Electronic Supplementary Information (ESI) for

Cerium oxide nanoparticles transformation at the root-soil interface of barley (*Hordeum vulgare* L.)

Cyren M. Rico,[1,2,4*] Mark G. Johnson,[2] Matthew A. Marcus,[3]

[1]National Research Council, Research Associateship Program, 500 Fifth Street, NW, Washington, DC 20001, USA

[2]US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Western Ecology Division, 200 SW 35th St., Corvallis, OR 97333, USA

[3]Advanced Light Source, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720, USA

[4]Missouri State University, Department of Chemistry, 901 S National Ave., Springfield, MO 65897, USA

[*]Corresponding author. Tel: 417 836 3304; Fax: 417 836 5507; E-mail address: CyrenRico@MissouriState.edu (C. M. Rico)

Journal: Environmental Science: Nano

Prepared on: March 1, 2018

9 pages in length, including 2 tables

CeO_2-NPs amendment to soil

Plastic pots (6 cm diameter × 6 cm high) were filled with 0.549 kg wet potting soil (MiracleGro® potting soil) to get an equivalent of 300 g dry weight soil. CeO_2-NPs were added to the soil to achieve a concentration of 250 mg CeO_2-NPs/kg soil. Briefly, 75 mg CeO_2-NPs were suspended in 100 mL Millipore water, sonicated for 30 min in a water bath (Branson Ultrasonics, Danbury, CT), and poured into the soil. The CeO_2-NPs amended soil was prepared three days before seedling transplantation. Control pots were amended with Millipore water only.

Plant growth conditions

Barley seeds were germinated in a growth chamber for nine days before two seedlings were transplanted in one pot. Sufficient water was added to each pot to keep the soil moist and to prevent loss of water from leaching. However, any leachate generated was collected and added back to the top of the pot. Barley seedlings were cultivated in soil for 60 days as previously described.[1] The plants were allowed to grow in the growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) with conditions maintained at 16-h photoperiod, 20/10˚C, 70% humidity, 300 µmol/m^2-s for the first 40 days, after which the conditions were kept at 16-h photoperiod, 25/15˚C, 70% humidity, 600 µmol/m^2-s until harvest. One hundred mL of Yoshida nutrient solution[2] was added to the pots on the day the seedlings were transplanted.

Preparation of root thin sections for synchrotron analysis

Samples for synchrotron analysis was prepared following the method described in Rico et al.[1] as adapted from Langer et al.[3] and Yamaguchi et al.[4]. Briefly, a soil core (column) was

collected by pounding a 2.5 cm × 6 cm (diameter × height) aluminum cylinder into pots. The cylinders were centered over the plant shoot and visible crown to insure collection of shoot, crown and root tissues. The ends of the aluminum tube containing the sample were wrapped in plastic wrap and frozen (-80°C) until used. The frozen soil/plant cores were thawed and embedded with Spurr's Resin, cut in half along the long axis, and 3-5 cm by 7 cm glass slides were glued to the cut surface to cover the entire cut surface on one half of each core. Further processing (i.e. cutting and polishing) produced intact root/soil thin-sections with a thickness of ~100 μm.

Preparation of Yoshida nutrient solution[2]

Stock solution:

Element	Reagent	Weight (g) 10L solution	1L solution	500mL solution	
N	NH_4NO_3	914.00	91.40	45.70	
P	$NaH_2PO_4 \cdot 2H_2O$	403.00	40.30	20.15	
K	K_2SO_4	714.00	71.40	35.70	
Ca	$CaCl_2$	886.00	88.60	44.30	
Mg	$MgSO_4 \cdot 7H_2O$	3240.00	324.00	162.00	
Mn	$MnCl_2 \cdot 4H_2O$	15.00	1.50	0.75	
Mo	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.74	0.07	0.04	
B	H_3BO_3	9.34	0.93	0.47	Dissolve separately, then combine with 50mL conc. H_2SO_4. Make up to required volume.
Zn	$ZnSO_4 \cdot 7H_2O$	0.35	0.04	0.02	
Cu	$CuSO_4 \cdot 5H_2O$	0.31	0.03	0.02	
Fe	$FeCl_3 \cdot 6H_2O$	77.00	7.70	3.85	
	citric acid (monohydrate)	119.00	11.90	5.95	

Preparation of 4L nutrient solution: Mix 5 mL of each of stock solution and make the volume to 4L. Adjust the pH to 5.0~5.1.

Localization and in situ speciation of Ce in intact root-soil profile

To acquire accurate elemental maps, 'multiple-energy' maps of Ce were collected at both 5710 eV (below the Ce L_{III} edge) and 5729 eV (the Ce L_{III} edge). The maps were collected by scanning each line of pixels twice, once at 5710eV and once at 5729eV, an energy chosen to give equal signals from Ce(III) and CeO_2. Energy calibration was such that the first peak for CeO_2 was at 5730.33 eV. Data processing was done using ALS BL10.3.2 software 'process multi-E chem maps' and 'Difference maps' programs, by which the 5710eV map was subtracted from the 5729eV map, and the Ce channel data from that difference combined with the other detector data channels to form a composite map in which Ce and Ti (whose K-edge is below the Ce L_3 edge and whose fluorescence energy is close to that of Ce) are accurately separated from each other. The resulting elemental maps were examined to locate areas and points of interest that contain Ce. Similarly, the chemical maps shown in Figures 2C,D were done by scanning at 5710, 5724.5 and 5746eV and processing using the spectra from CeO_2-NPs and $CeCO_3$ as references.

Cerium L-edge μ-X-ray absorption near edge structure (XANES) spectra were collected from points (spots) of interest in the thin section, based on the apparent presence of Ce at these locations as determined by the μXRF chemistry maps collected near the soil-root interface and on soil particles. Normalization and least squares combination fitting (LCF) were performed with ALS BL10.3.2 software. The proportion of Ce(III) and Ce(IV) at each spot was determined by LCF using ALS BL 10.3.2 software and the normalized intensities of the Ce(III) and Ce(IV) standards.

SI Table 1. Proportions of Ce(III) species transformed from CeO_2-NPs in plants. Reference citations are provided in the main text.

Plant	CeO_2-NPs	Concentration	Exposure media	Exposure duration	Plant parts	Ce LCF analysis	References
Soybean	8-10 nm	1000 mg CeO_2 NPs/kg	Farm soil	48 days	Root nodules	79% CeO_2-NPs + 23% Ce(III) hydroxide	5
					Pods	88% CeO_2-NPs + 12% Ce(III) acetate	
Mesquite	8-10 nm	500 mg CeO_2 NPs/L	Hydroponic	15 days	Root	81% CeO_2-NPs + 19% Ce(III) hydroxide	6
Kidney bean	8-10 nm	500 mg CeO_2 NPs/L	Hydroponic	15 days	Root	88% CeO_2-NPs + 12% Ce(III) acetate	7
Cucumber	6.9 nm	2000 mg CeO_2 NPs/L	Hydroponic	21 days	Root	66% CeO_2-NPs + 34% Ce(III) acetate	8
					Stems	86.4% CeO_2-NPs + 13.6% Ce(III) carboxylates	
					Leaves	78.5% CeO_2-NPs + 21.5% Ce(III) carboxylates	
Cucumber	Octahedral, cubic, commercial	2000 mg CeO_2 NPs/L	Hydroponic	14 days	Root	~80% CeO_2-NPs + ~20% Ce(III) phosphate	9
					Shoot	~80% CeO_2-NPs + ~20% Ce(III) oxalate	
	Rod				Root	~60% CeO_2-NPs + ~40% Ce(III) phosphate	
					Shoot	~60% CeO_2-NPs + ~40% Ce(III) oxalate	
Wheat	Diethylaminoethyl functionalized (CeO_2(+))	20 mg Ce/L	Hydroponic	8 hours	Root	86% CeO_2-NPs + 14% Ce(III)	10
	Dextran coated (CeO_2(0))			34 hours	Root	85% CeO_2-NPs + 15% Ce(III)	
	Carboxymethyl functionalized (CeO_2(+))			34 hours	Shoot	81-94% CeO_2-NPs + 6-21% Ce(III)	
Wheat	8-10 nm	500 mg CeO_2 NPs/kg	soil	91 days	Root	92-98% CeO_2-NPs + 3-7% Ce(III)	1
					Soil	86-94% CeO_2-NPs + 4-12% Ce(III)	
Lettuce	16.5 nm	2000 mg CeO_2 NPs/kg	Sand	21 days	Root	95.7% CeO_2-NPs + 4.3% Ce(III) phosphate	11
					Shoot	96.5% CeO_2-NPs + 3.5%	

-	30 nm	Agricultural soil	20 hours	-	Ce(III) carboxylates 52-99% CeO_2-NPs + 0.6-48% Ce(III) phosphate + 11% Ce(III) oxalate	12
-	78 nm	Agricultural soil		-	94-97% CeO_2-NPs + 2.8-5.8% Ce(III) phosphate	

SI Table 2. Linear combination fits (LCF) of Ce μXANES spectra obtained in barley roots. NSS is the normalized sum-square error of the fit $\sum (y - y_{fit})^2 / \sum y^2$ where y and y_{fit} are the XANES spectrum and fit, respectively.

Root	Spot	Ce LCF analysis	NSS
1	1	89.82% CeO_2-NPs + 11.85% Ce(III)	0.000571
2	1	89.30% CeO_2-NPs + 9.91% Ce(III)	0.000227
	2	84.07% CeO_2-NPs + 15.59% Ce(III)	0.000225
	3	87.10% CeO_2-NPs + 11.80% Ce(III)	0.000273
3	1	88.02% CeO_2-NPs + 11.32% Ce(III)	0.000103
	2	85.40% CeO_2-NPs + 13.29% Ce(III)	0.000130
	3	86.09% CeO_2-NPs + 15.89% Ce(III)	0.000192
	4	87.27% CeO_2-NPs + 12.73% Ce(III)	0.000168
	5	89.60% CeO_2-NPs + 10.40% Ce(III)	0.000286
	6	90.95% CeO_2-NPs + 9.05% Ce(III)	0.000697
	7	39.20% CeO_2-NPs + 60.80% Ce(III)	0.001147
	8	23.01% CeO_2-NPs + 76.99% Ce(III)	0.004574
	9	44.19% CeO_2-NPs + 55.81% Ce(III)	0.002690
	10	1.81% CeO_2-NPs + 98.19% Ce(III)	0.007292

References

1. C. M. Rico, M. G. Johnson, M. A. Marcus, C. P. Andersen, *Environ. Sci.: Nano,* 2017, **4**, 700.
2. S. Yoshida, D. A. Forno, J. H. Cock, K. A. Gomez, Laboratory Manual for Physiological Studies of Rice; International Rice Research Institute: Los Baños, Laguna, Philippines, 1976.
3. P. Langner, C. Mikutta, E. Suess, M. A. Marcus, R. Kretzschmar, *Environ. Sci. Technol.,* 2013, **47**, 9706.
4. N. Yamaguchi, T. Ohkura, Y. Takahashi, Y. Maejima, T. Arao, *Environ. Sci. Technol.,* 2014, **48**, 1549.
5. J. A. Hernandez-Viezcas, H. Castillo-Michel, J. C. Andrews, M. Cotte, C. Rico, J. R. Peralta-Videa, Y. Ge, J. H. Priester, P. A. Holden, J. L. Gardea-Torresdey, *ACS Nano,* 2013, **7**, 1415.
6. J. A. Hernandez-Viezcas, H. Castillo-Michel, J. R. Peralta-Videa, J. L. Gardea-Torresdey, *ACS Sustain Chem. Eng.,* 2016, **4**, 1187.
7. S. Majumdar, J. R. Peralta-Videa, S. Bandyopadhyay, H. Castillo-Michel, J. A. Hernandez-Viezcas, S. Sahi, J. L. Gardea-Torresdey, *J. Hazard. Mater.,* 2014, **278**, 279.
8. P. Zhang, Y. Ma, Z. Zhang, X. He, J. Zhang, Z. Guo, R. Tai, Y. Zhao, Z. Chai, *ACS Nano,* 2012, **6**, 9943.
9. P. Zhang, C. Xie, Y. Ma, X. He, Z. Zhang, Y. Ding, L. Zheng, J. Zhang, *Environ. Sci. Tech. Let.*, 2017, **4**, 380.
10. E. Spielman-Sun, E. Lombi, E. Donner, D. Howard, J. M. Unrine, G. V. Lowry, *Environ. Sci. Technol.,* 2017, **51**, 7361.
11. P. Zhang, Y. Ma, S. Liu, G. Wang, J. Zhang, X. He, J. Zhang, Y. Rui, Z. Zhang, *Environ. Pollut.,* 2016, **20**, 1400.

12. Y. Arai, J. T Dahle, *J. Agric. Food Chem.*, 2017, DOI: 10.1021/acs.jafc.7b01277.