

A standardized soil-based biotest to investigate the phytoavailability of nanoplastics

SUPPLEMENTARY MATERIAL

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Figure S1. HDPE pots, sealed at the bottom with a 30-μm pore-size stainless steel mesh, were used for the germination, growth and exposure of tomato plants

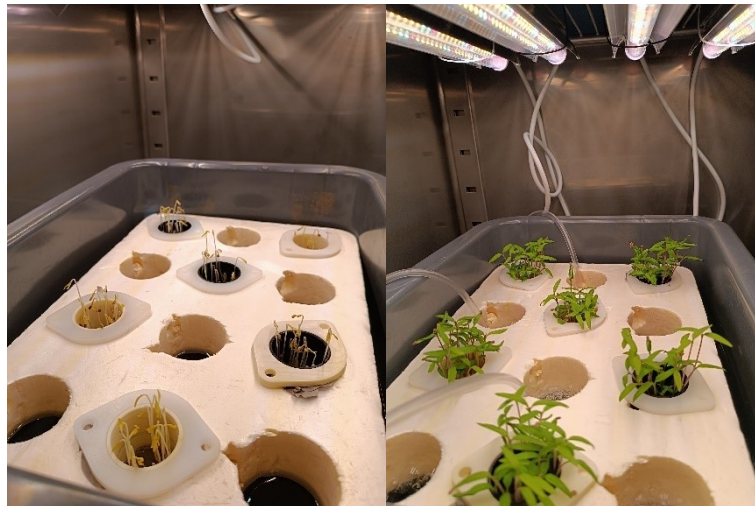


Figure S2. Preculture (7-days germination, left, and 14-days plantlet growth, right) under hydroponics. HDPE pots were placed on a floating platform, with their bottoms sealed with stainless steel mesh, in constant contact with the specific nutrient solutions contained in the grey boxes.

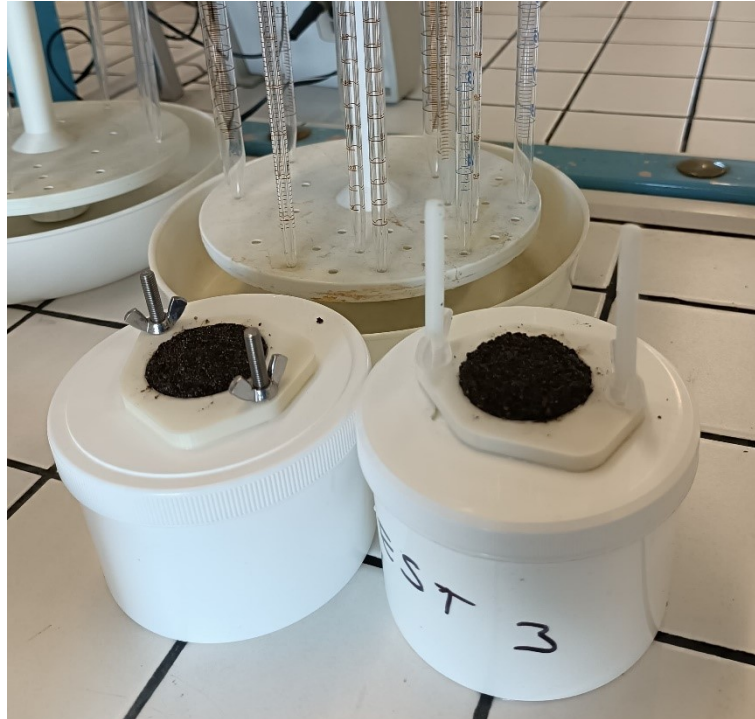


Figure S3. Details of the equipment used to place the 6-mm thick soil layer of (equivalent to 9 g dw). Soil test portions were in contact with the nutrient solution inside the white containers via filter paper wicks.



Figure S4. 8-days exposure phase with soil-plant contact.



Figure S5. Plants harvesting (left), shoot, root, and soil sample collection (middle) and samples drying (4-days at 40 °C, right) at the end of the exposure phase.

Table S1. Composition of standard soil (ISO 11269:2:2012)

Material	Content in standard soil (% d.w.)
Sphagnum peat moss	10
Quartz Sand	69
Kaolinite Clay	20
Calcium Carbonate	1

Table S2. Chemical and physical characteristics of the standard soil and related methods used for their determination. * = measured after 24h drying at 105°C.

Parameter	Value	Method
pH	7.3	ISO 10390:2021
Corg	43.5 g kg ⁻¹ dw	ISO 10694:1995
Salinity	8.1 meq/100 g dw.	Corwin, 2003
Water Content*	1.38 %	ISO 11465:1993
Water Holding Capacity	63 %	BS EN ISO 11269-2:2013 Met C
Total N	3.1 g kg ⁻¹ dw	ISO 11261:1995

Table S3. Elemental concentrations in the standard soil (determined by UNI EN 16174:2012 Met. A and UNI EN 16170:2016 methods). Measured Pd content is presented in Table S8.

Element	mg kg ⁻¹ dw
Sb	< 1
As	< 5
Be	< 2s
Cd	< 0.2
Co	4,4
Cr	< 5
Ni	9.2
Pb	< 5
Cu	20
Se	< 5
TI	< 2
V	12
Zn	21
Ca	44925
Mg	36369
K	1429
Na	41
P	635

Table S4. Composition of the Nutrient Solution Number 1 used in the RHIZOtest procedure for the first 7-days germination phase in hydroponics (EN ISO 16198, 2015)

Component	Concentration ($\mu\text{mol dm}^{-3}$)
CaCl_2	600
H_3BO_3	2

Table S5. Composition of Nutrient Solution Number 2 used in the RHIZOtest procedure for the second 14-days phase of plantlets growth in hydroponics (NF EN ISO 16198, 2015)

Component	Concentration ($\mu\text{mol dm}^{-3}$)
KH_2PO_4	500
KNO_3	2,000
$\text{Ca}(\text{NO}_3)_2$	2,000
MgSO_4	1,000
CuCl_2	0.2
H_3BO_3	10
MnCl_2	2
ZnSO_4	1
Na_2MoO_4	0.05
NaFe(III)EDTA	100

Table S6. Composition of Nutrient Solution Number 3 used in the RHIZOtest procedure for the last 8d exposure phase with soil-plant contact (EN ISO 16198, 2015)

Component	Concentration ($\mu\text{mol} \cdot \text{dm}^{-3}$)
KH_2PO_4	50
KNO_3	2,000
$\text{Ca}(\text{NO}_3)_2$	2,000
MgSO_4	1,000

Table S7. Characteristics of the stock dispersion of Pd-doped PS-NPs measured in this study.

Parameter	Value
Z-average diameter	210 nm
Polydispersity index (PDI)	0.1
Concentration of particles	$67.637 \pm 0.015 \text{ g L}^{-1}$
Pd concentration	$95.0 \pm 2.0 \text{ mg kg}^{-1}$
Pd content in particles	0.295 %

Table S8. Concentration of Pd ($\mu\text{g g}^{-1}\text{dw}$) – mean, standard deviations (Std. dev.) and coefficient of variation (CV) – measured in **soil** at the start (0 day) and end (8 days) of the exposure phase, at control and both spiking levels. Limit of quantification (LOQ): $0.01 \mu\text{g g}^{-1}\text{dw}$. Asterisks in the 8 days column indicate a significant difference between means of concentrations measured at the beginning (0 day) and at the end (8 days) of the exposure period.

Nominal initial concentration	Replicate n°	0 day $\mu\text{g Pd g}^{-1}\text{dw}$	8 days $\mu\text{g Pd g}^{-1}\text{dw}$
Control	1	<LOQ	<LOQ
	2	<LOQ	<LOQ
	3	<LOQ	<LOQ
	4	<LOQ	<LOQ
	5	<LOQ	<LOQ
	Mean	<LOQ	<LOQ
	Std. dev.	-	-
	CV (%)	-	-
400 mg NPs $\text{kg}^{-1}\text{ dw}$ 1.2 mg Pd $\text{kg}^{-1}\text{ dw}$	1	1.18	1.12
	2	1.17	1.04
	3	1.16	1.09
	4	1.17	1.06
	5	1.17	1.10
	Mean	1.17	1.08*
	Std. dev.	0.01	0.03
	CV (%)	0.5	3.2
4,000 mg NPs $\text{kg}^{-1}\text{ dw}$ 12 mg Pd $\text{kg}^{-1}\text{ dw}$	1	11.6	11.0
	2	11.8	11.1
	3	11.7	10.9
	4	11.8	11.2
	5	11.7	10.9
	Mean	11.7	11.0*
	Std. dev.	0.1	0.1
	CV (%)	0.6	1.2

Table S9. Concentration of Pd ($\mu\text{g g}^{-1}\text{dw}$) - mean, standard deviations (Std. dev.) and coefficient of variation (CV) - measured in the **roots** and **shoots** collected before (at the end of the pregrowth phase in hydroponics) and after the 8-day exposure phase, at control and both spiking levels . Limit of quantification (LOQ): $0.01 \mu\text{g g}^{-1} \text{dw}$.

Nominal initial concentration	Replicate n°	Roots $\mu\text{g Pd g}^{-1}\text{dw}$	Shoots $\mu\text{g Pd g}^{-1}\text{dw}$
Before exposure	1	<LOQ	<LOQ
	2	<LOQ	<LOQ
	3	<LOQ	<LOQ
	4	<LOQ	<LOQ
	5	<LOQ	<LOQ
	Mean	<LOQ	<LOQ
	Std. dev.	-	-
	CV (%)	-	-
Control	1	<LOQ	<LOQ
	2	<LOQ	<LOQ
	3	<LOQ	<LOQ
	4	<LOQ	<LOQ
	5	<LOQ	<LOQ
	Mean	<LOQ	<LOQ
	Std. dev.	-	-
	CV (%)	-	-
400 mg NPs $\text{kg}^{-1} \text{dw}$ 1.2 mg Pd $\text{kg}^{-1} \text{dw}$	1	0.24	0.06
	2	0.30	0.08
	3	0.24	0.08
	4	0.32	0.06
	5	0.29	0.04
	Mean	0.28	0.06
	Std. dev.	0.04	0.02
	CV (%)	13	27
4,000 mg NPs $\text{kg}^{-1} \text{dw}$ 12 mg Pd $\text{kg}^{-1} \text{dw}$	1	3.2	0.9
	2	3.7	0.9
	3	3.9	1.2
	4	3.5	0.7
	5	3.8	1.1
	Mean	3.7	0.9
	Std. dev.	0.3	0.2
	CV (%)	8	20

Table S10. **Mass of tomato plant parts** (g dw) – mean, standard deviations (Std. dev.) and coefficient of variation (CV) – collected before (at the end of the pregrowth phase in hydroponics) and after the 8-day exposure phase for each tested treatment and all replicates. Different letters next to the mean value in a given column indicate statistically significant differences (Tukey, p-value ≤ 0.05).

Nominal initial soil concentration	Replicate n°	Shoots g dw	Roots g dw
Before exposure	1	3,2	0,66
	2	3,4	0,62
	3	3,6	0,60
	4	3,1	0,61
	5	3,4	0,65
	Mean	3.3^a	0.62^a
	Std. dev.	0.2	0.02
	CV (%)	5	4
Control	1	3.9	1.02
	2	3.7	1.02
	3	4.0	0.95
	4	4.2	1.10
	5	4.0	1.00
	Mean	4.0^a	1.02^b
	Std. dev.	0.2	0.05
	CV (%)	4	5
400 mg NPs kg ⁻¹ dw 1.2 mg Pd kg ⁻¹ dw	1	4.0	0.84
	2	3.9	0.82
	3	4.0	0.96
	4	4.1	0.86
	5	3.5	0.95
	Mean	3.9^a	0.89^c
	Std. dev.	0.2	0.07
	CV (%)	6	8
4,000 mg NPs kg ⁻¹ dw 12 mg Pd kg ⁻¹ dw	1	3.8	0.75
	2	3.7	0.85
	3	4.2	0.63
	4	3.4	0.78
	5	4.3	0.70
	Mean	3.9^a	0.74^d
	Std. dev.	0.4	0.08
	CV (%)	10	11

REFERENCES

EN ISO 16198 2015. Soil quality - Plant-based test to assess the environmental bioavailability of trace elements to plants.