Electronic Supplementary Information

Accumulation, speciation and uptake pathway of ZnO nanoparticles in maize

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Nine pages, one table and nine Fig.s.

Characterization of ZnO nanoparticles. TEM was carried out with an H-7500 (Hitachi, Japan) at 80 kV, and samples were prepared by sonicating 50 mg L^{-1} ZnO NPs in ethanol solution for 30 min and loading 10 μL aliquots of the above suspension onto carbon-coated grid sample holders. X-ray diffraction (XRD) analysis of ZnO NPs was performed in reflection mode on an X'Pert PRO MPD (PANalytical) diffractometer using a Cu$K\alpha$ (λ = 0.154 nm) radiation in the scan range of 5-80°2θ. The pH values of the isoelectric point (IEP) of ZnO NPs were obtained from their zeta potentials as functions of pH ranging from 6.5 to 11.0 in 0.01 M $NaNO_3$ solution detected by Malvern Nano ZS (Malvern Instruments, Malvern, UK) and the hydrodynamic radii of ZnO NPs in solution were measured simultaneously.

Hydroponic cultivation. Seeds of maize (*Zea mays* L. cv. Zhengdan 958) were germinated on moist filter paper for 5 days after sterilization and soaking with water. Uniform seedlings were selected and transplanted in 250 mL crystallizing dishes containing 250 mL of 50 % Hoagland nutrient solution. The crystallizing dishes were wrapped with black paper to exclude light. Each crystallizing dish was covered by a plastic sheet with five holes and one seedling occupied each hole. The open areas between plastic and seedlings were sealed with sponge. Roots of the seedlings were submerged in the nutrient solution. Fresh air was supplied continuously to the dishes using an air pump. The seedlings grew in the nutrient solution for 1 week before the exposure study. The 50 % Hoagland nutrient solution was renewed every 48 h.

X-ray absorption spectrum analysis. Standard XAFS data reduction procedures were undertaken using the program package IFEFFIT and WinXAS v. 3.1was used for data fitting. Simply, background removal, normalization, cubic spline conversion, and forward Fourier transform of the $k^3\chi(k)$ spectra from 2.6 to 11.9 $Å^{-1}$ using Bessel window were performed to obtain the radial distribution function (RDF) in *R*-space.

Ab initio phase and amplitude functions were calculated using FEFF8.2 code to fit the spectra of samples with model structures in ZnO and hopeite ($Zn_3(PO_4)_2.4H_2O$). An amplitude reduction factor S_0 = 0.87 was determined by fitting of ZnO with fixed coordination numbers (CN).

Transmission electron microscopy analysis. Fresh maize tissues were thoroughly washed with deionized water. Sections about 2 × 2 mm at different parts of tissues were cut. These sections were prefixed in 2 % glutaraldehyde for 2 h, washed in 0.1 mol/Lphosphate buffer (PBS, pH 7.2), and postfixed in 1 % osmium tetroxide for 2 h. Samples were dehydrated with sequential treatment with 50, 70, and 90 % ethanol, 90 % ethanol and acetone mixture (1:1), and 90 and 100 % acetone for 15 min. The samples were then infiltrated and embedded in Quetol 812 epoxy resin (Nisshin EM, Tokyo, Japan) (treatment with 2:1, 1:2 of acetone/epoxy resin mixtures for 4 h and 8 h respectively, and 100 % Spurr's resin for 24 h). Polymerization was carried out for 12 h at 37 °C, 12 h at 45 °C, and 24 h at 60 °C. An ultramicrotome (Reichert OM U2) was used to slice the resin-fixed sample into thin sections (70 nm thick). The sections were deposited on a TEM grid and observation and EDS analysis were carried out using a JEOL JEM 1010 (Tokyo, Japan).

Table S1 Fitting parameters of Zn K edge XANES-LCF of plant samples.

	Zn-O(H) (%)	Zn-Phos		NSS (%)
		Zn phosphate (%)	Zn_{ads}-Phos	
Zn^{2+} root	5	26	69	0.008
Zn^{2+} shoot	6	70	24	0.008
ZnO root	24		76	0.011
ZnO shoot	6	83	12	0.015

Zn phosphate including $Zn_3(PO_4)_2$ and Zn phytate;Normalized sum-squares residual: NSS = $100*\sum[data_{exp} - data_{fit}]^2 / \sum[data_{exp}]^2$.

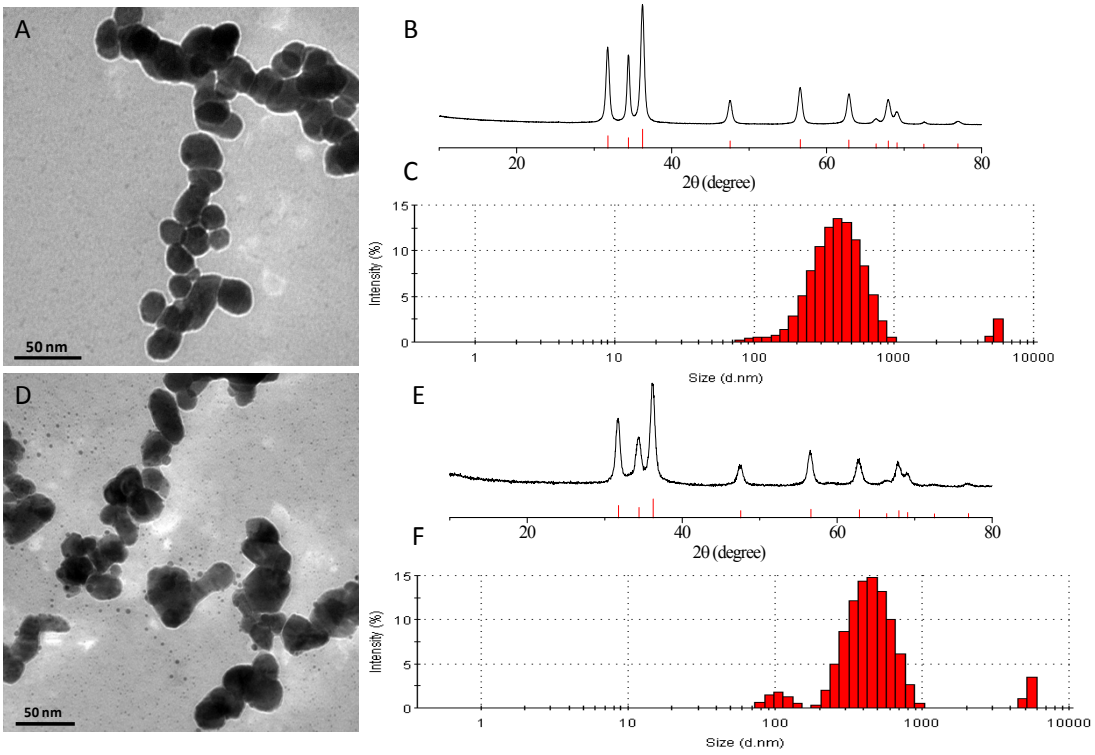

Fig. S1. TEM images, XRD patterns and hydraulic size distribution of ZnO NPs (A, B, C) and ARS-Zn O NPs (D, E, F) respectively.

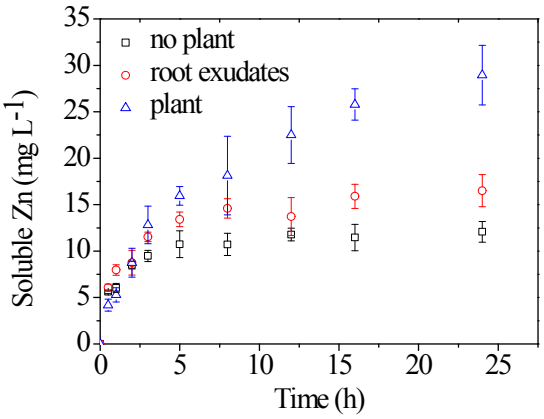

Fig. S2. Dissolution of ZnO NPs (100 mg L^{-1}) in hydroponic medium as a function of exposure time.

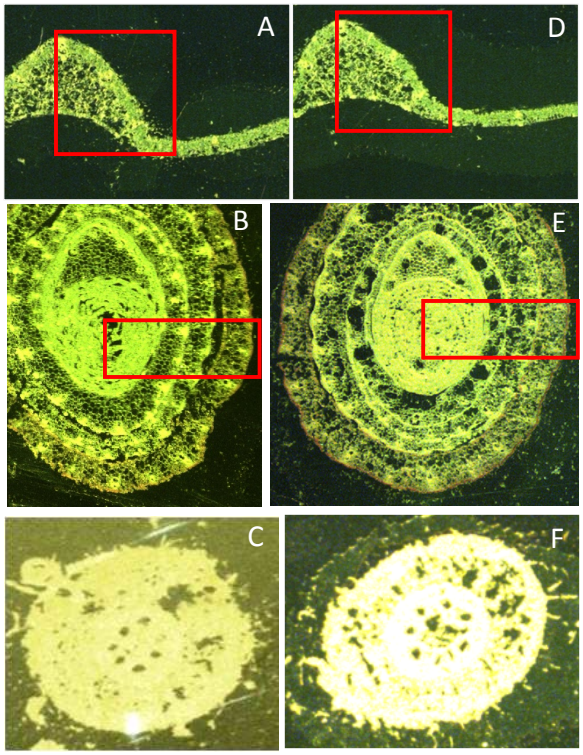

Fig. S3. Optical microscopy images of in leaf, stem, and root section of maize seedlings exposed to 30 mg Zn^{2+} L^{-1} (A, B, C) and 100 mg ZnO NPs L^{-1} (D, E, F), respectively. Red square is the relevant scan area of synchrotron μ-XRF microprobe imaging.

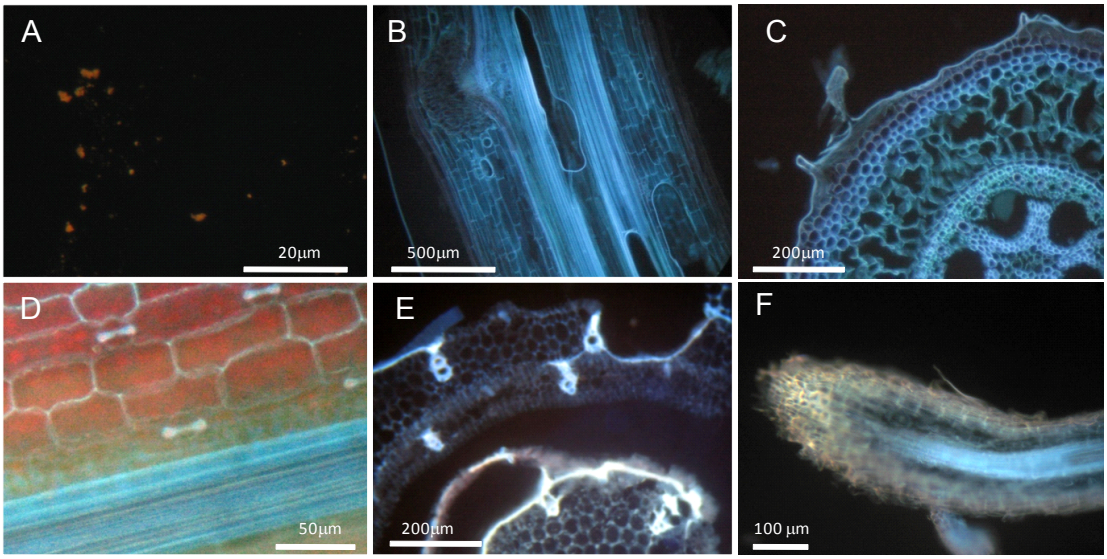

Fig. S4. Optical fluorescence microscopy images of ARS labeled ZnO NPs aggregates (A); longitudinal (B) and cross (C) sections of maize root exposed in ARS alone, purple represents the area of ARS accumulation; stem cross-section (D) , leaf (E) and root tip（F）of maize exposed in ARS labeled ZnO NPs and no ARS labeled ZnO NPs were found.

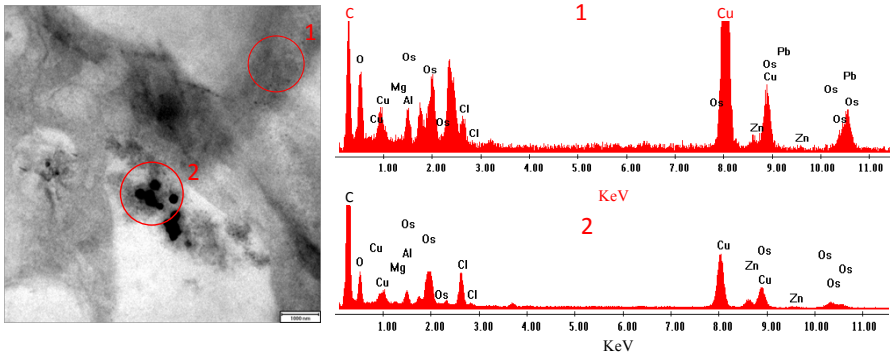

Fig. S5. Representative EDS spectra of background (1) and dense dots (2) in TEM images.

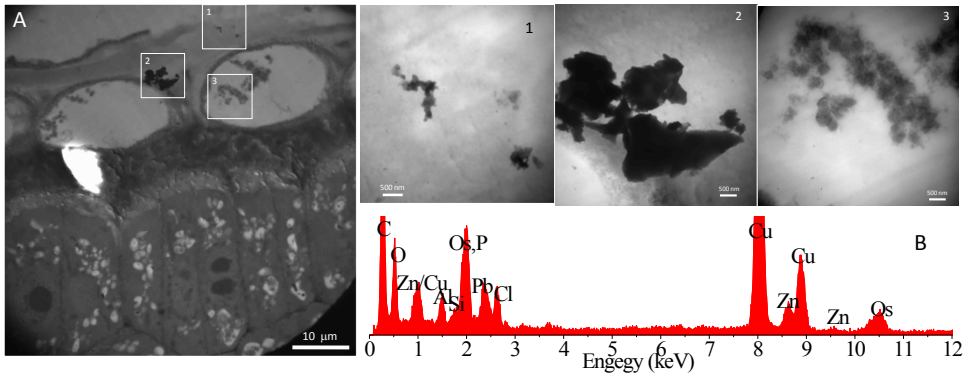

Fig. S6. TEM images of ZnO NPs and their aggregates observed in the surface and epidermis of root (A) and representative EDS spectra (B); 1, 2, 3 are enlargements of the panes in A.

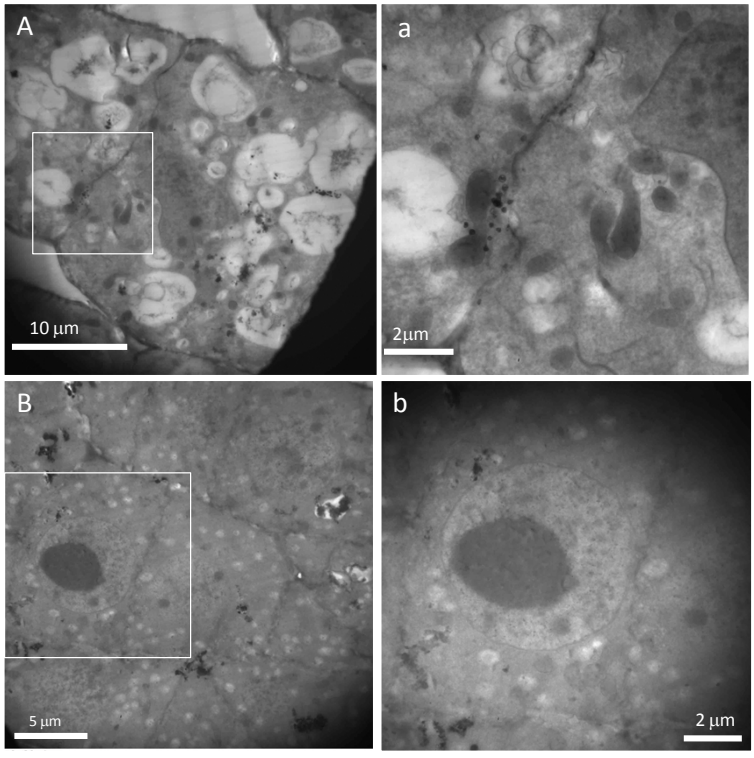

Fig. S7. TEM images of root tip sections under ZnO NPs treatments; a and b are enlargements of the panes in A and B.

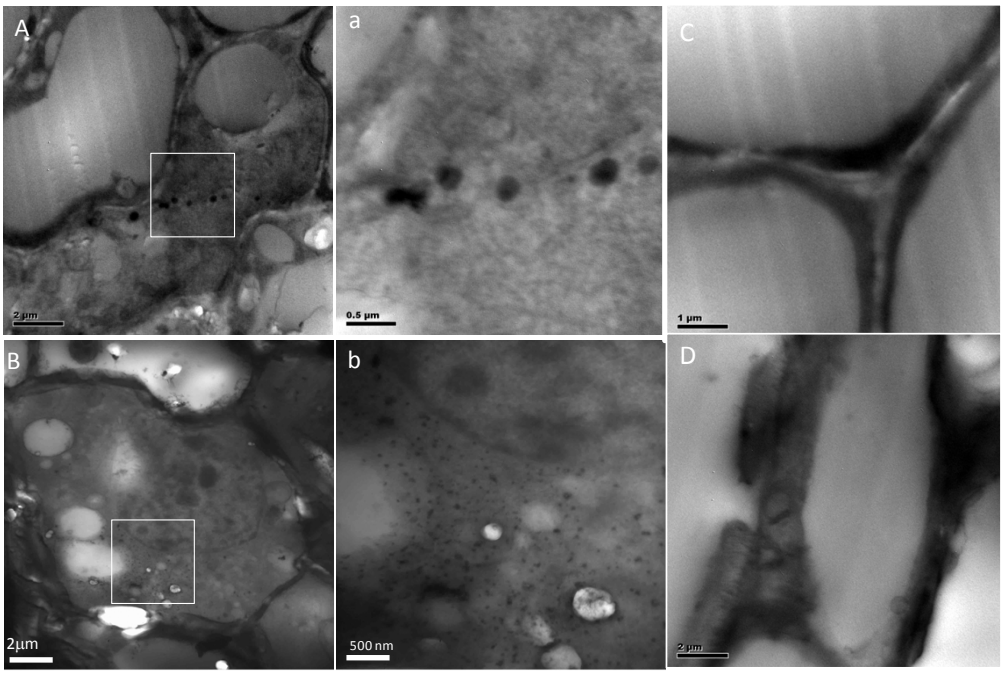

Fig. S8. TEM images of root (A, B) sections in maturation zone of root and stem sections (C, D) under 100 mg L^{-1} ZnO NP treatments; (A) is the image of cells in the cortex, (B) is image of cells near the area of main root-lateral root junction, a and b are enlargements of the panes in A and B.

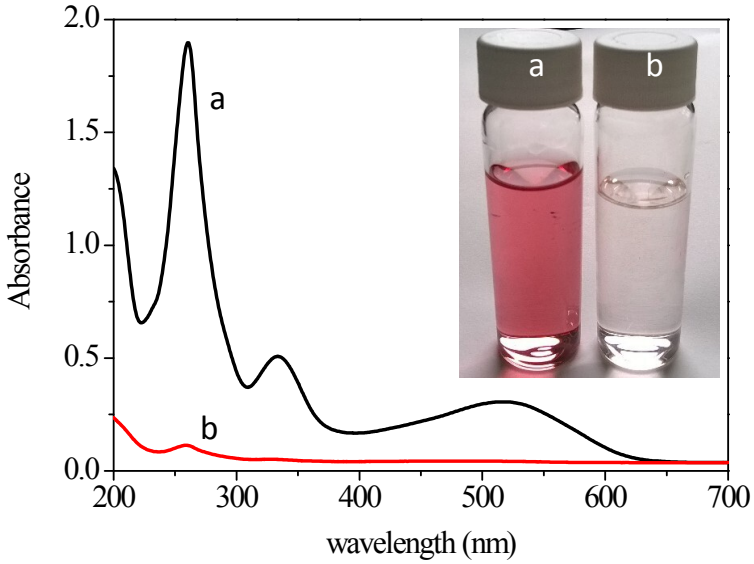

Fig. S9. UV-vis spectra of soluble ARS 3 mg L^{-1} (a), and ARS rleased from 100 mg L^{-1}ARS-ZnO NPs.