	PLA 125 – 150 µm	PLA 250 – 300 µm	PE 125 – 150 µm	PE 425 – 500 µm
25	81.3 ± 4.6a	80 ± 4b	82.7 ± 2.3a	86.7 ± 2.3c
50	81.3 ± 1.2a	82.7 ± 3.1b	81.3 ± 1.2a	91.3 ± 1.2c
100	86.7 ± 4.2a	86.7 ±1.5b	86.3 ± 1.2a	90 ± 1c
150	86.9 ± 1.7a	86.9 ± 1.7b	97.8 ± 1.9a	91.1 ± 1c
300	86.8 ± 1.7a	87.4 ±1.1b	86.2 ± 0.4a	96.8 ± 0.5c
	Treatment 2			
25	78.7 ± 4.6a	81.3 ± 2.3b	82.7 ± 2.3a	84 ± 4c
50	83.3 ± 2.3a	80.7 ± 3.1b	79.3 ± 1.2a	90 ± 4c
100	83 ± 3a	82.3 ±2.5b	86.7 ± 1.5a	89.3 ± 2.5c
150	84.4 ± 3.2a	87.3 ± 2.7b	86.4 ± 1a	91.8 ± 1c
300	86.3 ± 1.9a	89.1 ±1.1b	87.9 ± 2.4a	95.6 ± 0.5c

Values followed by the same letter within the same rows are not significantly different by LSD post-hoc test ($p \leq 0.05$)

Table 2 Recovery of the reference polymers in different treatments from Experiment 1

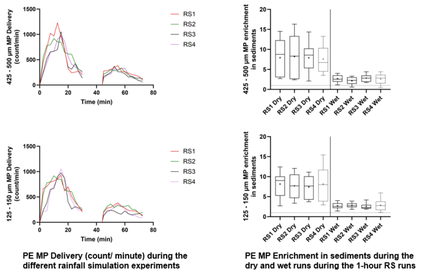


Mean recovery rates of microplastics decreased slightly with decreasing particle size ($P < 0.0005$, one-way ANOVA), showing significant differences between 125 -150 and 425 - 500 µm particles, as well as between 250 – 300 and 425 - 500 µm particles ($P < 0.05$ Fishers LSD), likely due to increased chances of losing smaller particles during processing, resulting in average losses of 17.4 ± 12.9, 16.8 ± 12, and 8.1 ± 3.9 particles for 125 – 150, 250 – 300, and 425 – 500 µm MP fractions, respectively, with no significant differences found between mean recovery rates of different fluorescent polymer types in the size range of 125-150 µm particles, nor between the two treatments.



The initial input concentration of MP correlated with the total area of particles for both PE and PLA size fractions, with observed regression coefficients (R2, $p < 0.01$) exceeding 95% from both treatments, suggesting that the regression models can serve as an alternative to estimate the MP weight.

RESULTS - FIELD EXPERIMENT



PE MP Delivery (count/ minute) during the different rainfall simulation experiments

PE MP Enrichment in sediments during the dry and wet runs during the 1-hour RS runs

- PE particles of a diameter between 425-500 µm and 125-150 µm were preferentially eroded and transported, leading to a mean enrichment ratio of 5.71 ± 4.18 (n = 4) and 5.15 ± 3.51 (n = 4) in the eroded sediment, respectively.
- 28% of induced MP was transported via surface runoff

CONCLUSION

- Our developed protocol slashes MP identification time to around 30 minutes per sample, enabling swift, repeated iterations allowing more sampling of transport experiments.
- We achieved recovery rates of 84 to 91% for fluorescent particles (100 – 500 µm), comparable to previous MP transport experiments and other extraction and identification methods employing Image J and/or machine learning approaches.
- No differences in recovery rates were observed between low-cost PLA filament-procured fluorescent microplastics and high-end PE fluorescent microbeads, indicating the protocol's versatility across different shapes and sizes of fluorescent MP particles.
- Wet sieving reduced sample volume by almost 90 to 95% in each sieve fraction (125 and 425 µm for PE, 125 and 250 µm for PLA microplastics), effectively decreasing sediment fractions and organic matter content, establishing uniform background conditions without requiring an extra extraction step.
- Unlike Nile Red, where sample quantification can be time-sensitive, we observed no such issues - sieved samples stored for three months and photographed by two operators three months apart showed an average particle loss of 4.8%, likely due to handling.

ACKNOWLEDGEMENTS

This study was supported by the project SOPLAS, which has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No 955334