Supporting Information

Stress Response and Tolerance of *Zea mays* to CeO_2 Nanoparticles: Cross Talk among H_2O_2, Heat Shock Protein and Lipid Peroxidation

Lijuan Zhao,[†,§] Bo Peng,[Δ] Jose A. Hernandez-Viezcas,[†,§] Cyren Rico,[†,§] Youping Sun,[⊥] Jose R. Peralta-Videa,[†,§], Xiaolei Tang,[Δ] Genhua Niu,[⊥] Lixin Jin,[#] Armando Varela-Ramirez,[Δ] Jian-ying Zhang,[Δ] Jorge L. Gardea-Torresdey[†,¶*,§]

[†]Chemistry Department, The University of Texas at El Paso, 500 West Univ. Av., El Paso, TX 79968

[¶]Environmental Science and Engineering PhD program, The University of Texas at El Paso, 500 West Univ. Av., El Paso, TX 79968

[Δ] Department of Biological Sciences, Border Biomedical Research Center, The University of Texas at El Paso, 500 West Univ. Av., El Paso, TX 79968

[#]Department of Geology Sciences, The University of Texas at El Paso, 500 West Univ. Av., El Paso, TX 79968

[⊥] Texas AgriLife Research Center at El Paso, Texas A&M University System, El Paso, TX 79927

[§]UC Center for Environmental Implications of Nanotechnology (UC CEIN), The University of Texas at El Paso, USA

CAT Assay: Extractions were performed in a cold room at 4°C and a ratio of 10% w/v of sample was homogenized with phosphate buffer (25 mM KH_2PO_4 at pH 7.4). The sample was centrifuged for 5 minutes at 2000 rpm on a bench centrifuge (Eppendorf AG bench centrifuge 5417 R, Hamburg, Germany). A sample of 50 µL of the supernatant and 950 µL of 10mM H_2O_2 was placed in a quartz cuvette to obtain a final volume of 1 mL. The mixture was shaken by hand (three times) and the absorbance was measured for three minutes at 240 nm using a Perkin Elmer Lambda 14 UV/Vis spectrometer (single beam mode, Perkin Elmer, Uberlinger, Germany). The absorbance values were obtained from the first linear section of slope between 0.5 and 2 min. The extinction coefficient for H_2O_2 was set at 23.148 mM^{-1} cm^{-1}.

APX Assay: Aliquots of 886 µL of 0.1 M KH_2PO_4 buffered at pH 7.4, 10 µL of 17 mM H_2O_2, 100 µL of the sample, and 4 µL of a 25 mM solution of ascorbate were placed in a quartz cuvette for a total volume of 1 mL. The change in absorbance was recorded for two minutes at 265 nm in a Perkin Elmer Lambda 14 UV/Vis spectrometer. The absorbance was recorded as described above and the extinction coefficient was experimentally set at 22.21 mM^{-1} cm^{-1}.

QB buffer preparation: A volume of 100 mL of the buffer solution was obtained by adding 5 ml of 2 M KPO_4 (pH 7.8), 200 µl of 0.5 M EDTA, 1 ml of Triton X-100, 12.5 ml of 80% Glycerol, and 81.1 ml of H_2O. Immediately before using, we added 100 µl of 1.0 M of DTT. The QB buffer was stored at -20°C.

Microscopy Observations: 20 day-old corn plants grown in soil were gently removed from de soil and thoroughly washed with deionized water. Subsequently, the plants were treated for 24 h with a suspension of CeO_2 NPs at 800 mg/L, harvested and prepared for microscopy observation. The samples were examined and photographed in a Zeiss EM-10 TEM, ACCF (Peabody, MA) at an accelerating voltage of 60 or 80 kV.

Root samples of 1 cubic mm were fixed in 2.0% (wt/vol) paraformaldehyde and 2.5% (v/v) glutaraldehyde in 0.06 M HEPES buffer, pH 7.4, for one h at room temperature, under continuous shaking. Sample specimens were washed three times for 15 min each with cold 0.06 M HEPES buffer and then post-fixed with 1% (wt/v) osmium tetroxide, 0.05 M potassium ferrocyanide in 0.12 M HEPES buffer for one h in the cold (-4^{o} C). Specimens were then washed in 0.06 M HEPES buffer followed by three washings (15 min each) with distilled water and then *en bloc* stained in the refrigerator with 0.5% (wt/v) aqueous uranyl acetate for one h. Specimens were then dehydrated with ethanol at increments of 10% (starting with 30% ethanol) followed by 100% acetone. Specimens were infiltrated with, and embedded in, Poly Bed 812 (Polysciences, Warrington, PA). The cross sections (100 nm thick), above the root tips, were cut for TEM examination using a microtome with a diamond knife.

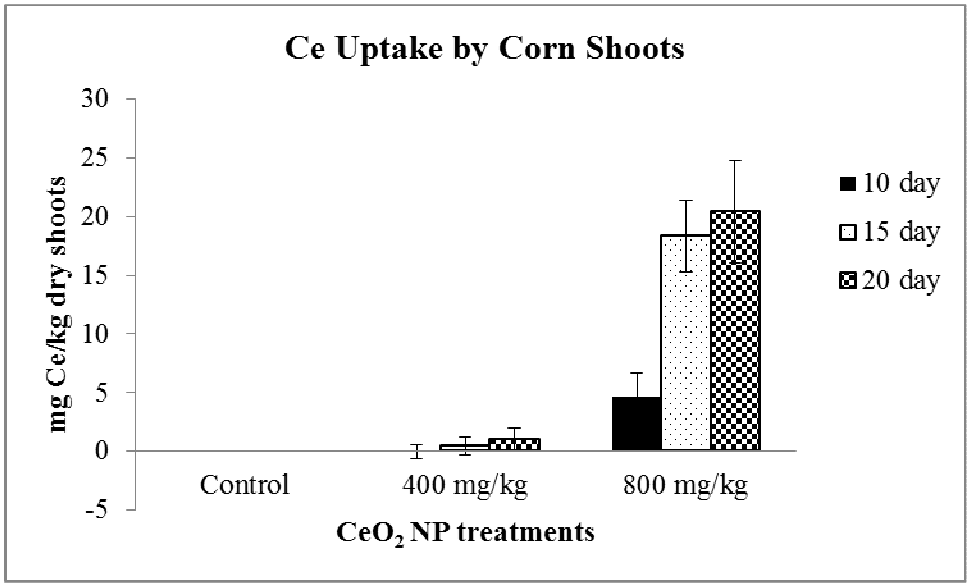

Figure S1. This figure shows the concentration of cerium in the shoots of soil grown corn plants, at different growth stages, exposed to CeO_2 NPs at 400 and 800 mg/kg.

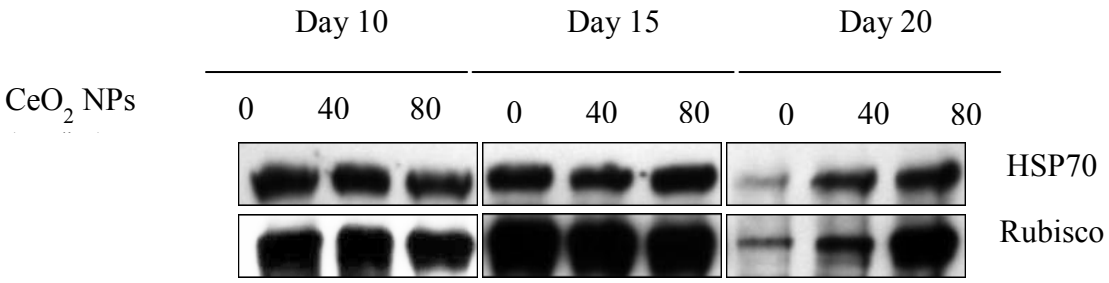

Figure S2. Western-blot analysis of HSP70 expression level in corn leaves. Leave proteins were extracted and aceton precipitated. Equal amount of proteins (20 μg) were loaded to each lane and resolved by SDS-PAGE. After being transferred to nitrocellulous membrane, HSP70 was detected with rabbit anti-cytoplasmic HSP70. Expression level of HSP70 was normalized to the expression level of Rubisco (internal control).

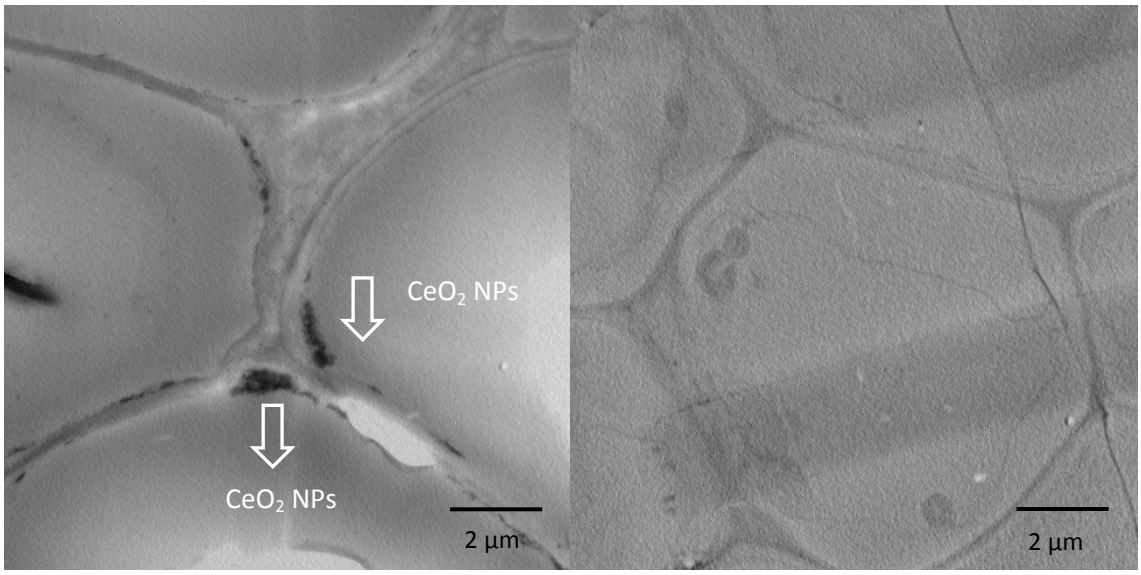

Figure S3. TEM images showing (**A**) CeO_2 NPs deposited on the internal part of the cell wall. (**B**) Control.

Previous X-ray microfluorescence studies (*J. Hazard. Mater.l* **2012**, 225, 131-138) have shown that CeO_2 NPs are stored without biotransformation within corn roots. Also, Ma et al. (*Chemosphere* **2010**, *78*, 273-279) did not mention dissolution of CeO_2 NPs.