Size-dependent accumulation and toxicity of PS-MPs in earthworms (*Eisenia foetida*)

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Microplastics are widely spread in terrestrial systems, while, at present, attention has been mostly focused on potential risk of microplastic in aquatic systems [1, 2]. In this study, earthworms *Eisenia fetida* were exposed to polystyrene microplastics (PS-MPs) of different size (70 nm, 1 μm and 10 μm) and exposure dose (0.5%, 5% and 10% w/w in food), aiming to achieve a comprehensive understanding of the accumulation and toxicological process of MPs.

Mortality, growth, oxidative stress and energy-metabolize were assessed to evaluate the toxicological effects of PS-MPs on *E. fetida*. Results showed that after 28 days of exposure, there was no significant influence of different PS-MPs treatments on mortality of earthworms. Organisms treated with 70 nm PS-MPs showed an obvious decrease of growth with increasing exposure dose. Compared to 1 μm and 10 μm PS-MPs treatments, 70 nm PS-MPs induced a more significant effect on the activities of peroxidase (POD), catalase (CAT), the content of malonaldehyde (MDA), and the concentration of ATP and non-esterified fatty acid (NFFA). These indicate that oxidative stress and energy influence are induced after exposure of MPs, especially under treatment of nano MPs. Furthermore, accumulation and distribution of PS-MPs in earthworms were analysed with laser confocal microscopy by exposing to fluorescent PS-MPs. Results showed different distribution patterns among PS-MPs of different size. Aggregation was observed for 70 nm and 1 μm PS-MPs in earthworm. The results of this study suggest that the size of MPs in terrestrial system is a key factor which should be considered for evaluating the toxicity of PS-MPs on soil organisms.

[1] Huerta Lwanga *et al*. (2016) *Environ. Sci. Technol* **50**, 2685-2691. [2] Anderson Abel De Souza Machado *et al*. (2018) *Glob Change Biol* **24**, 1405-1416.