Fate of ZnO nanoparticles in soils and cowpea (*Vigna unguiculata*)

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Supporting Information

Table S1. Characteristics of the two soils used in this study

Property	Oxisol	Ultisol
pH (1:5 water)	6.7	5.0
pH (1:5 $CaCl_2$)	6.1	4.1
Electrical conductivity (dS/m) (1:5 water)	0.15	0.02
Electrical conductivity (dS/m) (saturation extract)	1.1	0.2
Chloride (mg/kg)	23	10
Nitrate N (mg/kg)	24	3.5
Ammonium N (mg/kg)	4.5	1.7
Phosphorus - Colwell (mg/kg)	30	<5.0
Phosphorus - Olsen (mg/kg)	8.85	<2.00
Phosphorus Buffer Index (mg/kg)	560	66.2
Calcium ($cmol_{(c)}$/kg)	7.5	0.6
Potassium ($cmol_{(c)}$/kg)	0.61	0.21
Magnesium ($cmol_{(c)}$/kg)	4.7	0.3
Sodium ($cmol_{(c)}$/kg)	0.29	<0.022
Aluminium ($cmol_{(c)}$/kg)	0	1.2
Effective cation exchange capacity ($cmol_{(c)}$/kg)	13.1	2.33
Exchangeable sodium percentage (%)	2.2	<0.94
Aluminium saturation (%)		52
Copper (DTPA-extractable) (mg/kg)	0.66	0.026
Iron (DTPA-extractable) (mg/kg)	13	73
Manganese (DTPA-extractable) (mg/kg)	12	1.8
Zinc (DTPA-extractable) (mg/kg)	12	2.4
Total Zinc (aqua regia digestion) (mg/kg)	118	9.0
Boron (Hot $CaCl_2$) (mg/kg)	1.3	0.41
Sulfate Sulfur (KCl-extractable) (mg/kg)	52	2.3
Organic Carbon (%)	1.9	0.62
Color	Red-brown	Red-brown
Texture	Sandy Clay	Sandy Loam
BET Surface area (m^2/g)	8.1	12.0
XRD (Mineralogy)	55% Kaolinite 15% Quartz 12% Hematite 5% Maghemite	86% Quartz 12% Kaolinite 1% Hematite

Table S2. Biomass of various tissues of cowpea harvested after growth for 4 weeks in either soils or solutions containing no added Zn, or Zn added as ZnO-NPs or soluble $ZnCl_2$

Treatment	**Biomass of various tissues of cowpea (g/per plant, dry mass)**					
	Solution culture			Soil culture		
	Root	Stem	leaf	Root	Stem	leaf
Control	0.11	0.25	0.11	0.15	0.21	0.11
ZnO-NPs	0.08*	0.21	0.08*	0.14	0.24	0.12
$ZnCl_2$	0.04*	0.13*	0.04*	0.14	0.22	0.11

Asterisks (*) denote statistically significant ($p < 0.05$) biomasses of each tissue compared to control (one-way analysis of variance, ANOVA).

Table S3. Concentrations of Zn in various tissues of cowpea grown in either solution culture or soil culture and exposed to Zn added as either ZnO-NPs or soluble $ZnCl_2$

Treatment	**Zn concentration, μg/g dry tissue**				**Transfer coefficient**
	Root	stem	leaf	seed	Zn concentration ratio of leaf/root
Solution culture					
ZnO-NPs	44740	583.9	118.6		0.003
$ZnCl_2$	9653*	487.6	139.3		0.014
Soil culture					
ZnO-NPs	1003	118.1	155.0	43.3	0.155
$ZnCl_2$	1175	108.4	180.8	55.7	0.154

Asterisks (*) denote statistically significant ($p < 0.05$) Zn concentrations between ZnO-NPs or $ZnCl_2$ treatment (one-way analysis of variance, ANOVA).

Table S4. Results of principle component analysis (PCA) performed for the 18 bulk-XANES and 18 EXAFS spectra of Zn in soils. The PCA was applied to the whole experimental spectra set of soil samples in order to evaluate the number of relevant components indicated by the indicator function (IND) reaching a minimum. The first four components accounted for 99% of the total variance of the XANES spectra and 79% of the total variance of the EXAFS spectra, and therefore, a maximum of four reference components were permitted for each fit.

Component	Eigen value		Variance		Cumulative Variance		IND* Value	
	XANES	EXAFS	XANES	EXAFS	XANES	EXAFS	XANES	EXAFS
1	129.3	129.1	0.962	0.598	0.962	0.598	0.00308	0.03363
2	2.355	20.87	0.017	0.096	0.979	0.695	0.00176	0.02805
3	1.116	11.07	0.008	0.051	0.988	0.746	0.00108	0.02846
4	0.512	9.872	0.003	0.045	0.992	0.792	0.00083	0.02858
5	0.317	7.449	0.002	0.034	0.994	0.826	0.00066	0.03030
6	0.164	5.852	0.001	0.027	0.995	0.853	0.00066	0.03371
7	0.123	5.338	0.000	0.024	0.996	0.878	0.00069	0.03797
8	0.090	4.741	0.000	0.021	0.997	0.900	0.00078	0.04327
9	0.077	3.881	0.000	0.017	0.997	0.918	0.00091	0.05321
10	0.066	3.427	0.000	0.015	0.998	0.934	0.00111	0.06789
11	0.061	3.244	0.000	0.015	0.998	0.949	0.00137	0.09000
12	0.047	2.865	0.000	0.013	0.999	0.962	0.00189	0.12926
13	0.034	2.878	0.000	0.012	0.999	0.975	0.00320	0.19603
14	0.033	1.903	0.000	0.008	0.999	0.984	0.00662	0.42260
15	0.030	1.766	0.000	0.008	0.999	0.992	0.02153	1.61092
16	0.021	1.610	0.000	0.007	1.000	1.000	NA	NA

*: Indicator value.

Table S5. Target Transformation SPOIL values of selected reference spectra obtained by PCA analysis. Reference spectra are classified as excellent (SPOIL < 1.5), good (1.5-3.0), acceptable (3.0-4.5), poor (4.5-6.0), or unacceptable (> 6.0). The standard compounds with SPOIL values < 3 in EXAFS TT and < 2.5 in XANES TT were included in the subsequent LCF analyses.

References	SPOIL VALUES	
	EXAFS	XANES
ZnAl-LDH nitrate	1.5593	0.7806
Hemimorphite, $Zn_4Si_2O_7(OH)_2 \cdot H_2O$	2.1569	0.7555
ZnAl-LDH silicate	1.7620	0.8604
Hopeite, $Zn_3(PO4)_2$	2.8074	2.2065
Zn substituted ferrihydrite	2.7260	1.1561
Zn humic acid	2.7731	1.5084
ZnAl silicate	2.9285	0.8604
ZnO-NPs	2.4423	0.8727
Zn-phosphate	3.0880	0.7580
Zn silicate	3.3604	0.1627
Zn cysteine	2.2959	2.1743
Willemite, Zn_2SiO_4	3.4338	1.4766
Zn citrate	2.6828	2.8783
Zn sorbed on ferrihydrite	2.8109	1.1072
Zn sulfide	2.6996	5.0716
Smithsonite, $ZnCO_3$	2.9246	2.2551
Zincite, ZnS	2.9582	1.8191
Zn sulfate	2.9616	2.1288
Franklinite	2.7568	1.1339
Wurzite, (Zn,Fe)S	3.4168	3.0510
Sphalerite (Zn,Fe)S	2.9307	6.4164
Zn aqueous	4.5651	1.2864
Gahnite, $ZnAl_2O_2$	4.7398	2.4615
Zn acetate	5.1442	3.0652

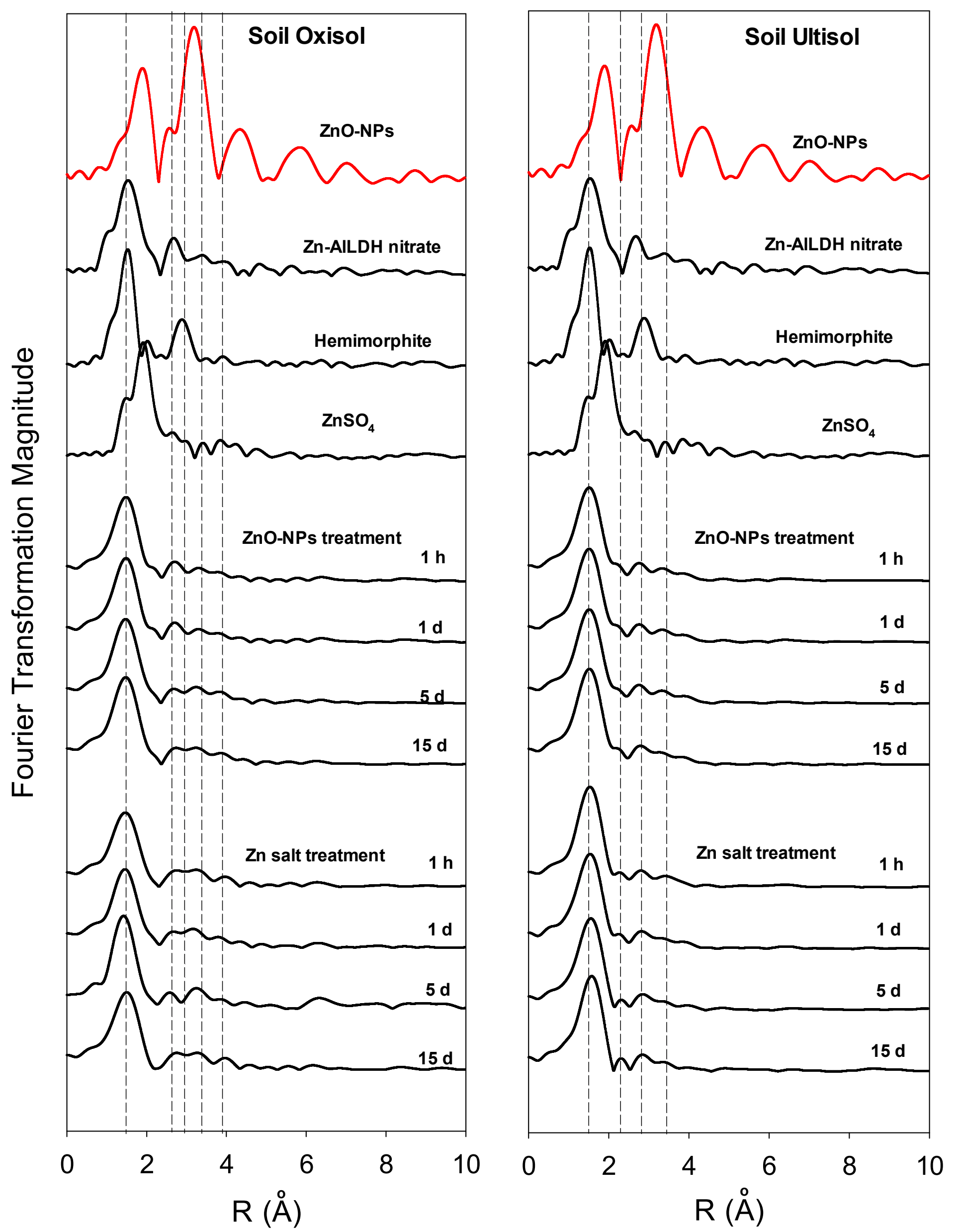

Figure S1. Comparison between the Fourier Transform for EXAFS spectra of Zn standards (ZnO-NPs, ZnAl-LDH nitrate, hemimorphite, and $ZnSO_4$) and soil samples amended with 500 µg kg^{-1} ZnO-NPs or $ZnCl_2$ followed by incubating for 1 h, 1 d, 5 d, 15 d.

The LCF results for EXAFS spectra were also reinforced by the Fourier Transform EXAFS spectra, in which similarities were observed for both soils amended with $ZnCl_2$ and ZnO-NPs. This suggests that the local molecular environments of Zn in both soils were similar and the added ZnO-NPs were transformed to the same species as found in the $ZnCl_2$ treatment. The main shells present in all experimental spectra are similar to the shells for ZnAl-LDH and hemimorphite, but substantially different from the shells for ZnO-NPs.