Elucidating the Mechanisms of Plant Uptake and *in-planta* Speciation of Ce in Radish (*Raphanus sativus* L.) Treated with Cerium Oxide Nanoparticles

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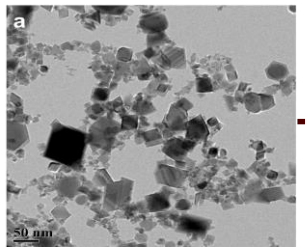
Possible Human Exposure Pathways to CeO_2NPs



CeO_2NPs production

Applications

Surface water

Sewage treatment

Irrigation water

Biosolids

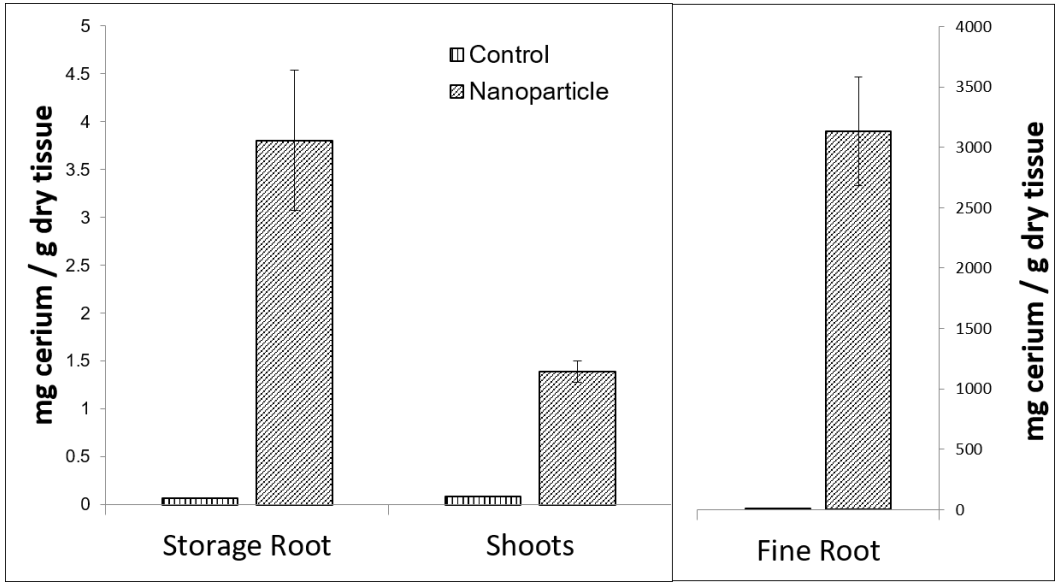
Crops

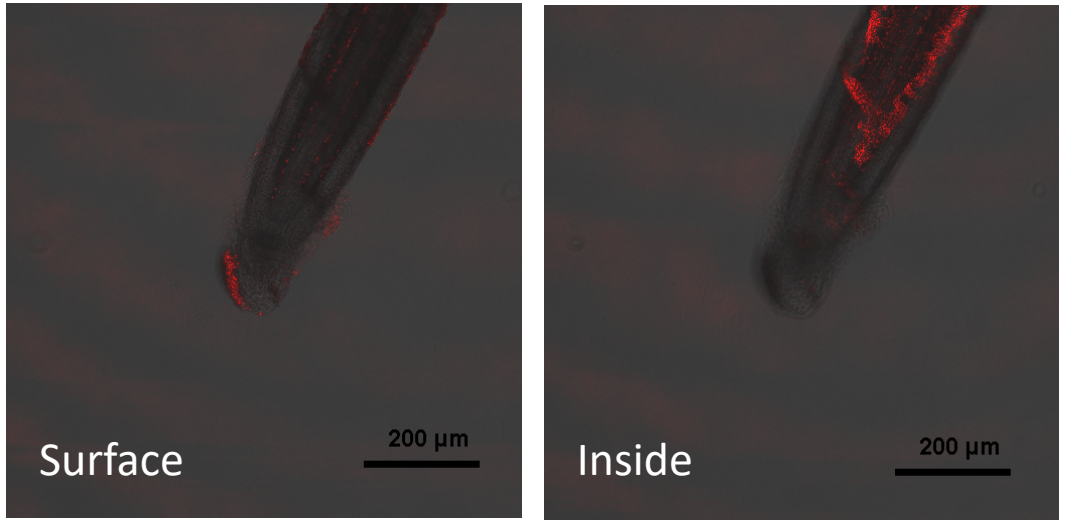
In soil

Transport

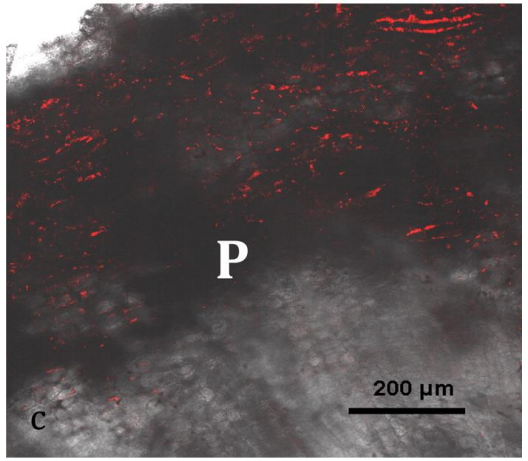
Dietary exposure

Previous Results





Confocal images of fine roots exposed to CeO_2NPs

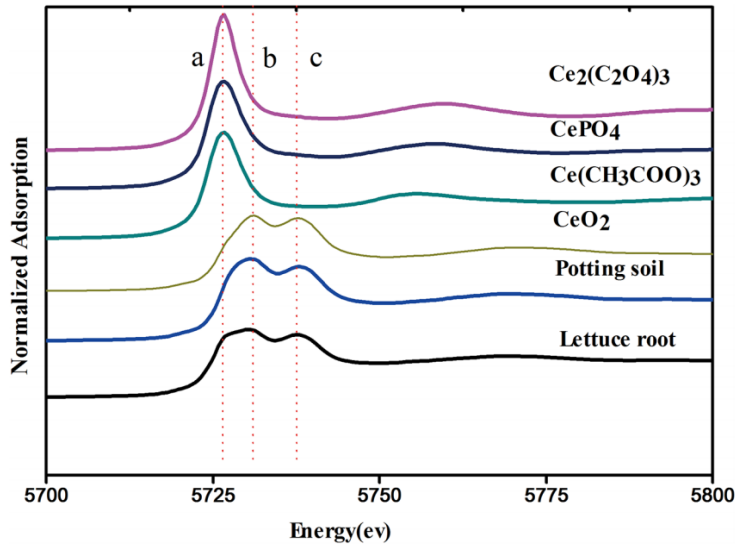


P: periderm
VT: vascular tissues

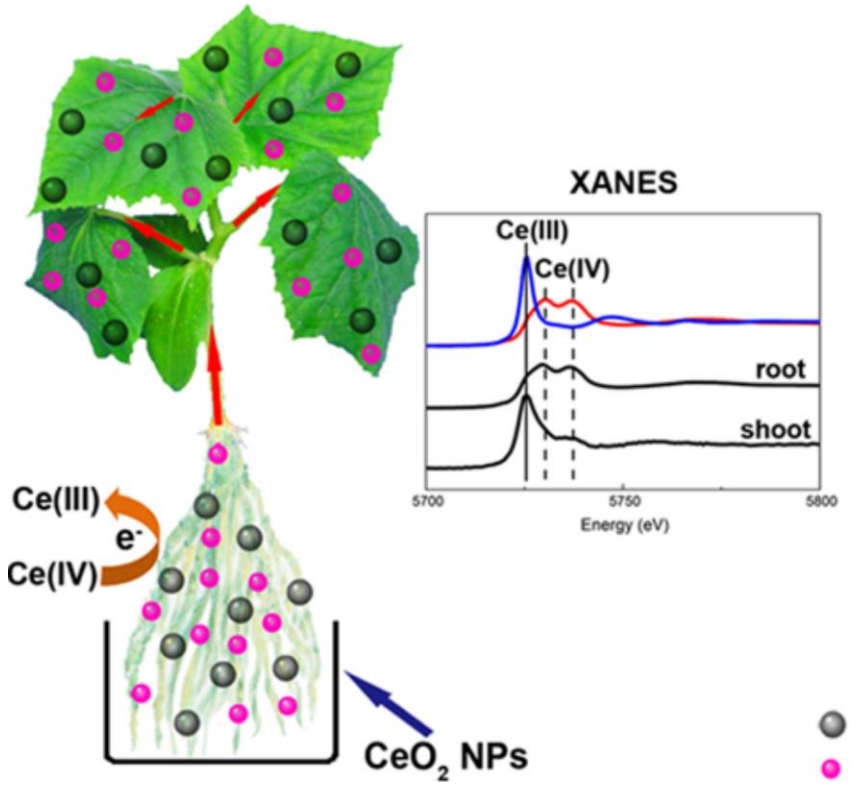
Previous Results

The presence of Ce in the plant tissue was usually interpreted as mainly CeO_2NPs due to

- Low solubility of CeO_2NPs
- The presence of Ce(IV)



XANES Ce LIII-edge spectra (5723 eV) in roots of butterhead lettuce treated with CeO2 NPs. (Gui et al. 2015)



Ma, Y., et al. (2015). "Where does the transformation of precipitated ceria nanoparticles in hydroponic plants take place?" Environmental science & technology **49**(17): 10667-10674.

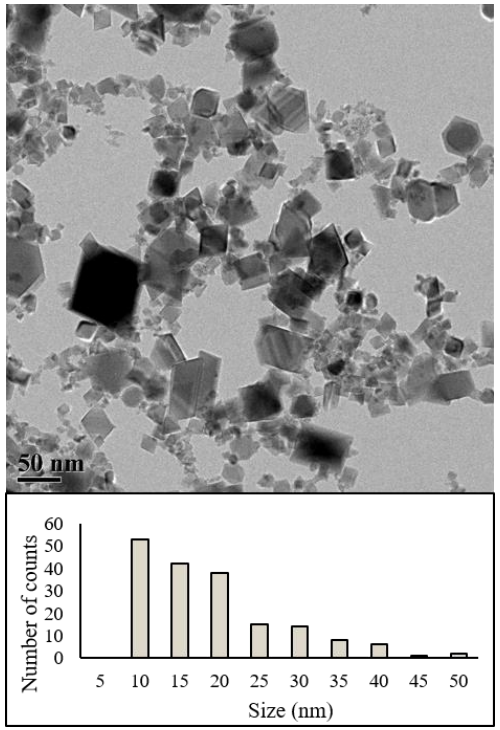
OBJECTIVES

- To determine the speciation of Ce in radish following plant exposure to CeO_2NPs.
- To further investigate the mechanisms for CeO_2NPs transformation and radish uptake of CeO_2NPs.

Experimental Setup

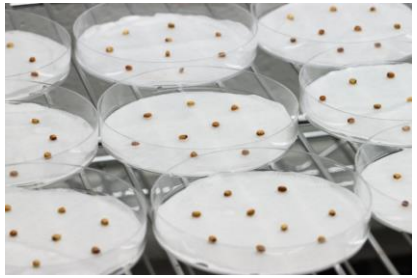
Treatments

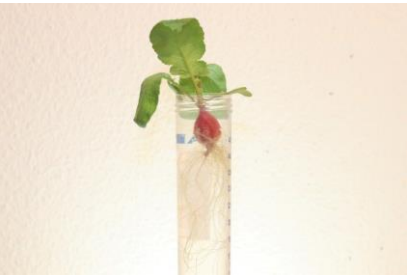
- Control
- CeO_2NPs: 10ppm



Day 1 – Day 5 | Day 6 – Day 19 | Day 20 – Day 24 | Day 24

Germination | Development | Ce exposure | Harvest



Experimental Setup

Radish roots will be washed by $CaCl_2$ (5 mM) solution 5 times.

Washing solution | **Growth medium**

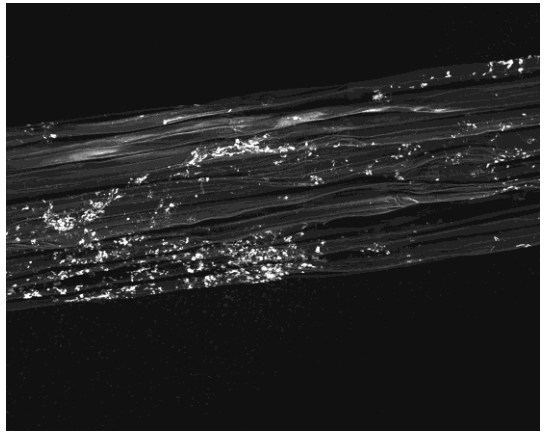
10 kDa Amicon Ultra-4 Centrifugal Filter unit

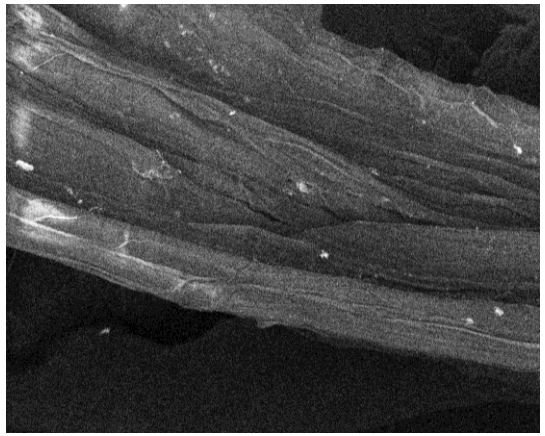
$HNO_3 + H_2O_2$ Strong acid digestion

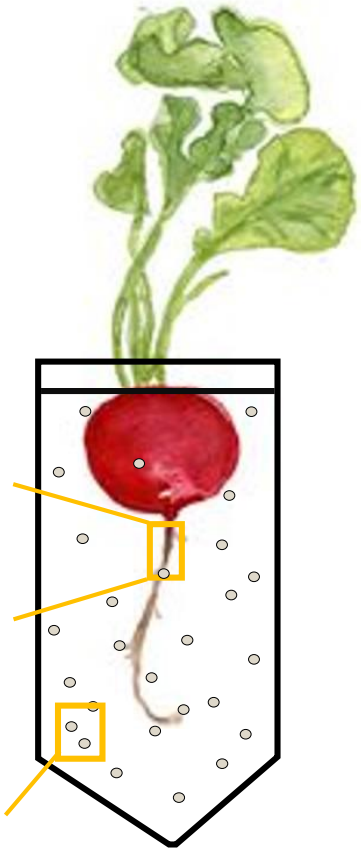
Dissolved Ce on root surface and in growth medium

Total Ce on root surface and in growth medium

Root surface: Dissolved/Total VS Growth medium: Dissolved/Total





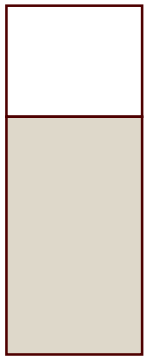


Ce Extraction Method

Strong Acid Digestion

HNO_3+H_2O_2

- Completely dissolve the element.
- Forms of Ce in the plant tissues are unknown.

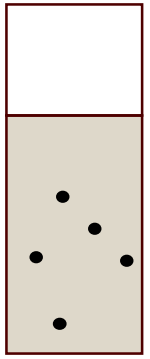


Dissolved salt

Enzymatic Digestion

Macerozyme R-10 enzyme

- Degrade of proteins, and digest plant cell walls and membrane.
- Keep ENPs physicochemical properties.



Particulate forms
Dissolved salt

Experimental Setup

Macerozyme R-10 enzyme

+

0.5 g of plant tissues

Enzyme concentration: 3 mg/mL

Mixture will be shaken at 37 °C for 24 hours

10 mL of Digestate

4 mL

10 kDa Amicon Ultra-4 Centrifugal Filter unit

Dissolved Ce in plant tissues

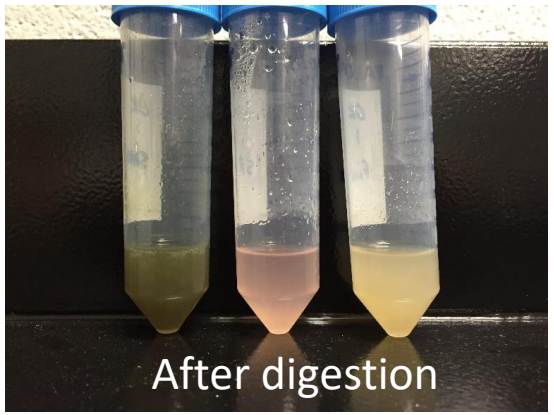
4 mL

$HNO_3 + H_2O_2$ Strong acid digestion

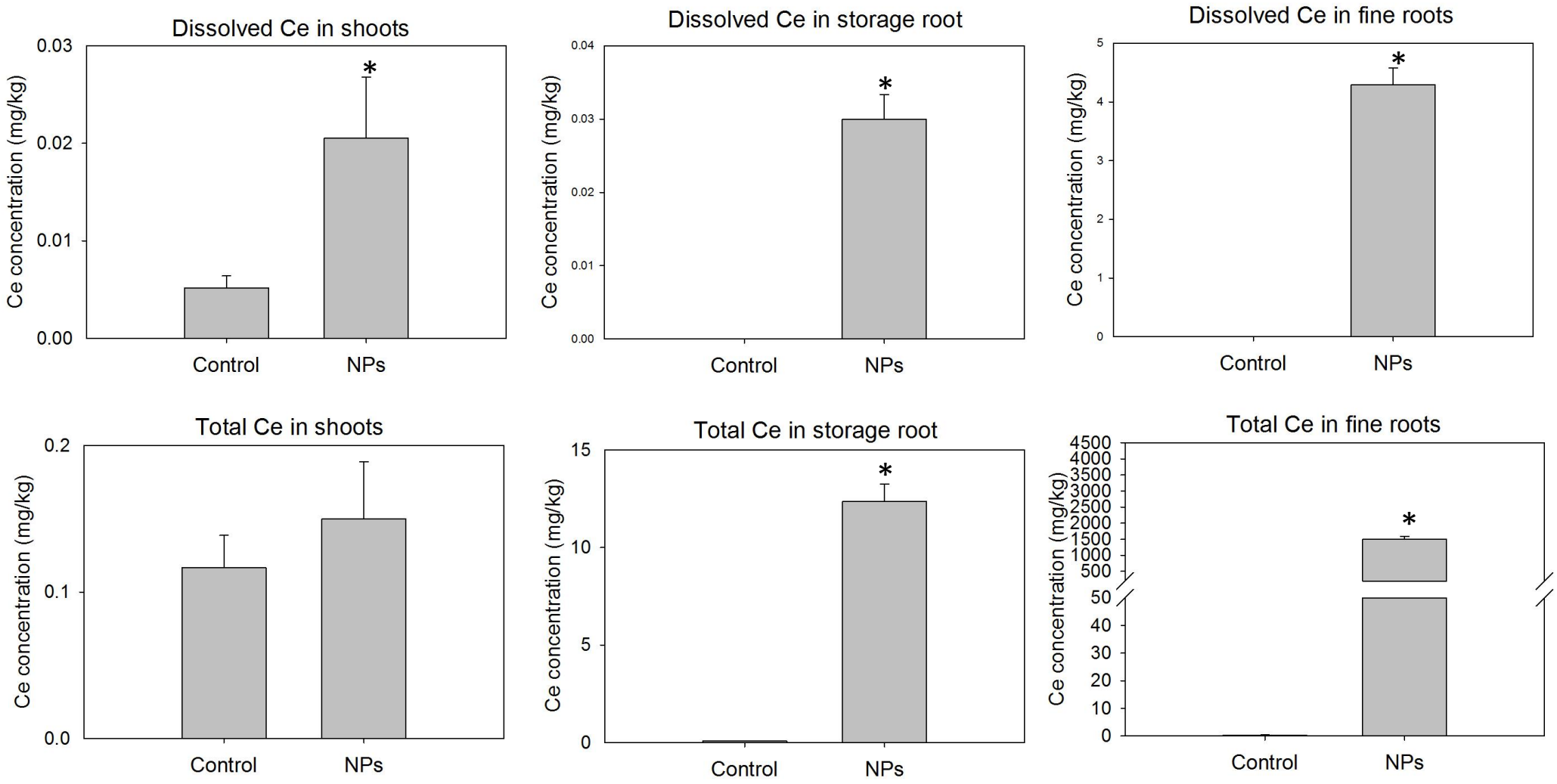
Total Ce in plant tissues

2 mL

TEM Observation



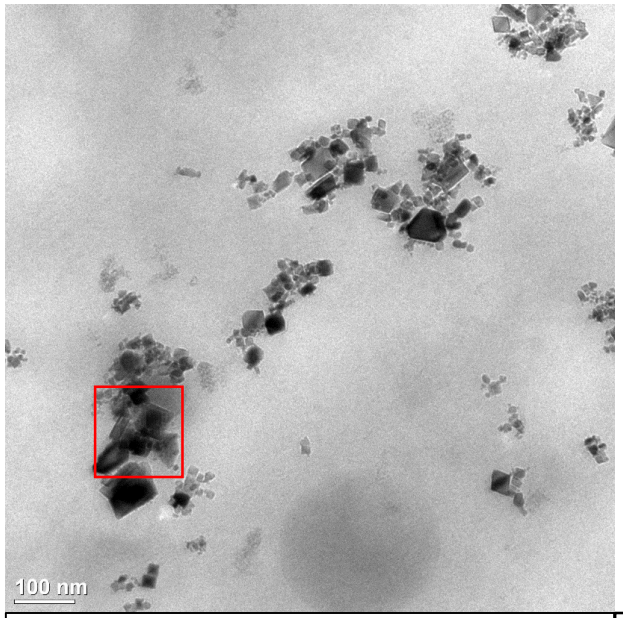
Ce Concentrations in Plant Tissues

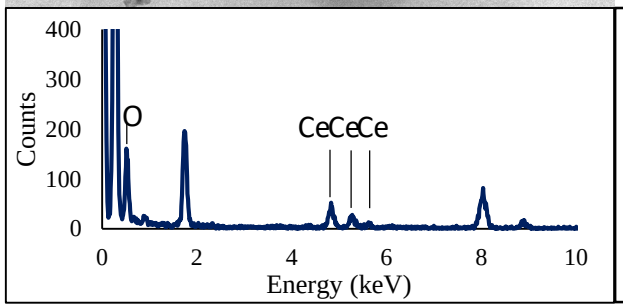


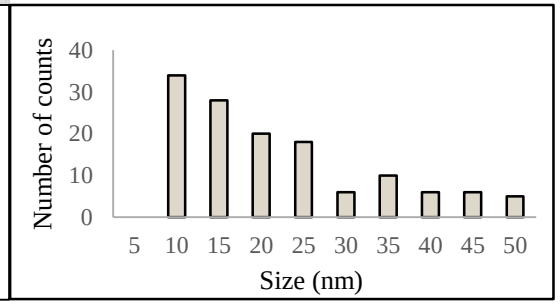
Asterisks indicate significant differences between two bars by conducting student's t-test ($p < 0.05$)

TEM Images of Enzymatic Digestate (CeO_2NPs)

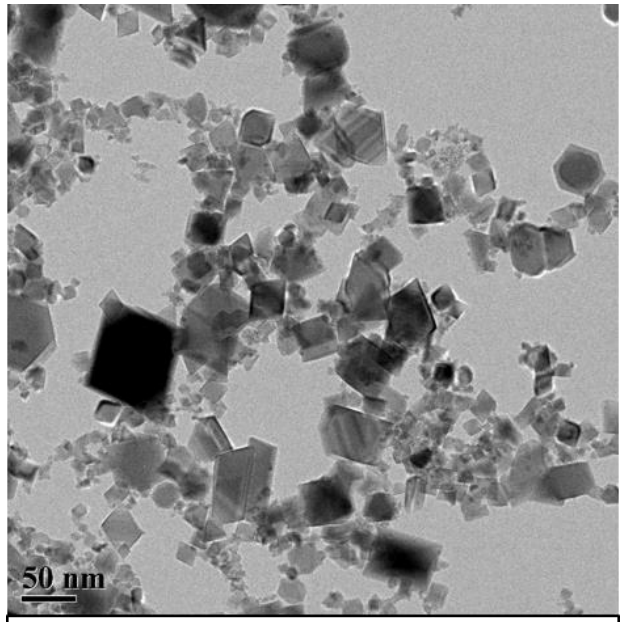
Digestate of fine roots exposed to CeO_2NPs

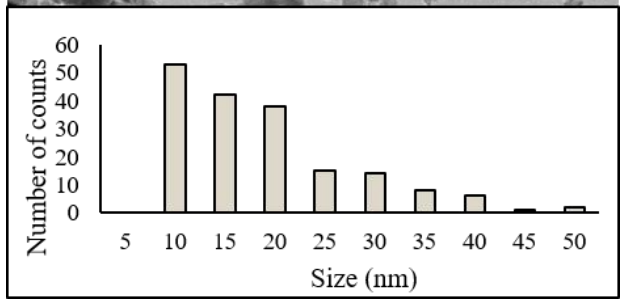






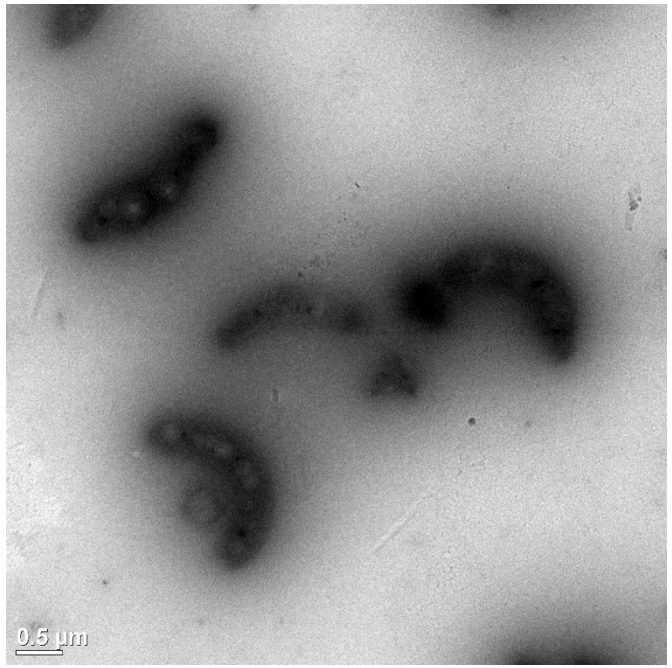
Primary CeO_2NPs in growth medium

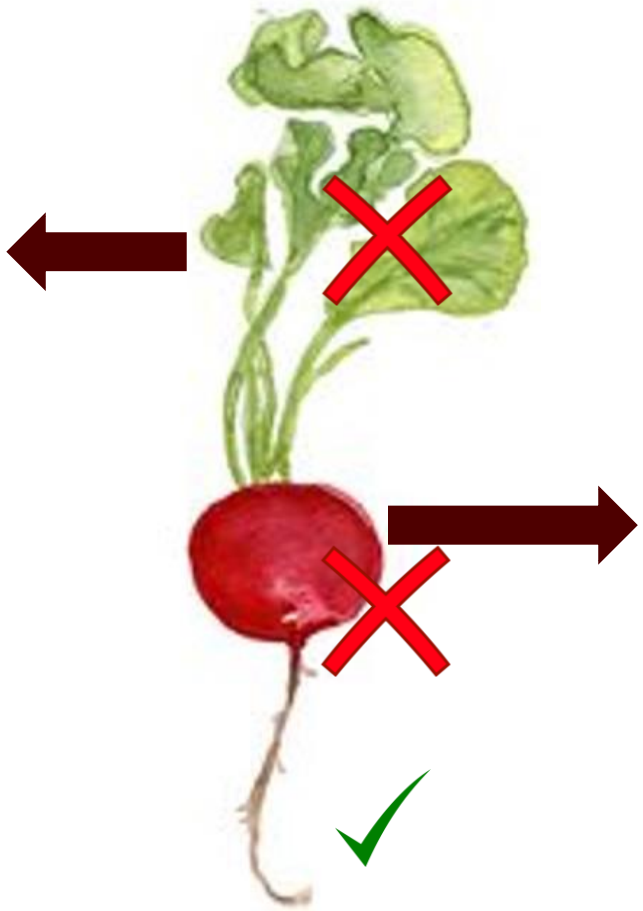




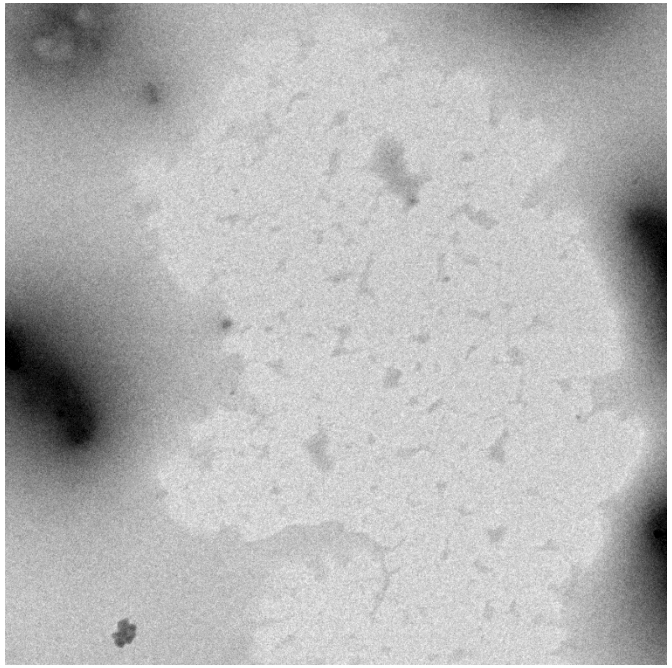
TEM Images of Enzymatic Digestate (CeO_2NPs)

Digestate of shoots

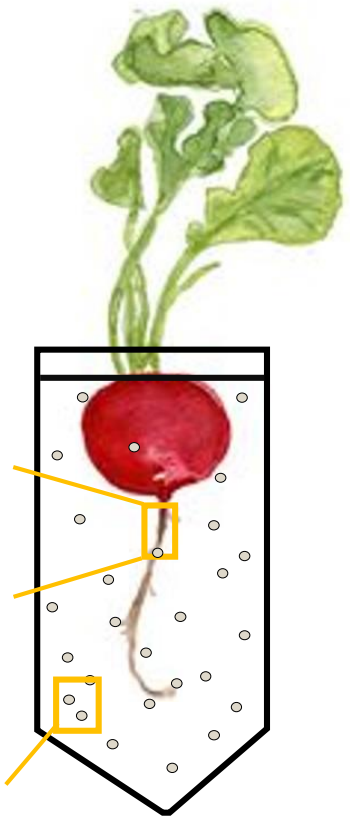




Digestate of storage root



Ce on Root Surface and Growth Medium

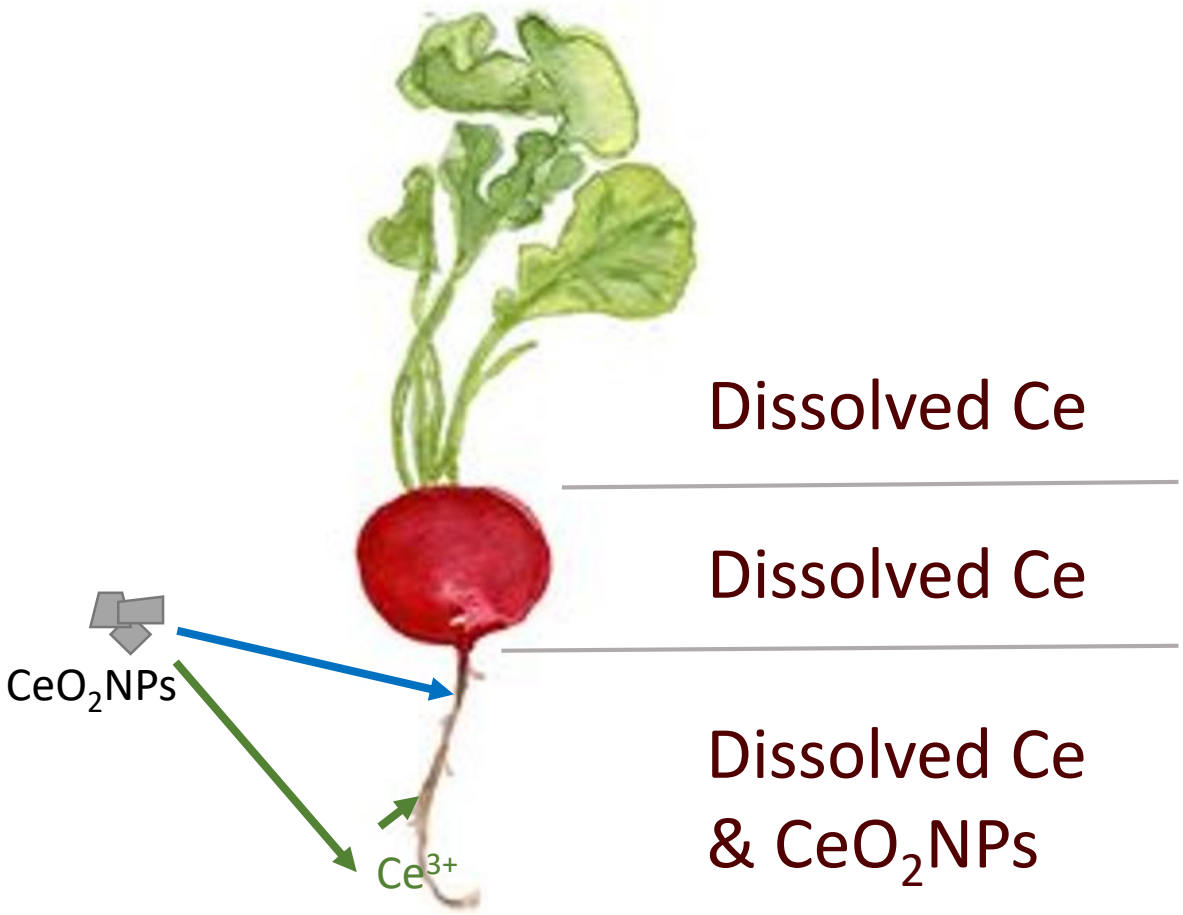


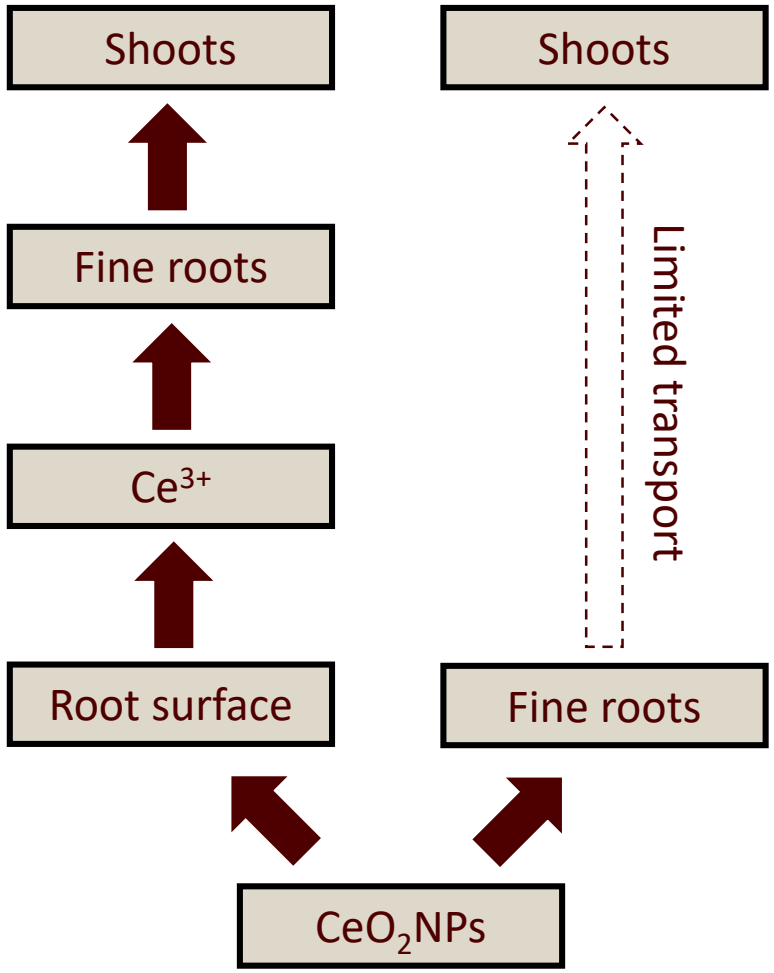
Dissolved Ce	Control	NPs
Washing solution (µg/L)	0.16±0.08	23.03±2.42
Growth media (µg/L)	0.22±0.11	0.21±0.14

Dissolved Ce / Total Ce	Control	NPs
Washing solution (%)	-	4.21±0.55
Growth medium (%)	-	0.024±0.01

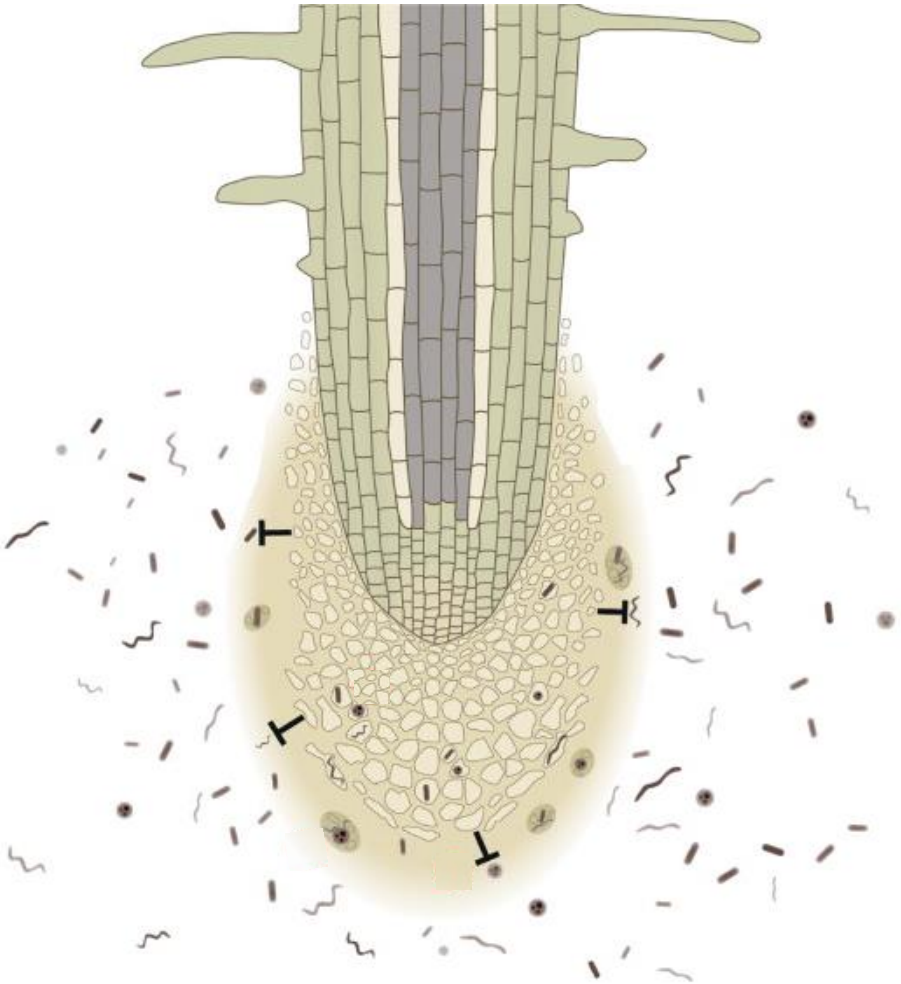
Root surface deposit > Fine roots > Storage root > Shoots

Ce Transformation and Transport in Plant Tissues





Root Exudate



Sugars

- Glucose
- Fructose
- Galactose

Organic acids

- Succinic acid
- Malic acid
- Citric acid

Amino acids

- Serine
- Proline
- Glycine

⋮

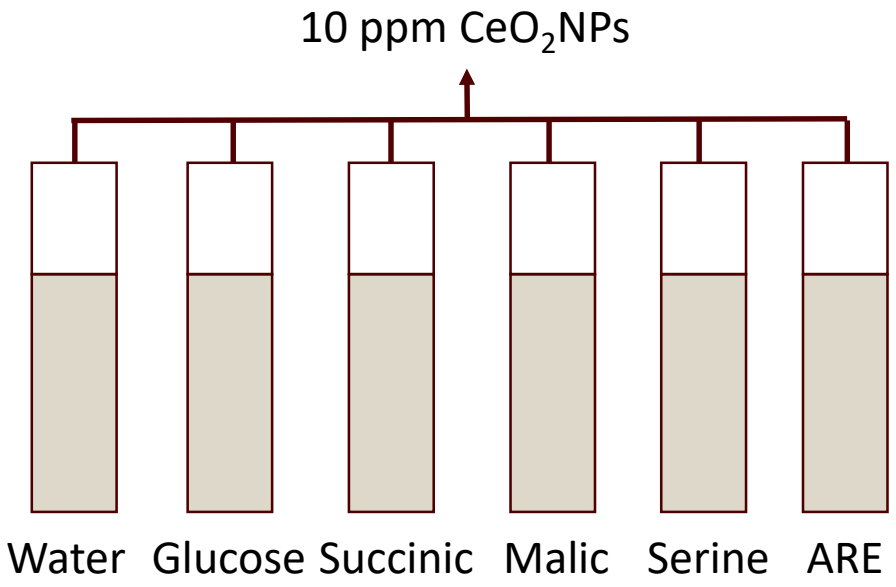
Simplified Artificial Root Exudate

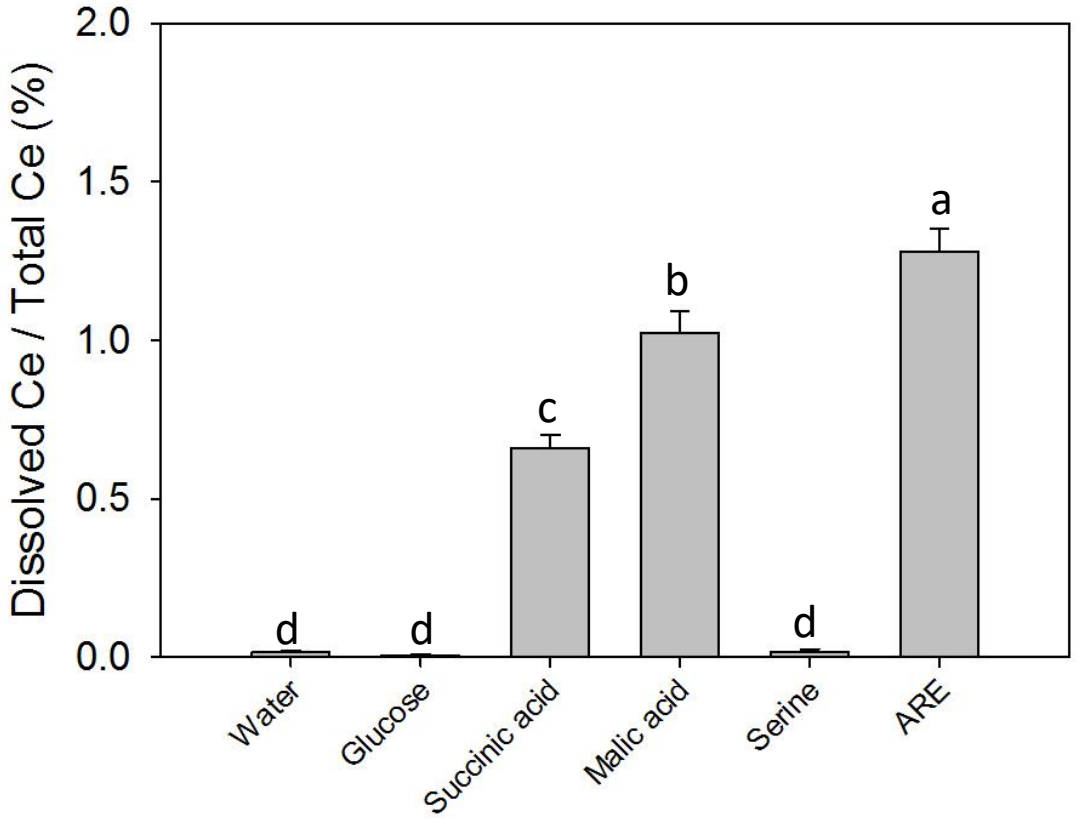
Glucose	50 mM
Succinic acid	25 mM
Malic acid	25 mM
Serine	12.5 mM

Root Exudate

ARE

- Glucose 50 mM
- Succinic acid 25 mM
- Malic acid 25 mM
- Serine 12.5 mM





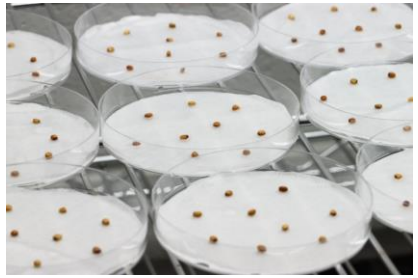
pK_{a1} = 4.2 pK_{a1} = 3.4

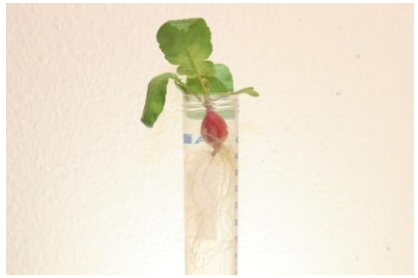
Experimental Setup

- Treatments
  - Control
  - CeO_2NPs: 10ppm
  - Ce^{3+}: 10ppm

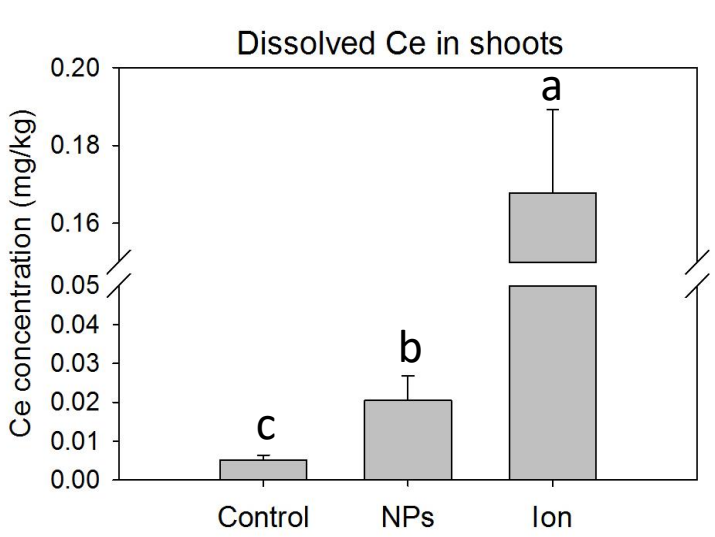
Day 1 – Day 5 | Day 6 – Day 19 | Day 20 – Day 24 | Day 24

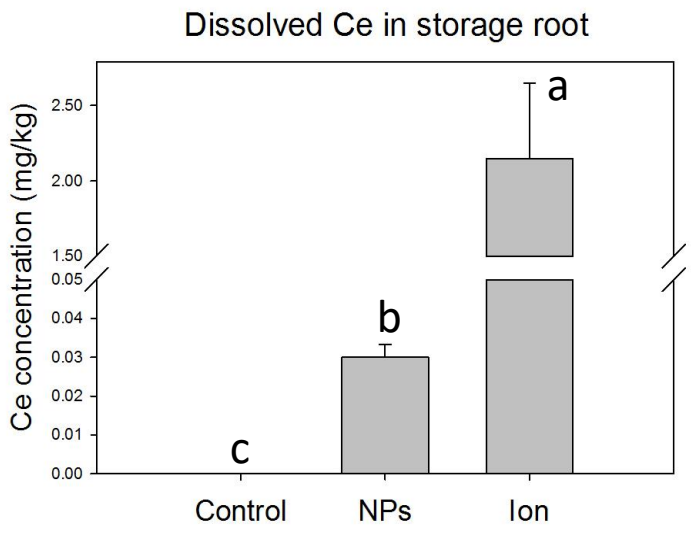
Germination | Development | Ce exposure | Harvest

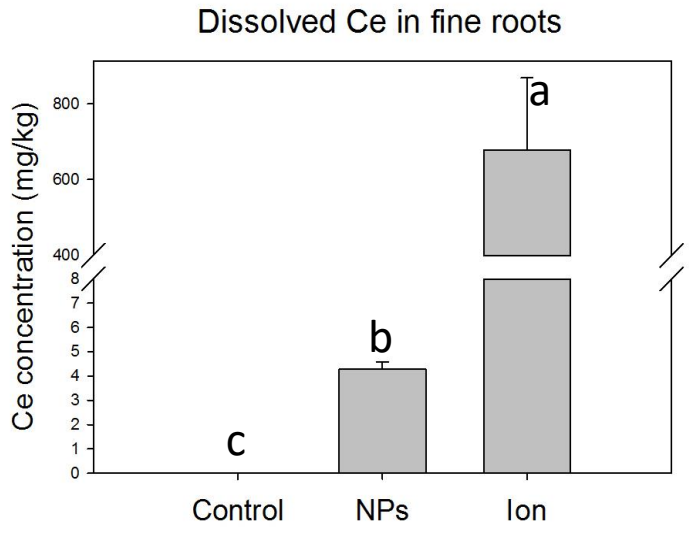


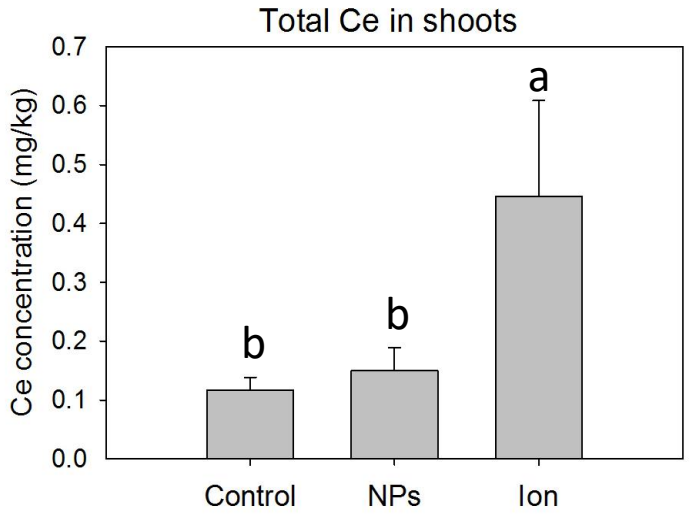



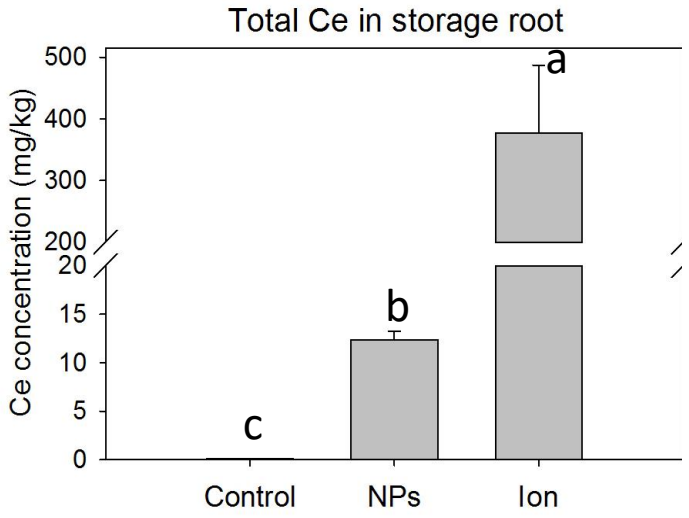
Ce Concentrations in Plant Tissues

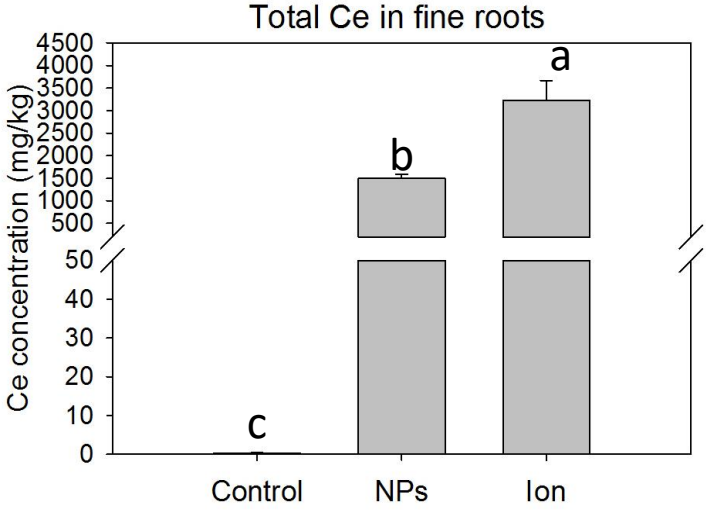






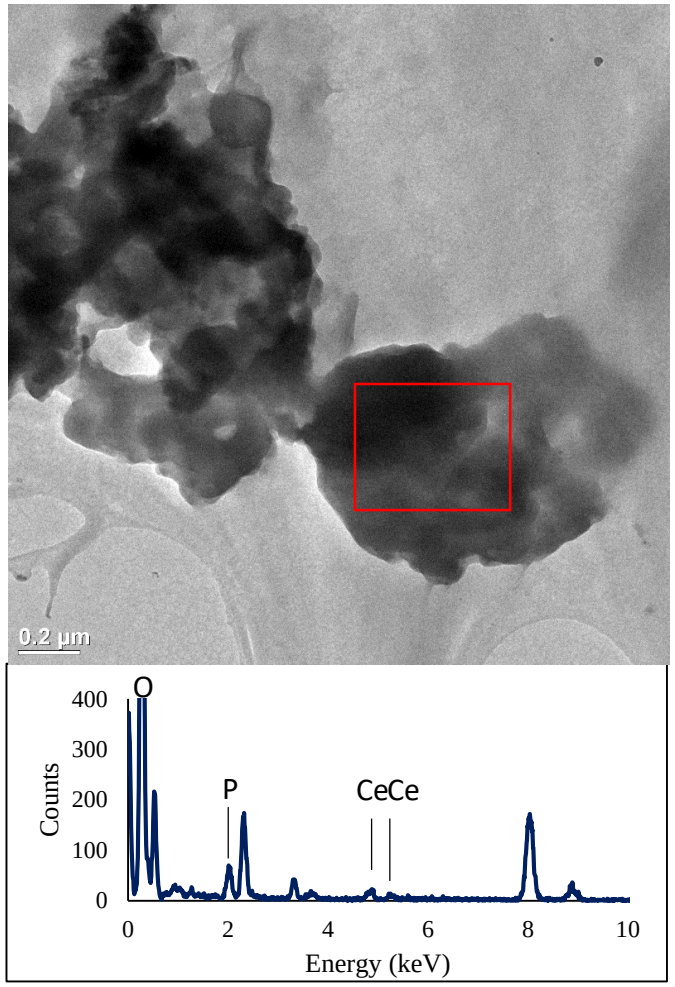




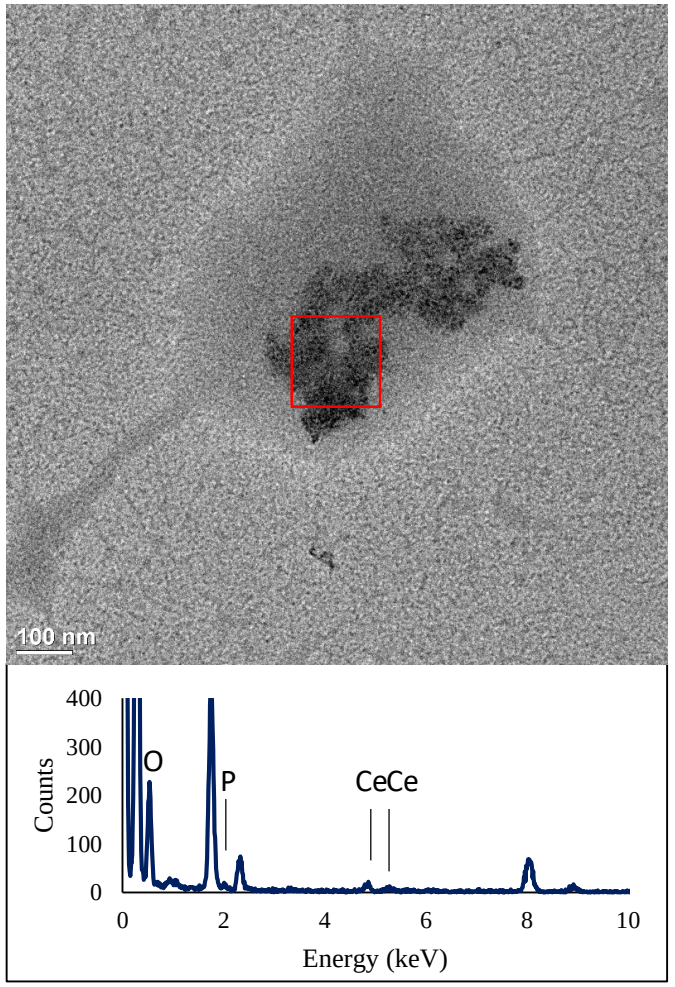


TEM Images of Enzymatic Digestate (Ce^{3+} ion)

Digestate of storage root

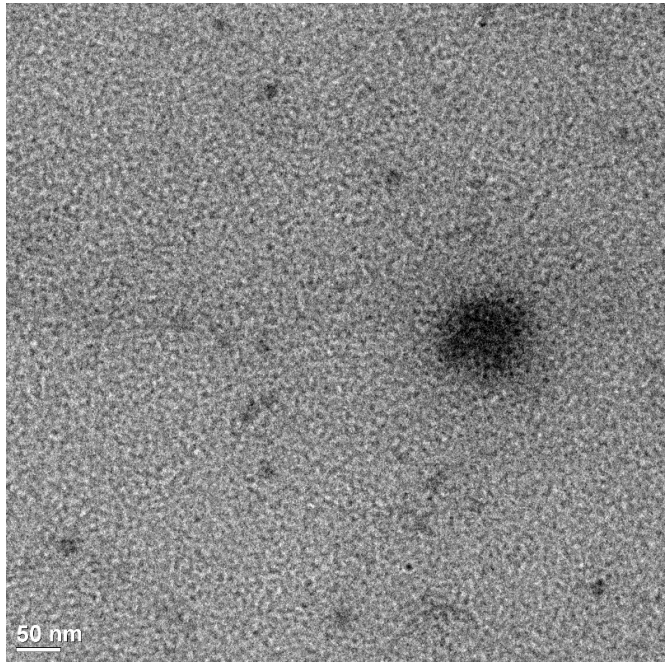


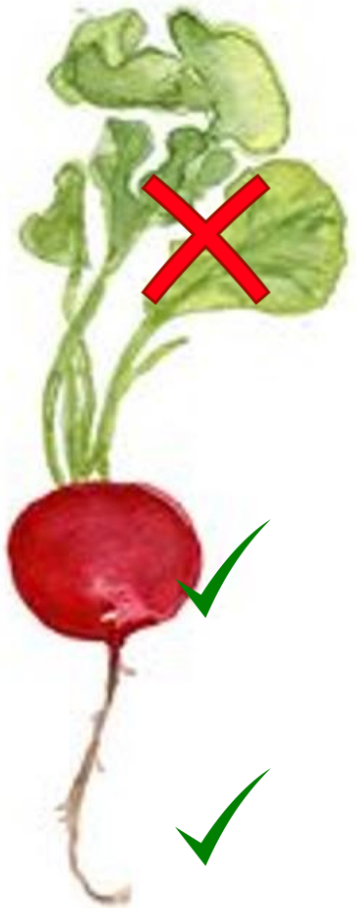
Digestate of fine roots



TEM Images of Enzymatic Digestate (Ce^{3+} ion)

Digestate of shoots





Conclusions

- Transformation of CeO_2NPs to dissolved Ce occured on the root surface. The radish root exudate played a critical role on the transformation of CeO_2NPs.
- CeO_2NPs can be taken up by radish fine roots as both intact NPs and dissolved ions, while only dissolved Ce demonstrated high upward transport.
- Particulate Ce that might be $CePO_4$ could be formed from Ce^{3+} in the radish roots.

Acknowledgements

- Sustainable Nanotechnology Organization
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- Dr. Samuel Ma, Dr. Honglan Shi, and the research team.

Sustainable Nanotechnology Organization
Research | Education | Responsibility

USDA

Missouri S&T
University of Science & Technology

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Thank you!

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