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Earthworms ingest microplastic fibres and nanoplastics with effects on egestion rate and long-term retention

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Supporting Information

Material synthesis

The total metal content was acquired by microwave (ultraCLAVE, MLS GmbH, Leutkirch, Germany) assisted acid digestion (see details below) followed by inductively coupled plasma mass spectrometry (ICP-MS) analysis (253.6 mg Pd/L) and the particle size and electrophoretic mobility was measured with the Malvern Zetasizer (z-average: 187 nm, polydispersity index: 0.04, zeta-potential (derived from the electrophoretic mobility): -43 mV).

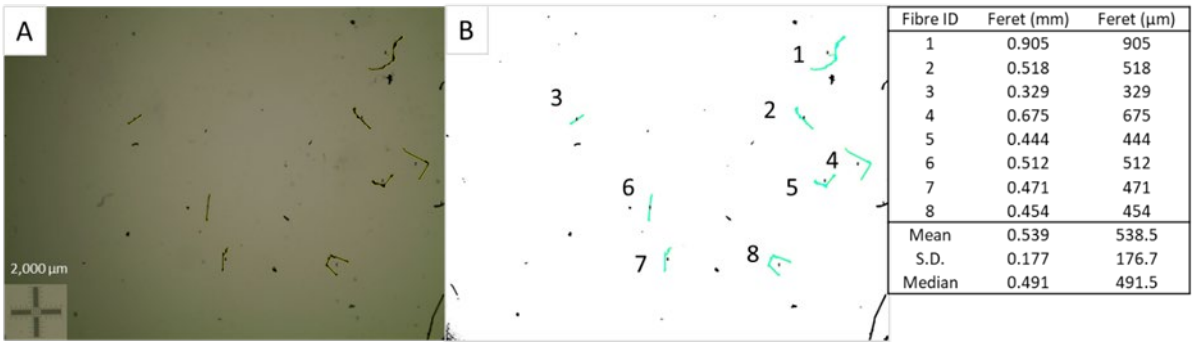
Microplastic fibre (MPFs) characterisation

Digital images were taken on an EVOS core XL photo microscope (Thermo Fisher Scientific, Massachusetts, USA) (Figure S1A). Analysis of MPFs was performed in ImageJ. Images were converted to 8-biy and the threshold adjusted manually to remove the background. A global scale was set defined using a microscope slide scale bar at the same magnification and used for particle sizing in all images. Only particles >0.01 mm and with a circularity between 0.00 – 0.3 were analysed and reported on to remove dust and imperfections on the glass slides or areas where multiple particles formed a bundle of overlying entangled fibres from also being counted in the analysis (Figure S1B). Fibres on the edges of the field of view were also excluded from analysis. An example of the summary results for a single image are provided in the table in Figure S1. A total of 140 fibres were measured to calculate the mean, median and standard deviation Feret length in mm and μm.

ICP-MS calibration

Ionic palladium (Pd) and Indium (In) standard solutions (10,000 mg Pd/l and 1,000 mg In/l, respectively, Sigma Aldrich) were diluted and used for calibration solutions, made fresh daily, at concentration of 0, 0.5, 1, 2.5 and 5 μg/l. An ionic rhodium standard (10,000 mg/l, Sigma Aldrich) was diluted to approximately 100 μg/l as an internal standard during all sample measurements.

Supplementary figures and tables



Fibre ID	Feret (mm)	Feret (µm)
1	0.905	905
2	0.518	518
3	0.329	329
4	0.675	675
5	0.444	444
6	0.512	512
7	0.471	471
8	0.454	454
Mean	0.539	538.5
S.D.	0.177	176.7
Median	0.491	491.5

Figure S1: *Example image analysis of fibres using optical microscopy (A) and analysis in ImageJ of individual fibres (B) to generate summary data for the Feret length (in millimetres and micrometres) of individual microplastic fibres.*

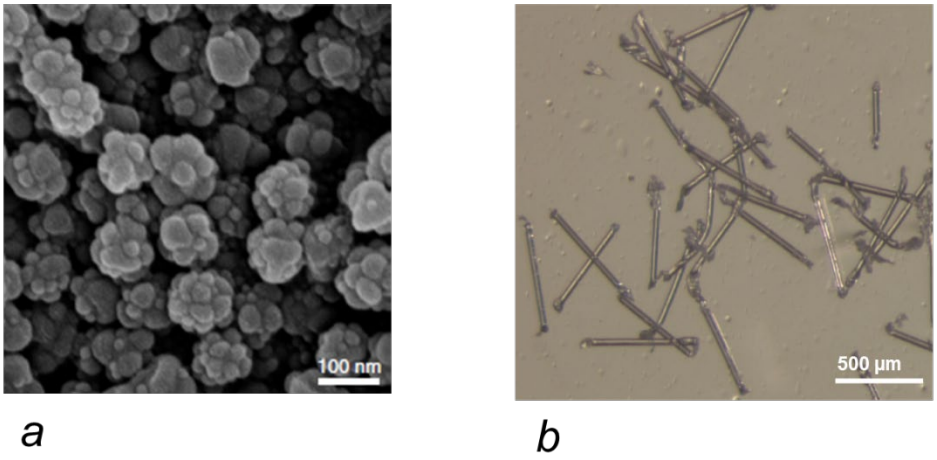


Figure S2: *The palladium doped polystyrene nanoplastics (a) and indium doped polyethylene terephthalate microplastic fibres (b) used in the earthworm accumulation assays.*

Figure S3: *Indium doped polyethylene terephthalate microplastic fibres following direct dry spiking in the soil in preparation to be used in the earthworm accumulation assays. The red arrows indicate fibres as an example. Concentration of 5000 mg MPFs/kg dry weight soil.*

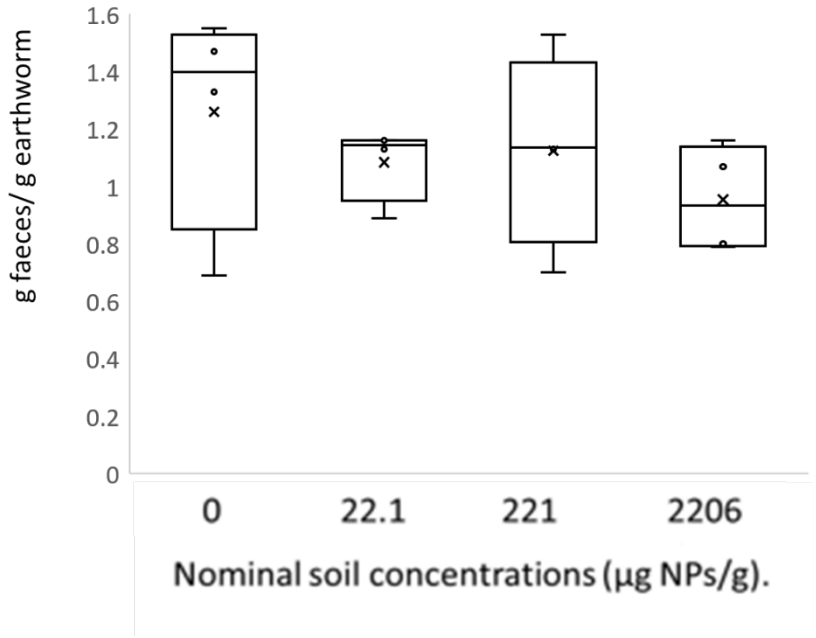


Figure S4: *The biomass of faeces produced per gram earthworm during 48 hours depuration following 7 days exposure to three concentrations of Pd-doped nanoplastics. Earthworms were also exposed in soil not spiked with NP (0 mg/kg). The x denotes the average concentration and the error bars show the standard deviation.*

Table S1: *The mass of soil estimated to be in the earthworm following depuration if all the In or Pd measured in the earthworm was due to the measurement of retained soil (Sr) and the relative earthworm body mass after exposure relative to initial body mass. (mean ± standard deviation, n=4).*

Nominal MPFs concentration (µg MPFs /g dry weight soil)	***Sr* = Estimated soil mass in earthworm following depuration (mg soil d.w.)[b]**	**Relative earthworm mass after exposure (%)**
0	-	93.1 ± 4.1
50	24.3 ± 12.5	96.6 ± 7.4
500	30.0 ± 8.1	101.8 ± 6
5000	17.1 ± 26.5	102.3 ± 4.4
Nominal NPs concentration (µg NPs/g dry weight soil)	***Sr* = Estimated soil mass in earthworm following depuration (mg soil d.w.)[b]**	**Relative earthworm mass after exposure (%)**
0	-	101.2 ± 2.12
22.1	87.7 ± 203	91.1 ± 5.4
221	60.8 ± 14.6	94.8 ± 6.9
2206	47.4 ± 6.4	93.3 ± 4.6

[b] calculated based on Eq1.

Table S2: *The mass of earthworms measured at the start (Day 0) and end (Day 7 or Day 21) of each exposure (wet weight, mean ± standard deviation) and the overall % change in mass between start and end of the experiments.*

NP 7 days	***Day 0***	***Day 7***	***% change***
0	*5.39 ± 1.453*	*5.6 ± 1.43*	*3.9*
22.1	*6.02 ± 0.984*	*5.87 ± 0.909*	*-2.4*
221	*6.02 ± 0.899*	*5.96 ± 1.07*	*-1*
2206	*4.66 ± 1.839*	*4.57 ± 1.53*	*-2*
MPF 7 days	***Day 0***	***Day 7***	***% change***
0	*5.33 ± 0.71*	*4.96 ± 0.677*	*-6.9*
50	*3.94 ± 0.874*	*3.81 ± 0.842*	*-3.5*
500	*4.28 ± 0.466*	*4.34 ± 0.349*	*1.5*
5000	*5.10 ± 0.444*	*5.23 ± 0.660*	*2.6*
NP 21 days kinetic	***Day 0***	***Day 21***	***% change***
0	*4.385 ± 1.2*	*5.03 ± 0.695*	*14.7*
464	*4.88 ± 0.973*	*4.41 ± 1.5*	*-9.6*

Table S3: *The derived model parameters for the two one-compartment models (A) without an inert fraction and (B) with an inert fraction. BAF = bioaccumulation factor (k_1/k_2), Half life = ($ln[2]/k_2$). Fixed parameters : C_0 = 0.02 µg Pd/g earthworm, C*exp *= 1.97 µg Pd/g soil.*

Model parameter	**One compartment model A (without inert fraction)**	**One compartment model B (including inert fraction)**
k_1 (g soil/g earthworm/day)	0.0098 ± 0.0047	0.0176 ± 0.0124
k_2 (d^{-1})	0.210 ± 0.103	0.432 ± 0.312
F_i	-	0.0159 ± 0.0089
BAF	0.043	0.041
Half-life (days)	3.3	1.6