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

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Standardized methods are essential for generating reliable and reproducible data to support risk assessment and decision-making related to soil contamination by environmental pollutants, including nanoplastics (NPs). This study evaluated the ability of the RHIZOtest method, a standardized soil–plant exposure system, in providing a high-throughput testing platform for investigating NP phytoavailability. As a proof of concept, tomato plants were exposed to artificial soil spiked with model NPs at concentrations of 400 and 4000 mg kg^{-1} dm. Palladium (Pd)-doped polystyrene particles (PS-P) (a Z-average diameter of 210 nm, a surface charge zeta potential of −45.20 ± 032 mV, a polydispersity index of 0.1, and a Pd doping ratio of 0.295% w/w Pd to PS-P) were used as surrogates for NPs. Pd content was measured as a proxy for quantifying PS-P uptake. After eight days of exposure, Pd was detected in both the roots and shoots of plants grown on both spiked soils, confirming PS-P uptake and translocation. On average, 5 ± 1% of the spiked PS-P were taken up by the plants across spiking levels. Root concentration factors varied slightly between the lower and higher levels (31 ± 2% and 24 ± 3%, respectively), while translocation factors remained similar (~25%). Root biomass was significantly reduced compared to controls, suggesting possible concentration-dependent PS-P rhizotoxicity. Notably, the limited variability in concentration values measured in roots (±11%) and shoots (±23%), along with near-complete mass balance recovery (97–100%), demonstrated the reliability of the RHIZOtest in accurately and consistently quantifying NP uptake while accounting for rhizosphere processes.

Environmental significance

Reliable data on nanoplastic uptake by crops are urgently needed to assess potential health risks; however, current studies lack standardized soil-based methods and often suffer from poor comparability and low reproducibility. This study is the first to demonstrate the applicability of the RHIZOtest (ISO 16198) as a standardized method for assessing nanoplastic phytoavailability in soil–plant systems. Using 210 nm Pd-doped polystyrene particles and tomato plants, high experimental repeatability and efficient mass balance closure were achieved, demonstrating its adaptability to a wide range of nanoplastics, diverse crops, soil types, and treatments. These findings establish the RHIZOtest as a robust, high-throughput platform for systematic NP uptake studies, supporting its wider adoption in both research and regulatory risk assessment frameworks.

1. Introduction

The presence of nanoplastics (NPs) in soils has been widely acknowledged and is considered a particular concern due to their potential to enter the human food chain through plant uptake.[1,2] However, the actual exposure *via* the soil–plant transfer (*i.e.*, the bioavailability of NPs for plants or NP phytoavailability) remains far from being fully understood, due to several challenges persisting in this field. One key limitation is the insufficient analytical sensitivity to the direct quantification of NPs (*e.g.*, through Py-GC-MS) at environmentally relevant concentrations in plant tissues.[3,4] In addition, due to the absence of reference materials representative of naturally occurring NPs, researchers often relied on metal-doped model plastic nanoparticles, which allow more accurate quantification in plant tissues, even at low concentrations, using tracer-based techniques such as ICP-MS.[5,6] While useful and convenient for fundamental

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† Electronic supplementary information (ESI) available: Additional information on materials (Tables S1–S7), including photographs of the experimental setup (Fig. S1–S4), as well as the complete dataset on measured concentration values and plant biomass at the end of the test (Tables S8–S10) (DOC). See DOI: https://doi.org/10.1039/d5en00328h

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environmental studies, these model particles poorly reflect the heterogeneity of NPs occurring in real soil systems, thereby limiting the transferability of results to environmentally relevant scenarios.[7] In particular, differences in size and surface charge (zeta potential) may affect their interactions with plant root systems, promoting or inhibiting crop uptake and ecotoxicity.[8] Potential phytotoxic effects of additives or surfactants used as processing aids in the production of model nanoparticles should also be considered. Consequently, the behavior and fate of surrogate plastic nanoparticles may differ significantly from those of real NPs.

In this context, many studies have investigated model NP uptake in plants,[9] the majority reporting findings from hydroponic experiments.[10–12] Hydroponic systems provided highly reproducible exposure conditions for investigating the mechanisms of NP absorption, translocation, and toxicity in plants,[13–15] as well as for assessing novel quantification methodologies.[16] However, these results lack environmental relevance for risk assessment due to oversimplified conditions determining greater NP mobility.[12] Soil-based studies instead, though more complex and variable, provide a more realistic assessment, accounting for model NP homo- or heteroaggregation with soil organic matter and minerals as influenced by root activities (*e.g.*, nutrient uptake, organic acid exudation, and proton and hydroxyl excretion).[10] Comparing the results derived from the few existing soil-based studies is challenging due to substantial variations in experimental designs, including differences in growth media, exposure durations, plant species, and model NP compositions.[7–9,17] This highlights the urgent need for a standardized soil-based biotest to support the interoperability and transferability of results for risk assessment and decision-making related to NP regulations.[18,19] The RHIZOtest, a standardized biotest, has been developed to routinely investigate the phytoavailability of soil contaminants while accounting for rhizosphere processes.[20,21] The Rhizotest design is based on the root-mat approach, which establishes contact between a dense, planar mat of roots and a few millimetre-thick soil layer without allowing the roots to penetrate down to the soil, thanks to a fine mesh placed in between.[21] Initially applied to enable a standardized and reliable estimation of uptake for major nutrients and trace elements,[22–25] its scope has since expanded to include engineered metallic nanoparticles,[26] pesticides,[27] pharmaceuticals and personal care products,[28] brominated flame retardants,[29] and more recently to a model NP consisting of 50 nm polystyrene nano-beads, spiked in a Cambisol soil.[30] However, in this former study, the quantification of nano-beads was limited to shoot tissues and only at the highest exposure concentration (300 mg kg^{-1} dm), likely due to constraints associated with the sensitivity of the Py-GCMS analytical method.[6,30] Moreover, the absence of a mass balance assessment within the soil–plant system hinders a comprehensive evaluation of the RHIZOtest's reliability and the accuracy of the reported results. In this regard, inductively coupled plasma mass spectrometry (ICP-MS) along with metal-doped NPs has been demonstrated to be promising in dealing with this critical aspect.[6] Addressing these gaps, this study evaluated the applicability of the RHIZOtest for investigating the fate and phytoavailability of NPs in the soil–plant system. Among the plant species validated in the standard method, tomato (*Solanum lycopersicum* L.) was selected as the model species, while palladium-doped polystyrene particles (Pd-doped PS-NPs) were employed as surrogates to enable precise and sensitive quantification in plant tissues.

2. Materials and methods

2.1. Materials

Commercially available seeds of tomato (*Solanum lycopersicum* L. cv. 'Cuore di bue') were selected within the list of species validated in the standard method, as they are known to be well adapted to the RHIZOtest setup.[21,29] An artificial standard soil sample was prepared in the laboratory following ISO 11269-2.[31] Artificial soil was selected to ensure controlled conditions and enhance the reproducibility of the experiment and to isolate plant uptake processes without interference from possible biodegradation or sorption variability. This approach is consistent with previous work using the RHIZOtest system to assess the bioavailability of flame retardants in artificial soil.[29] The soil composition and its main characteristics are provided in Tables S1–S3 of the ESI.†

The composition of the three nutrient solutions used in the RHIZOtest procedure is detailed in Tables S4–S6.† A stock dispersion of Pd-doped polystyrene particles (PS-P), with a Z-average diameter of 210 nm, a polydispersity index of 0.1, and a doping ratio of 0.295% w/w Pd to PS-P, was used for soil spiking. Full characterization data are provided in Table S7.† Particle sizing was derived from SEM images, while STEM–EDX analysis confirmed the homogeneous metal distribution within the particles. Details on the synthesis and characterization methods are available elsewhere.[32]

2.2. Soil spiking with Pd-doped PS-NPs

Pd-doped PS-P were spiked into the soil test portions to achieve nominal concentrations of 400 and 4000 mg kg^{-1} dry matter (dm), corresponding to 1.2 and 12 mg Pd kg^{-1} dm, respectively. While the selected concentration of 400 mg kg^{-1} dm reflects the upper boundary of values reported in the literature for microplastics in agricultural soils, it is consistent with the order of magnitude (*i.e.*, hundreds of mg kg^{-1} dm) used in soil-based studies investigating the uptake of model nanoparticles.[1,30,33–36] Given the current lack of data on NP concentrations in real soil samples, it is generally assumed that their levels are lower than those of their micro counterparts. In this context, and considering the low expected phytoavailability of NPs and the relatively short exposure duration of the RHIZOtest, higher spiking concentrations were assumed necessary to enable the

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quantification of uptake and to assess the sensitivity of the RHIZOtest system. The higher concentration of 4000 mg kg^{-1} dm was instead tested to explore potential correlations between contamination levels, plant uptake and translocation, and possible phytotoxicity.

To enhance the repeatability of the soil spiking process and to ensure uniform initial concentrations across soil portions, Pd-doped PS-P were added through diluted working suspensions, obtained from the initial stock suspension. Working dispersions were prepared one day before spiking by diluting the stock suspension of the Pd-doped PS-P in an aqueous soil extract, following an adapted procedure previously proposed for other nanomaterials.[37] This step allowed us to better simulate realistic scenarios of soil contamination by avoiding the use of additional solvents with unknown effects. The aqueous soil extract was obtained by stirring 40 mg of soil in 100 mL of the nutrient solution used during the RHIZOtest exposure phase (Table S6†) for 1 h, followed by filtration through cellulose membranes (15–20 μm pore size). The prepared working suspension was kept under constant stirring before and during spiking to guarantee uniform distribution of Pd-doped PS-P into each soil portion. Prior to spiking, soil test portions (9 g dm) were prepared in aluminum containers and adjusted to 70% of the soil water holding capacity (Table S2†) using the same nutrient solution employed in the RHIZOtest exposure phase (Table S6†). Unspiked soil test portions were maintained as the control. Both spiked and unspiked soil test portions were prepared at the start of the hydroponic phases of the RHIZOtest, mixed, and allowed to equilibrate for three weeks before exposure under the same climatic conditions as those used for the germinated plantlets (see below for climatic conditions).

2.3. RHIZOtest experiments

The procedure adhered to the requirements of the international standard ISO 16198,[21] with specific adjustments for application to NPs as detailed in the ESI† (Fig. S1–S5). Tomato seeds were surface-sterilized in a 5% NaClO solution for 30 min and then rinsed with sterile water. Forty seeds were placed in each HDPE pot, sealed at the bottom with a 30 μm pore size stainless steel mesh (Fig. S1†). The seeds were germinated for 7 days, followed by a 14-day hydroponic growth phase (Fig. S2, Tables S4 and S5†). This hydroponic preculture period allowed the plants to develop a dense and planar root mat along the inner surface of the mesh in each pot. At the end of the pre-culture phase, fifteen plant pots, visually exhibiting a homogeneous biomass, were selected to be exposed to the control (unspiked) and spiked artificial soil portions at the two nominal concentrations (five replicates for each), by putting the root mat in contact with a 6 mm thick layer (equivalent to 9 g dm) of soil (Fig. S3†) while maintaining the 30 μm stainless steel mesh in between. The exposure phase lasted for 8 days (test culture period – Fig. S4†). Each soil layer was connected to a nutrient solution (Table S6†) *via* hardened ashless filter paper wicks. The entire RHIZOtest experiment was conducted in a growth chamber (Caron, Avantor, VWR International, USA) under the following conditions: 25 ± 3 °C, relative humidity of 75 ± 5%, and photosynthetically active radiation of 200–400 μmol photons m^{-2} s^{-1}, with a 16 : 8 hour light/dark cycle. At the end of the preculture and test culture periods, root and shoot replicates were harvested separately. No visible soil particles adhered to the root particles. However, root samples were gently rinsed to remove any adhering soil particles that could have passed through the 30 μm mesh. Soil, root, and shoot samples were then oven-dried at 40 °C for 4 days, weighed, and prepared for Pd analysis (Fig. S5†).

2.4. Measurement of Pd concentrations

After fine milling, total Pd determination was carried out using an adapted procedure previously developed for NP quantification.[32] Approximately 100 mg of the milled sample was placed into a Teflon digestion tube, followed by the addition of 1 mL of H_2O_2 (30% vol.). After 1 h, 4 mL of HNO_3 (68% vol.) were added and left to stand for another hour. Finally, 0.5 mL of H_2SO_4 (95% vol.) was added, and the samples were mineralized at 253 °C under 100 bars for 25 min in a microwave digester (ETHOS X, Milestone Srl, Italy). Pd concentrations in the digests were determined by ICP-MS (iCAP RQplus, Thermo Fisher Scientific) using external calibration with In as the internal standard. The average recovery values calculated for Pd concentrations ranged from 81% to 119% across all types of samples analyzed. The LOQ was estimated as ten times the standard deviation of the procedural blanks analyzed alongside the samples.

2.5. Statistics

The complete dataset on plant biomasses and Pd concentrations in soils and plants is available in the ESI† (Tables S8–S10). A two-sample *t*-test (α = 0.05) was used to assess significant differences in mean Pd concentrations within and between the roots and shoots across exposure levels. Data normality and homoscedasticity were verified using Kolmogorov–Smirnov and Levene tests, respectively. The root concentration factor (RCF) and translocation factor (TF) were calculated for each exposure level. The RCF was determined as the ratio of Pd-doped PS-P concentration in roots to the initial soil concentration (mg g^{-1} dm), while the TF was calculated as the ratio of Pd-doped PS-P concentration in shoots to that in roots at the end of exposure. The software OriginPro 2025 was used to create all figures and conduct statistical analyses.

3. Results and discussion

3.1. The RHIZOtest allows closure of the mass balance of NPs in the soil–plant system

Initial soil Pd concentrations closely matched nominal values, with a coefficient of variation (CV) under 1% (Table

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S8†), suggesting a negligible effect of the homo-aggregation of Pd-doped PS-P in the working dilution or hetero-aggregation with soil solution components. Thus, nominal concentrations were used for result interpretation.

After exposure, the CV values of the measured Pd concentrations were low for soils (3.2%) and within an acceptable range for biological samples—roots and shoots—at 13% and 27%, respectively (Table S9†), considering the inherent variability typical of plant-based assays.[38,39] Consistently, Ryzhenko *et al.* (2024) measured PS-P concentrations in shoots with low variability (around 5%) after exposure to a similar soil concentration of 50 nm PS-P using the RHIZOtest. Furthermore, the background Pd concentrations in control soils were below the method's limit of quantification (LOQ, *i.e.*, 0.01 μg g^{-1} dm), indicating that Pd was an appropriate tracer.

In the soil–plant system spiked with Pd-doped PS-P, the mass balance of Pd and consequently of Pd-doped PS-P, was neatly closed, with a recovery rate of 97 ± 2.4% and 100.1 ± 0.1% at both initial soil concentrations, respectively (Table 1). Accordingly, potential losses of Pd-doped PS-P due to sorption onto the HDPE pots, stainless steel mesh or diffusion into the nutrient solution through the filter paper wicks were negligible. The resulting concentrations in all replicates were thus calculated in terms of PS-P by multiplying the measured Pd concentrations by the measured doping ratio (Table S7†). This calculation assumes that no Pd release occurred from the PS-P throughout the test, as demonstrated in simulations of plant–water interactions using the same Pd-doped PS-P.[13] Although soil solutions may be more aggressive than hydroponic conditions, the conservative leaching conditions reported were considered sufficient to assume that no significant Pd leaching occurred under the experimental conditions used.[13]

3.2. Concentration-dependent uptake and translocation of Pd-doped PS-P in plants

After exposure, Pd was quantified in spiked soils as well as in the roots and shoots of all tomato plants exposed to both concentrations of Pd-doped PS-P (Fig. 1a and Tables S8 and S9†). On average, across spiking levels, Pd-doped PS-P taken up by plants accounted for approximately 5.35 ± 1.03% of the initial quantity initially introduced into the soil.

Uptake and translocation of nanoparticulate materials have already been observed in previous studies using different exposure systems, plant species, and alternative analytical methods for detecting the particles.[8] Uptake through root cracks, root intercellular pathways and retention on the root epidermis are commonly observed through field and fluorescence SEM imaging.[10,11,14,40] Imaging techniques could help to visualize the localization of PS–Ps at the root surface or within root tissues. Future studies could adopt a complementary desorption step prior to tracer analysis, using appropriate washing solutions to selectively remove loosely bound PS–Ps from the root surface. Analyzing the desorption solution for the tracer content would allow for a quantitative differentiation between surface-retained and internalized particles, thus improving the understanding of uptake pathways.

However, data on NP concentrations in plant tissues remain limited and are predominately derived from hydroponic experiments. In these studies, the type of exposure medium (hydroponic, sand, or soil) and the size of nanoparticles drove plant absorption. In particular, hydroponic conditions resulted in overall higher NP uptake than soil-based systems, likely due to increased NP mobility, fewer root barriers, and a stronger transpiration driven uptake.[12,14] Higher concentrations of Pd-doped PS-P have been reported in cucumber roots (6.89 ± 0.42 mg g^{-1} dm), leaves (1.08 ± 0.08 mg g^{-1} dm), and stems (0.16 ± 0.01 mg g^{-1} dm) after 14 days of exposure to 50 mg L^{-1} NPs.[16] Similarly, wheat plants exposed for three weeks to 30 mg L^{-1} of the same NPs used in this present study exhibited concentrations of approximatively 4 mg g^{-1} dm in roots and 0.3 mg g^{-1} dm in shoots, higher than those recorded in this RHIZOtest at the highest soil spiking level.[13] At lower exposure doses (3 mg L^{-1}), concentrations were 0.5 mg g^{-1} dm in roots and 0.1 mg g^{-1} dm in shoots, in between to those observed in this study at the lower spiking level.[13]

Notably, Ryzhenko *et al.* (2024) reported 0.4 mg g^{-1} of PS-P in barley shoots using a modified RHIZOtest procedure with a soil concentration of 300 mg kg^{-1}.[30] This concentration is 20 times higher than, and comparable to, the concentration measured in this study in the shoots of tomato plants exposed to soil spiked with 400 and 4000 mg kg^{-1} Pd-doped PS-P, respectively. This difference may be attributed to the longer exposure time (13 days *vs.* 8 days) and the smaller nanoparticle size (50 nm *vs.* 200 nm) used by Ryzhenko *et al.*,

Table 1 Mass balance of the RHIZOtest experiment. Results are expressed as the mean content (μg) ± standard deviation (n = 5) of Pd in soil, roots, and shoots in individual replicates measured before and after the 8-day exposure phase to the soil spiked with Pd-doped PS-P at nominal concentrations of 1.2 and 12 mg Pd kg^{-1} dm, respectively, corresponding to 400 and 4000 mg PS-P kg^{-1} dm

		1.2 mg Pd kg^{-1} dm (400 mg PS-P kg^{-1} dm)		12 mg Pd kg^{-1} dm (4000 mg PS-P kg^{-1} dm)	
Soil spiking level		Before exposure	After exposure	Before exposure	After exposure
Soil	(μg Pd)	10.62 ± 0.10	9.83 ± 0.33	105.60 ± 0.80	99.26 ± 1.20
Roots	(μg Pd)	<LOQ	0.24 ± 0.03	<LOQ	2.70 ± 0.31
Shoots	(μg Pd)	<LOQ	0.25 ± 0.07	<LOQ	3.67 ± 1.04
TOTAL	**(μg Pd)**	**10.62 ± 0.10**	**10.32 ± 0.29**	**105.57 ± 0.77**	**105.62 ± 0.71**

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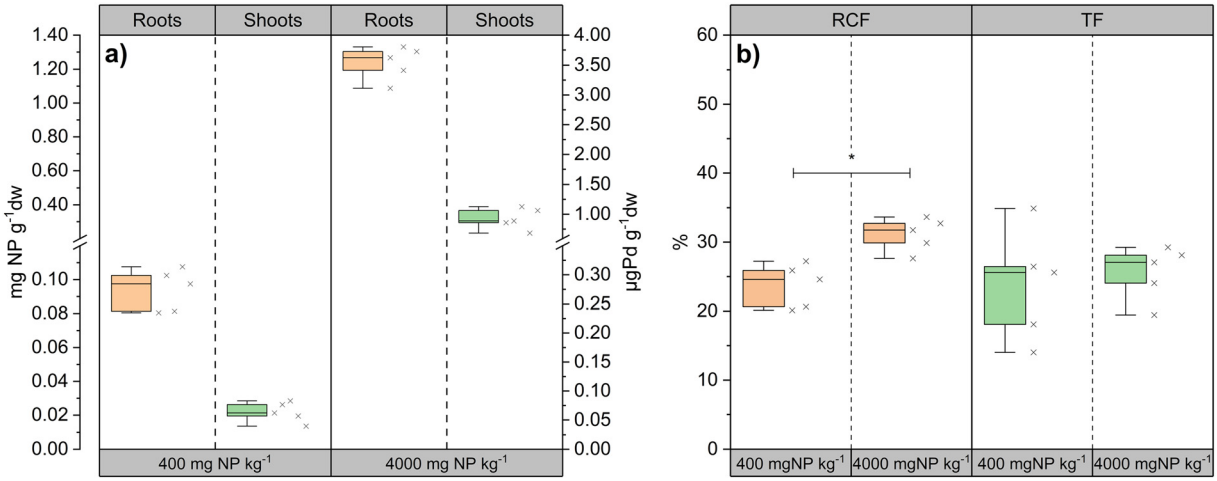

Fig. 1 a) Measured concentrations of Pd-doped PS-P in the roots and shoots of tomato plants after 8 days of exposure to soil spiked with 400 and 4000 mg NPs kg^{-1} dm; b) values calculated for the RCF and TF at both initial soil concentrations of Pd-doped PS-P. The asterisk indicates a significant difference (*t*-test $p < 0.05$).

(2024), which likely enhanced root absorption and translocation.[30,41] The average RCF for the lower spiking level (31 ± 2%) was slightly but significantly higher (p = 0.004) than that for the higher level (24 ± 3%) (Fig. 1b). However, the absolute difference was minimal, suggesting that RCF values were generally comparable between the two concentrations. The TF values also showed no significant difference between the two exposure levels (24 ± 8% and 26 ± 4%). This suggests that root uptake and translocation mechanisms for Pd-doped PS-P are consistent across the two concentrations. Expanding the study to include a broader range of soil concentrations could help determine whether these mechanisms remain stable across different exposure levels.

Even though these TF values are numerically in line with those reported by del Real *et al.* (2022) for wheat exposed hydroponically to 3 mg L^{-1} of the same Pd-doped PS–Ps, such comparisons should be interpreted with caution due to fundamental differences in exposure conditions and involved plant species.[7,13] Specifically, differences in the root physiology and physical behaviour of NPs at the root surface (*i.e.*, aggregation and overall higher mobility in liquid media) can influence their available quantity for transfer to the shoots.

While the results of this study may contribute to raise concern about human exposure to nanoplastics transferred from soil to the food chain and associated risks, it is important to acknowledge the limitations of the tested conditions. Specifically, the model nanoparticles used may differ markedly from the heterogeneous nanoplastics typically found in real soils in terms of their size, polymer composition, and surface chemistry and consequent interactions with the rhizosphere. Furthermore, the use of standard artificial soil may have limited the influence of processes occurring in real soil samples, potentially modifying the concentrations during the exposure.[42] Together with the use of a single plant species, these assumptions limit the transferability of these results to other polymers, plant species, and real-world soil environments. Nonetheless, they represent a necessary first step toward a more realistic assessment of nanoplastic transfer in soil–plant systems using the RHIZOtest procedure. In this regard, the RHIZOtest exposure platform has the potential to investigate systematically the influence of environmentally relevant variables (assessed alone or in combination) on the NP uptake process. Still, longer-term monitoring and experimental studies are essential to generate realistic risk estimates regarding the potential translocation of nanoplastics into the human food chain *via* crop uptake.

3.3. Concentration-dependent rhizotoxicity of PS-P

Meeting the validity criteria of the method, the plant mass in each replicate and treatment significantly increased between the start and end of the exposure period, with low variability (*i.e.*, CV up to 11%, Table S10†). Moreover, the mean root mass in plants exposed to control soil (1.02 ± 0.05 g dm) was significantly higher ($p < 0.05$) than that of roots exposed to both 400 mg Pd-doped PS-P kg^{-1} (0.89 ± 0.07 g dm) and 4000 mg Pd-doped PS-P kg^{-1} dm (0.74 ± 0.08 g dm), suggesting inhibited root growth, influenced by the increasing soil concentration of PS-P. In contrast, no significant differences were observed in shoot development in exposed plants (Table S10†). Here, potential negative effects due to Pd leached from the NPs or surfactants/residues from the synthesis of the stock dispersion had been previously ruled out.[13] Future studies comparing the doped and undoped PS–Ps of comparable size are recommended to further isolate the contribution of the model particle matrix itself to the observed phytotoxic effects.

Previous studies have reported both the presence and absence of phytotoxicity in hydroponic systems, with

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outcomes strongly dependent on the tested dose, NP composition and the ability of plant species to regulate the antioxidant level and express genes related to oxidative stress.[13,17,43–45] In addition, toxicity might also be induced by the physical blockage of root pores.[46] In this regard, while the RHIZOtest is not specifically designed for long-term phytotoxicity assessment due to its simplified design and short exposure period, the observed reduction in root biomass even after short-term exposure is noteworthy. The early manifestation of toxic effects suggests that physiological responses were already active at the onset of uptake, thereby adding relevance and realism to the quantification of Pd-doped PS-P phytoavailability under these conditions.

4. Environmental relevance

When investigating the phytoavailability of NPs for plants, it is essential to account for rhizosphere processes to ensure environmental realism. However, most available exposure studies have relied on hydroponic systems, which, while allowing precise control over exposure concentrations, lack key features of natural soil–plant interactions. Soil-based experiments offer more realistic exposure scenarios. However, they are often not well mastered as limited by experimental complexity, including difficulties in achieving homogeneous soil test portions (particularly in ensuring even distribution across replicates when using model particles), synchronizing plant developmental stages, maintaining adequately sized soil pots to support healthy long-term growth, and preventing contamination during root sampling.[47] In particular, root sampling from soil can damage fine root structures or leave residual soil particles, potentially causing an underestimation or overestimation of the NP content, respectively, and increase the overall variability of the results.

The RHIZOtest method effectively integrates the advantages of both hydroponic and soil-based approaches. It ensures reliable results through hydroponic pre-growth, which standardizes the plant developmental stage across replicates, and the use of a 30 μm mesh that enables clean root–soil separation at harvest, minimizing mechanical damage and contamination risks. These features improve the reproducibility and accuracy of NP quantification among replicates and comparability between treatments. Furthermore, the method is environmentally relevant as it enables exposure to soil while retaining rhizosphere processes that are excluded in hydroponics.

The present study demonstrated the method's applicability for testing model plastic nanoparticle uptake in artificial soil by tomato plants confirming good mass balance recovery and limited variability across replicates, showing the occurrence of root uptake and some degree of phytotoxicity. The method does not require a specific analytical technique for subsequent NP determination, though the use of metal-doped PS nanoparticles, in this case, greatly eased their quantification in the system compared to other analytical detection methods.

Besides, the use of a standardized artificial soil, specific model nanoparticles, and a single crop species limits the broader applicability of the outcomes provided by this study. Nevertheless, the proposed procedural adaptation of the RHIZOtest shows potential as a standardized exposure platform to support the investigation of NP uptake and translocation in plant–soil systems and their influencing factors, by variating parameters such as site-specific soils, plant species, or different NPs, either model or real, and differing in the composition and morphology, as well as the introduction of additional soil stressors.

Furthermore, it is important to highlight that the short-term exposure of the RHIZOtest might not be comparable to more realistic long-term tests that cover a whole vegetation period. Similarly to what has been observed for microplastics, aging in soil can also likely modify the chemical and physical properties of NPs, thus influencing their mobility and phytoavailability in the rhizosphere.[42] The short-term exposure time usually used in RHIZOtest experiments is imposed by the geometry of the system. The root-mat approach, despite the above mentioned inherent advantages, is artificial by design and does not allow us to grow plants for more than about ten to twenty days. Accordingly, it is clearly impossible to envisage growing plants with this procedure for several weeks to months up to crop maturity (*e.g.* mature fruits or grains). This inherent limitation is the counterpart of its many advantages and notably of the ability to standardize the exposure of the whole plants to soil contaminants while accounting for and measuring rhizosphere and plant physiological processes involved as phytoavailability drivers.

In this context, the RHIZOtest is not intended to replace long-term field experiments, which remain the most environmentally relevant scale of investigation. However, it can serve as a complementary tool, either as a preliminary screening method to identify conditions most suitable for field-scale testing, or as a follow-up to field trials, which are often limited in scope, to extend the range of environmentally relevant scenarios assessed.

Within this scope, based on the results of this study and thanks to its discussed inherent advantages, its adaptability to conditions that retain ecological relevance, and standardization under ISO guidelines, the RHIZOtest can be considered as a promising platform for the routine high-throughput investigation of NP plant uptake that can be widely implemented in research and in regulatory applications, ultimately improving risk assessment frameworks for nanoplastics in terrestrial ecosystems.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its ESI.†

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Conflicts of interest

There are no conflicts to declare. In the interest of transparency, M. N. Bravin wishes to disclose that his research on the environmental assessment of soil contamination using the RHIZOtest is marginally funded through a partnership between Cirad and Ginger Burgeap, a company that employs the RHIZOtest for commercial purposes.

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