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Supplementary Material

Isotopic labelling for sensitive detection of nanoparticle uptake and translocation in plants from hydroponic medium and soil

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Characteristics of soil used for plant cultivation

Soil from the Weizmann Institute of Science, Rehovot, Israel was used for cultivation of tomato plants. The soil was characterized as loamy sand (Goykhman et al. 2018) according to standard procedures (Gavlak et al. 2005); the results are summarized in Table S1.

Table S1
Properties of soil from the Weizmann Institute of Science, Israel.

Properties	Methods	Results
Composition	Hydrometer method	89% ± 3% sand 7% ± 4% silt 3% ± 1% clay
pH	DDW	7.97 ± 0.01
	DDW+$CaCl_2$	7.41 ± 0.02
Porosity	Saturated column method	0.39 ± 0.03
Organic matter (%)	Loss on ignition	0.5% ± 0.2%
CEC composition [meq/100g]	Na^+	0.27
	K^+	0.13
	Mg^{2+}	1.21
	Ca^{2+}	8.71
Total CEC [meq/100g]	Ammonium replacement method	10.32
Soil carbonates (CO_3-C%)	Gravimetric method	0.14 ± 0.02
Dissolved Organic Carbon [ppm]	UV at 254 nm	± 0.06

Possible toxicity of components in ^{65}Cu-NP suspension to plants

Due to high toxicity of ^{65}Cu-NPs on growth of some plants, the concentration of boron in freshly synthesized ^{65}Cu-NP suspensions was measured with ICP-MS. Sodium borohydride is a prime ingredient in the process of synthesis of ^{65}Cu-NP. The concentration of PEI (another prime ingredient in synthesis of ^{65}Cu-NP) was not possible to estimate quantitatively. Also, according to the literature, PEI must not be present in original form after synthesis of a ^{65}Cu-NP suspension. PEI is the binding agent that stabilizes ^{65}Cu-NP in the suspension and is not present as a free compound in the suspension. Fig. S1 shows the concentration of boron in suspensions of ^{65}Cu-NP at different concentrations.

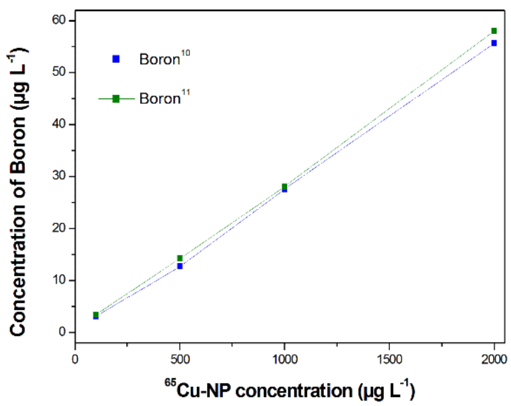

Fig. S1. Concentration of boron in suspensions of synthesized ^{65}Cu-NPs, at concentrations of 100, 500, 1000 and 2000 µg L^{-1}, analyzed with ICP-MS.

EDS spectra analysis

Fig. S2 shows the EDS spectrum for the ^{107}Ag-NPs, ^{65}Cu-NPs and ^{70}ZnO-NPs observed with TEM analysis in remnant media (25% Hoagland's medium) after harvesting the plants, and confirms the presence of the respective metals. Fig. S3 shows the presence of Ag in tomato plant root tissue matrix, along with other biogenic background elements. The peak of Ag is notably smaller than other elements in the spectrum, as the Ag was identified as a tiny particle amongst all other elements present in abundance in the complex plant matrix.

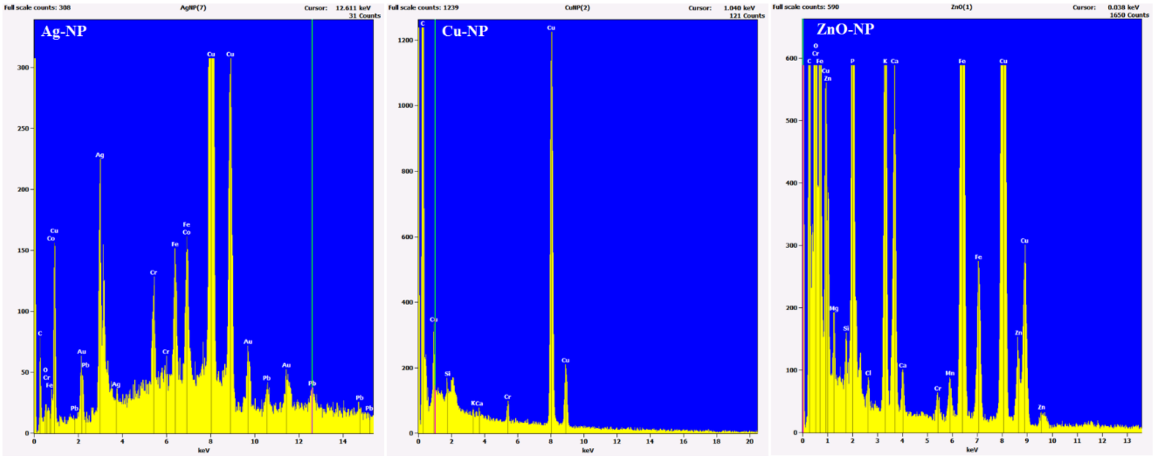

Fig. S2. EDS spectra showing presence of Ag, Cu and Zn in the spotted particles of ^{107}Ag-NPs, ^{65}Cu-NPs and ^{70}ZnO-NPs found scattered in remnant hydroponic medium.

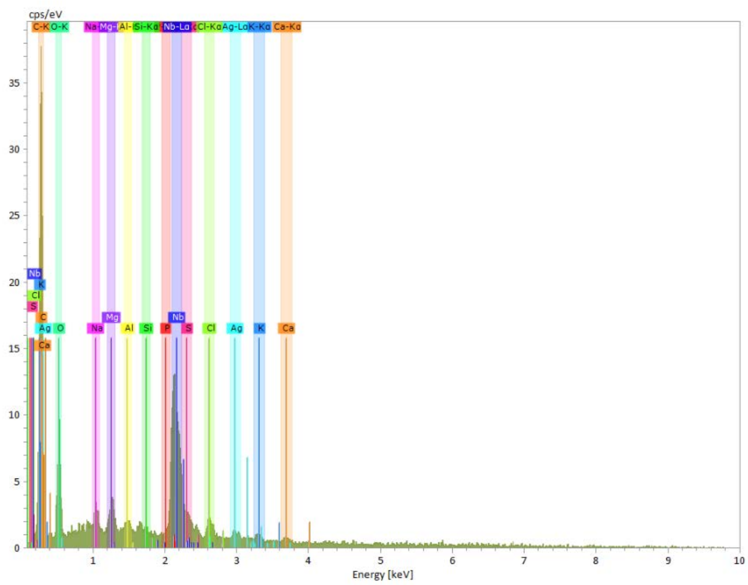

Fig. S3. EDS spectrum showing presence of Ag in tomato plant root tissue matrix, along with other more abundant biogenic background elements.

Total metal concentration in plants under different conditions

Uptake of NPs/metals in different plants under hydroponic cultivation and in soil cultivation is discussed in the main manuscript. Figures 1 and 4 shown in the main manuscript enable comparison of the total metal concentration in different plants and different parts of plants. However, for reference to the exact concentrations of the respective metals/isotopes found in the plants in these studies, the data are shown in Tables S2 and S3.

Table S2. Total concentration of respective metal isotopes (mg kg^{-1} biomass) in shoots and roots of three different plants exposed to isotopically-labeled nanoparticles under hydroponic conditions.

The data represent mean and standard error (n = 3).

Plants	Sample	Total concentration of respective metals (in mg kg^{-1})		
		^{107}Ag	^{65}Cu	^{70}Zn
Arabidopsis thaliana	Shoots- control	0.269±0.071	0.982±0.150	2.236±0.364
	Shoots treated with NPs	0.952±0.345	15.609±0.833	1279.320±63.847
	Roots- control	0.281±0.063	2.548±0.321	3.651±0.223
	Roots treated with NPs	1705.656±124.35	6539.853±365.291	3858.242±165.383
Solanum lycopersicum (tomato)	Shoots- control	0.295±0.056	1.215±0.256	2.329±0.867
	Shoots treated with NPs	1.927±0. 182	11.381±1.534	194.993±41.280
	Roots- control	0.437±0.021	3.410±0.492	4.479 ±0.346
	Roots treated with NPs	348.228±34.058	4944.278±526.447	1166.906±104.341
Phragmites australis (common reed)	Shoots- control	0.240±0.061	1.996±0.215	2.926±0.824
	Shoots treated with NPs	2.797±0.557	9.643±1.524	182.207±24.583
	Roots- control	0.324±0.092	3.628±0.356	3.803±0.532
	Roots treated with NPs	432.099±23.985	2835.520±16.229	1058.041±27.406

Table S3. Concentration of metals/isotopes from respective nanoparticles in different parts of tomato plants and soil (in mg kg^{-1} of biomass) analyzed with ICP-MS after acid digestion. Tomato plants were grown in soil under greenhouse conditions. Soil Layer A represents the top layer of soil (~0-2.8 cm), Soil Layer B represents the middle layer of soil (~2.8-5.6 cm), Soil Layer C represents the bottom layer of soil (~5.6-8.5 cm). The data represent mean and standard error (n = 4 for control; n = 6 for plants exposed to NPs).

Part of plant	Sample	Conc. of ^{107}Ag (mg kg^{-1})	Conc. of ^{65}Cu (mg kg^{-1})	Conc. of ^{70}Zn (mg kg^{-1})
Roots	Control	1.26±0.12	10.9±0.7	7.11±0.71
	Treated with NPs	5.76±0.29	24.9±0.5	15.28±0.82
Stems	Control	0.82±0.07	1.08±0.71	5.06±0.82
	Treated with NPs	0.96±0.15	1.36±0.45	7.78±0.69
Leaves	Control	0.84±0.13	2.78±0.40	5.01±0.44
	Treated with NPs	0.86±0.06	3.54±0.41	18.94±1.02
Fruits	Control	0.88±0.09	0.84±0.12	4.94±0.72
	Treated with NPs	1.26±0.22	1.17±0.29	5.58±0.56
Soil Layer A	Control	0.04±0.02	3.60±0.84	2.65±0.82
	Treated with NPs	1.29±0.08	4.39±0.42	6.71±0.78
Soil Layer B	Control	0.04±0.02	2.22±0.63	2.76±0.53
	Treated with NPs	0.15±0.01	2.51±0.42	4.18±0.81
Soil Layer C	Control	0.04±0.01	2.34±0.75	2.74±0.42
	Treated with NPs	0.06±0.02	1.98±0.89	2.99±0.35

Analysis of NPs suspended in plant media

The hydrodynamic diameter (average size) and polydispersity index (PDI) of the NPs suspended in 25% Hoagland's media and 50% Murashige-Skoog media were determined by dynamic light scattering (DLS) at 25 °C in a Malvern Zetasizer Nano ZSP. The NPs suspended in plant media were analyzed for changes at the start of each experiment (before exposure to plants) and at the end (after exposure to plants), where samples were filtered with Millex-GV PVDF filter unit, size 4.5 µm, before analysis. Overall surface charge and stability of the NPs suspended in the plant media were also estimated through ζ-potential measurements in Malvern, Zetasizer Nano ZSP. Changes in pH were also recorded initially and at the end of experiments. The results of both types of media exposed to different plants are shown in Tables S4 and S5, respectively.

Table. S4. The average size and ζ-potential measurements of NPs suspended in 25% Hoagland's medium at the beginning of experiments (before exposure to *Arabidopsis* plants) and at the end of experiments (after exposure to *Arabidopsis* plants). The data represent mean and standard error (all measurements were done in triplicate).

Sample		DLS		ζ-potential	pH
NPs suspended in Hoagland's media		Average size (nm)	PDI		
Control	Initial	256.78 ± 118.9	1.0	-15.77 ± 0.4	6.229
	End of experiment	275.45 ± 12.63	0.56	-15.23 ± 1.09	6.909
^{107}Ag-NP	Initial	167.9 ± 98.9	0.46	-11.4 ± 0.9	6.507
	End of experiment	192.2 ± 14.5	0.33	-12.2 ± 1.08	6.950
^{65}Cu-NP	Initial	172.9 ± 109.6	0.59	-5.3 ± 0.4	6.323
	End of experiment	612.1 ± 78.4	0.65	-13.4 ± 0.9	6.566
^{70}ZnO-NP	Initial	141.5 ± 80.05	1.00	-16.1 ± 0.5	6.295
	End of experiment	452.2 ± 128.33	0.53	-11.03 ± 2.32	6.813

Table. S5. The average size and ζ-potential measurements of NPs suspended in 50% Murashige-Skoog medium at the beginning of experiments (before exposure to common reed plants) and at the end of experiments (after exposure to common reed plants). The data represent mean and standard error (all measurements were done in triplicate).

Sample		DLS		ζ-potential	pH
NPs suspended in Murashige-Skoog media		Average size (nm)	PDI		
Control	Initial	387.33 ± 89.4	0.42	-3.85 ± 0.98	5.785
	End of experiment	273.35 ± 11.24	0.39	-4.47 ± 2.3	3.418

^{107}Ag-NP	Initial	270.15 ± 70.64	1.0	-5.77 ± 3.4	5.997
	End of experiment	166.96 ± 10.43	0.47	-6.05 ± 0.66	4.119
^{65}Cu-NP	Initial	301.33 ± 58.2	0.58	-6.87 ± 2.27	5.838
	End of experiment	196.03 ± 43.22	0.61	-4.65 ± 0.74	3.159
^{70}ZnO-NP	Initial	212.4 ± 10.9	0.26	-10.8 ± 0.4	6.154
	End of experiment	195.3 ± 59.0	0.88	-4.27 ± 0.38	3.632

References

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