Microplastics in *Lumbricus terrestris* middens/casts and surrounding urban soil

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INTRODUCTION

Microplastics pollution in terrestrial ecosystems has been poorly investigated and the impact on soil-dwelling organisms is not well understood. The aim of this study was to investigate whether *Lumbricus terrestris* concentrates the microplastics in its casts, and how particles quantity and size compare to those in the surrounding soil. Also, we investigated microplastics quantity and size on agricultural land and in green urban areas to assess the differences from the aspect of different land uses.

MATERIALS AND METHODS

soil and casts sampling within an urban area

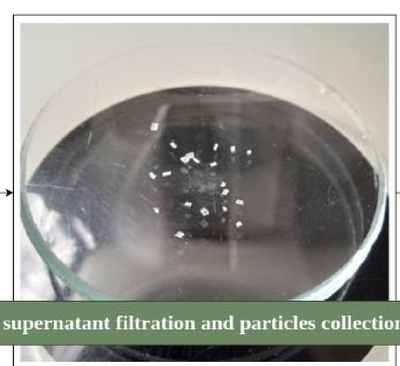
sample collection

20 sampling locations included agricultural fields and urban parks. Soil is sampled at the depth of 10 cm with metal auger. Composite soil sample consisting of 5 sub-samples was collected. Earthworm casts are sampled manually at each location. Samples are dried, sieved, and stored in a glass jars.

soil/casts flotation in 5M $ZnCl_2$

density separation

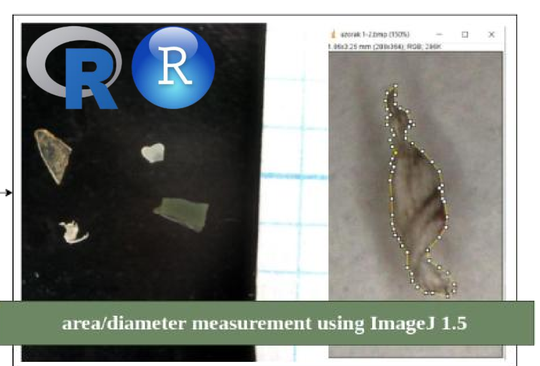
Density separation includes digestion of organic matter using 30% H_2O_2 at 60°C, mixing environmental sample with 5M aquaeous $ZnCl_2$ solution, and sedimentation period of 2 h. After that, mineral component of the sample accumulates at the bottom, and microplastics are floating at the top of the solution.



supernatant filtration and particles collection

extraction

Supernatant containing microplastics is decanted and filtered. Filters were dried and inspected under the stereomicroscope. Individual microplastic particles are separated with tweezers, put on a microscope slide next to a milimeter paper, and then photographed using digital microscope camera.

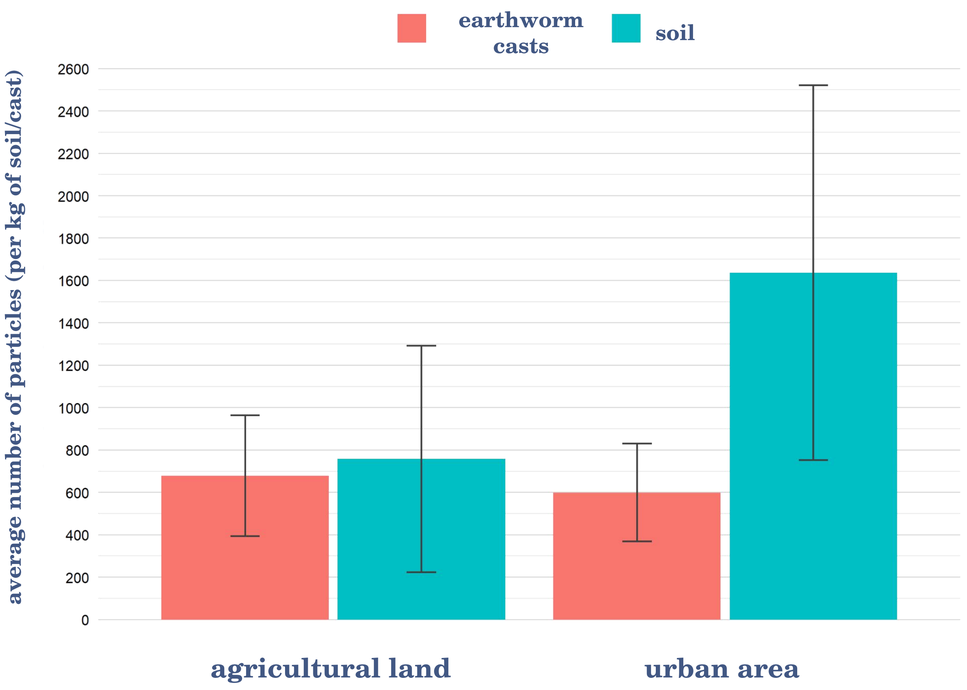


area/diameter measurement using ImageJ 1.5

quantitative and qualitative analysis

Images are analyzed using ImageJ 1.5 software. The scale of the individual image is calibrated using milimeter paper. For size parameters measurement, a polygon is created around each microplastics particle. Feret's diameter and surface area of each particle is calculated and stored for further statistical analysis. Statistical analysis was conducted in R programming environment.

RESULTS



Mean microplastics count in soil was 977.76 ± 726.39 particles kg_{soil}^{-1} and in earthworm casts 655.89 ± 265.11 particles kg_{cast}^{-1}. No statistically significant difference in number of microplastic particles between soil and earthworm casts was found (p=0.081).

	Earthworm cast	Soil
Surface area (mm^2)	0.529 ± 0.660	0.482 ± 0.765
Diameter (mm)	1.205 ± 0.705	1.102 ± 0.703

*mean values ± standard deviation

Mean surface area of microplastics isolated from soil samples was 0.482 ± 0.765 mm^2 and from earthworm casts 0.529 ± 0.660 mm^2. On average, diameter of microplastic particles was 1.102 ± 0.703 mm in particles isolated from soil and 1.205 ± 0.705 mm in particles from earthworm casts. Of all the isolated particles in the soil samples, there were 91.01% fragments and 8.99% fibers. The proportion of fragments in the earthworm casts was 86.67%, and the proportion of fibers was 13.33%. The χ 2-test did not reveal any statistically significant differences in the particle shape ratio between soil samples and earthworm casts (p=0.237).

CONCLUSIONS

Previous studies have shown that earthworms can accelerate biodegradation of microplastics in the soil by ingestion. Therefore, it was expected that the count of microplastics in earthworm casts would exceed the count of microplastics in soil samples and that the particles would be smaller compared to those found in soil. However, this research has shown the opposite, which suggests potential preferential retention of smaller microplastics within the earthworm organism. Retention of small-size microplastics could lead to bioaccumulation of microplastics in earthworm organism, and therefore higher mortality and lower growth rates, as previous research showed.