New insight into naturally occurring nanosilver: Role of plant root exudates

Huiyuan Guo
Ph.D. candidate
Advisor: Prof. Baoshan Xing

Environmental soil and chemistry program
University of Massachusetts Amherst

Sustainable Nanotechnology Organization
Research | Education | Responsibility

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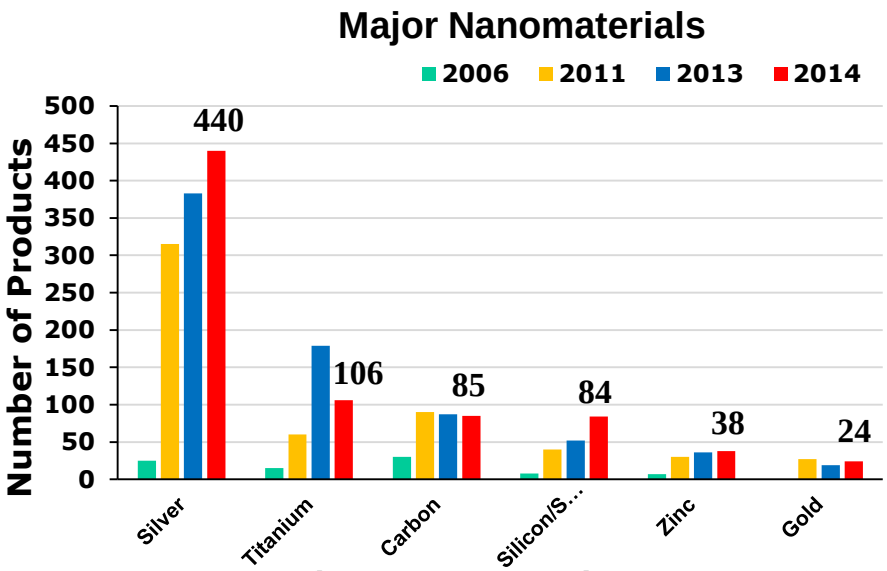
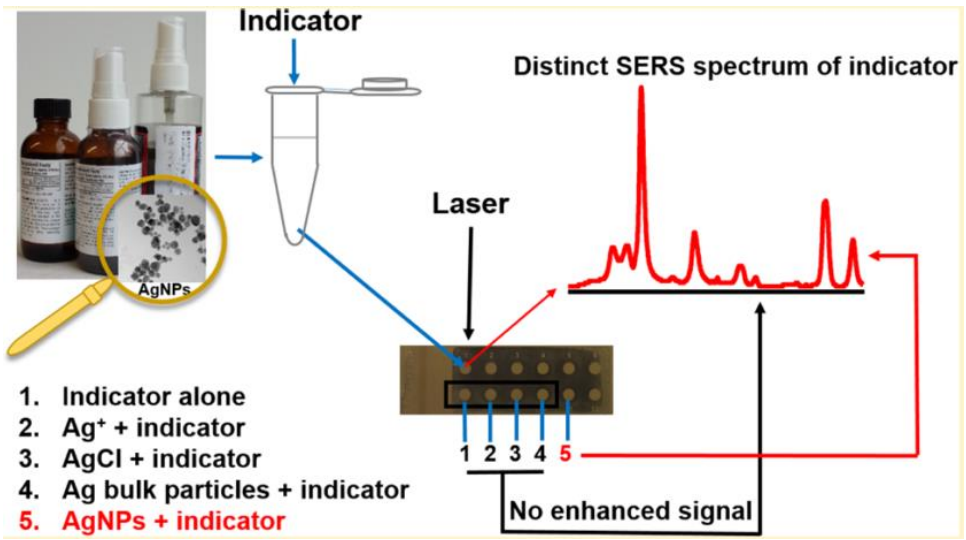
Background: sources of nanosilver (nAg)

- Engineered nAg from consumer products



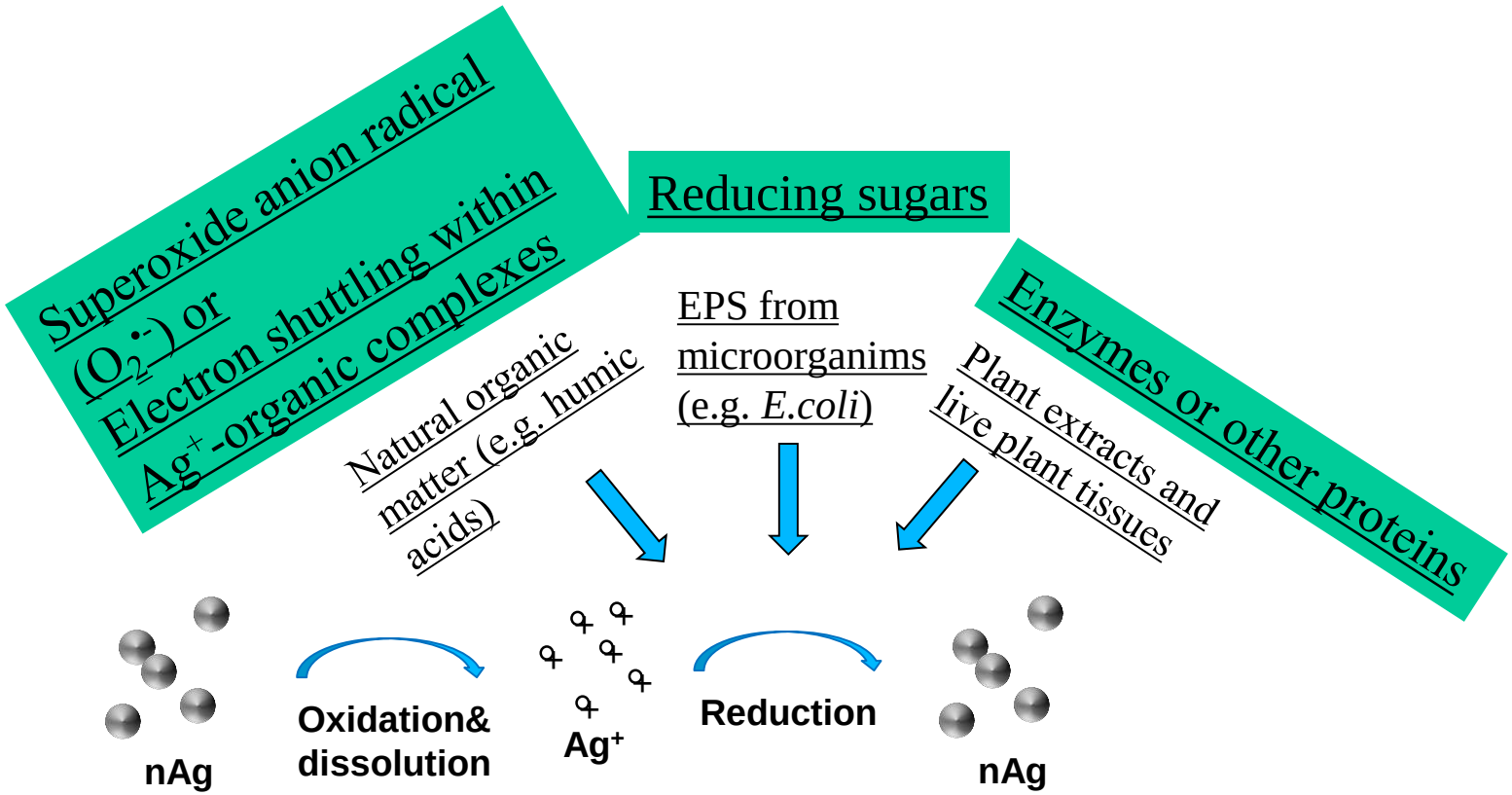
(http://www.nanotechproject.org/cpi/)



(Guo et al. 2015 Environ. Sci. Technol)

Background: sources of nanosilver (nAg)

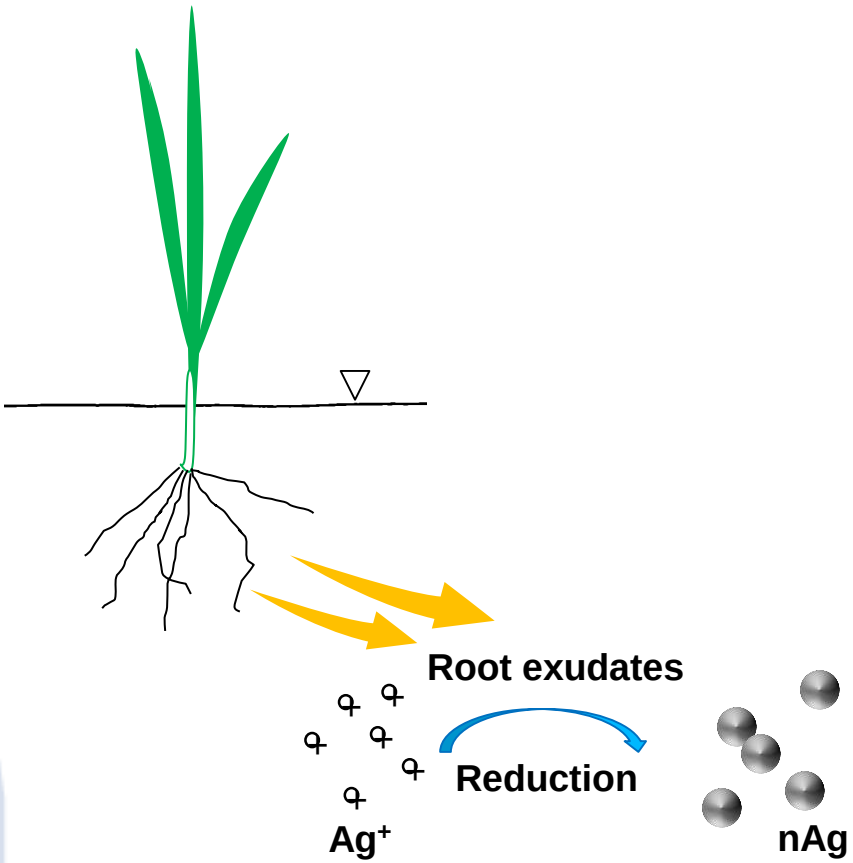
- Naturally formed nAg



EPS: extracellular polymeric substances

Root exudates (RE)

- Ubiquitous in soil and aquatic environments;
- A cocktail of organic substances and inorganic ions;
- Unknown role in the formation and stability of naturally occurring metal nanoparticles.



Research goals:

- ❑ **Investigate if RE own the ability to transform Ag^+ to nAg**
- ❑ **If yes, identify the key factors that control the reduction of silver ions by RE and figure out the underlying mechanisms**

Experimental design

Reduction of Ag^+ by root exudates (RE) from wheat plants

RE collection and characterization

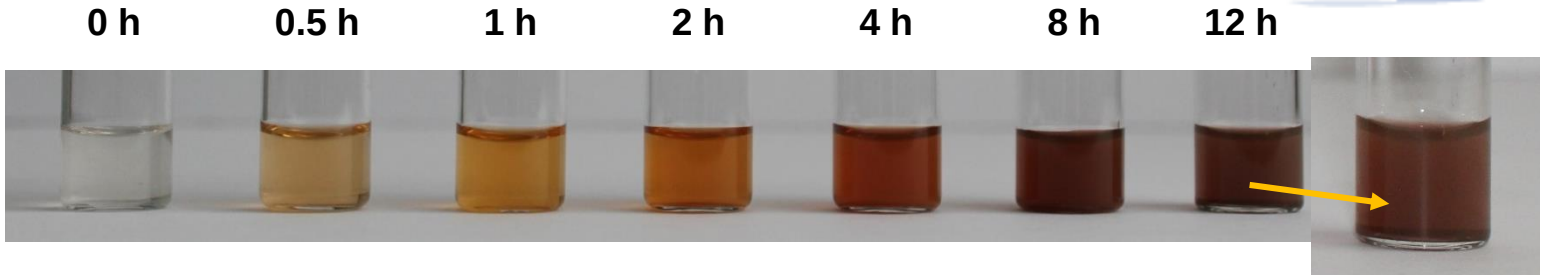
- Greenhouse: 25 ℃ with 16h/8h (light/dark) cycle, light intensity ~32400 lux.
- RE were collected from ~1 week old of live wheat roots after 24 h incubation in water.
- TOC: 134±2 mg/L, pH 6.7±0.1. GC-MS

Interaction of RE with Ag^+

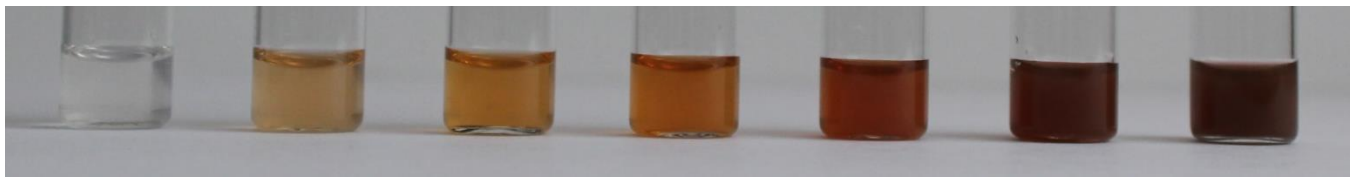
- The extracted RE interacted with Ag^+ (1 mM) in a chamber room with light intensity of 8288 lux.

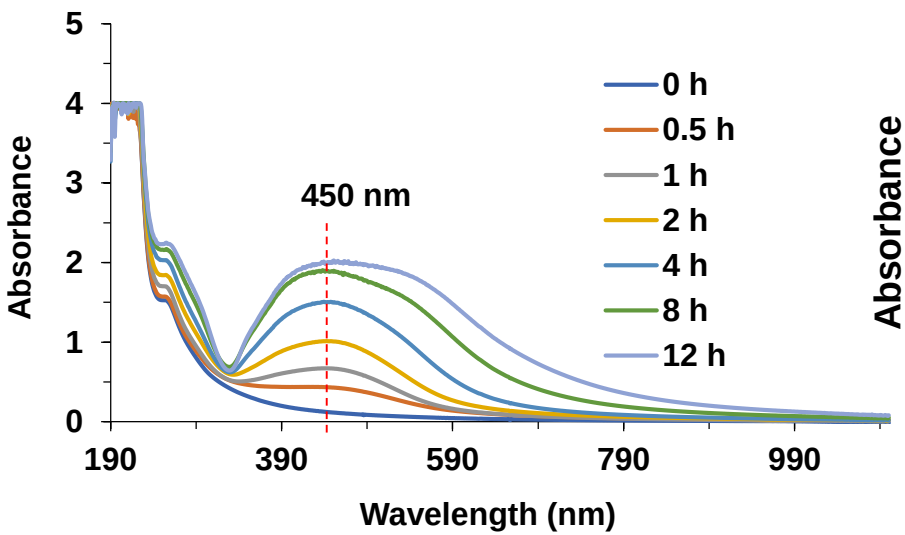
Characterization and determination of the formed particles

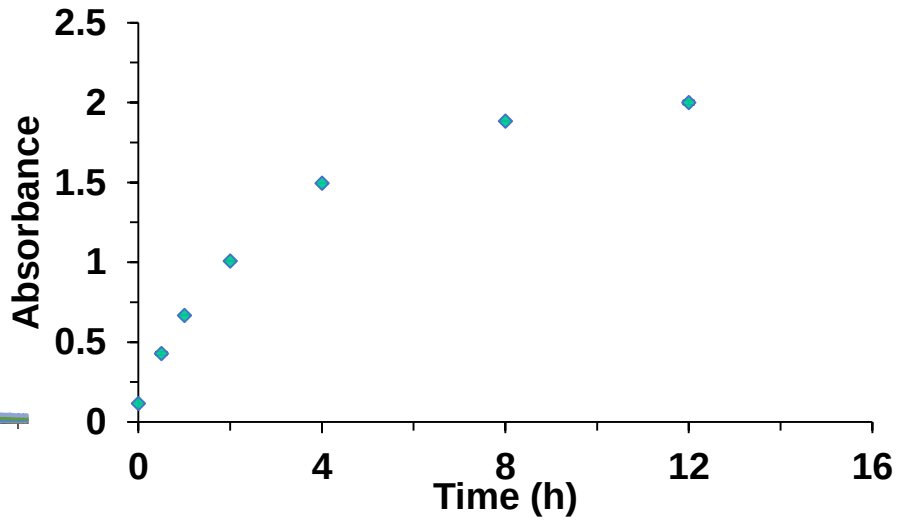
- UV-vis spectroscopy
- Surface-enhanced Raman spectroscopy (SERS)
- SEM-EDS
- XRD



after well mixing

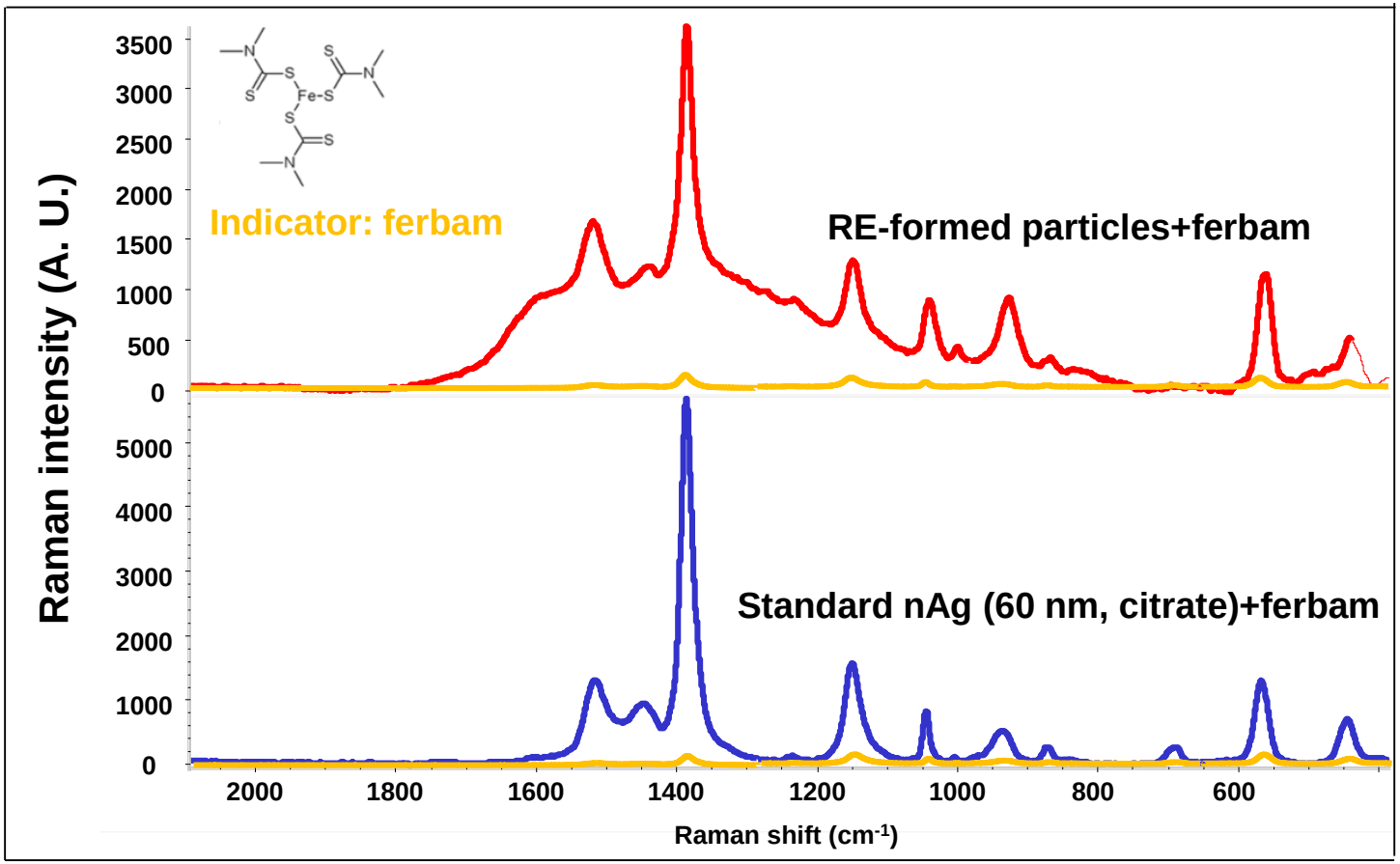






- Root exudates can reduce Ag^{+} to nAg.
- The AgNP concentration increased in 8 h of irradiation and leveled off thereafter.

Surface-enhanced Raman spectroscopy (SERS) data

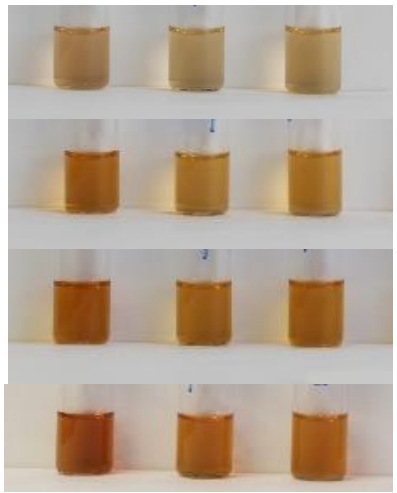


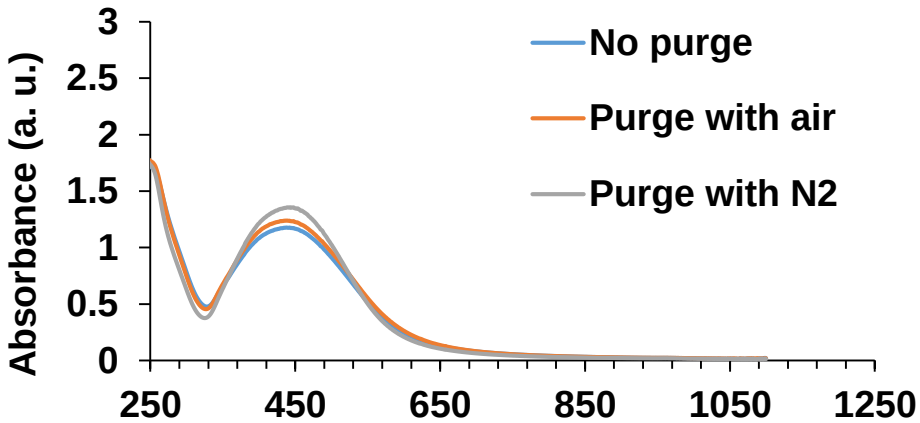
- **SERS data confirmed the formation of nAg.**

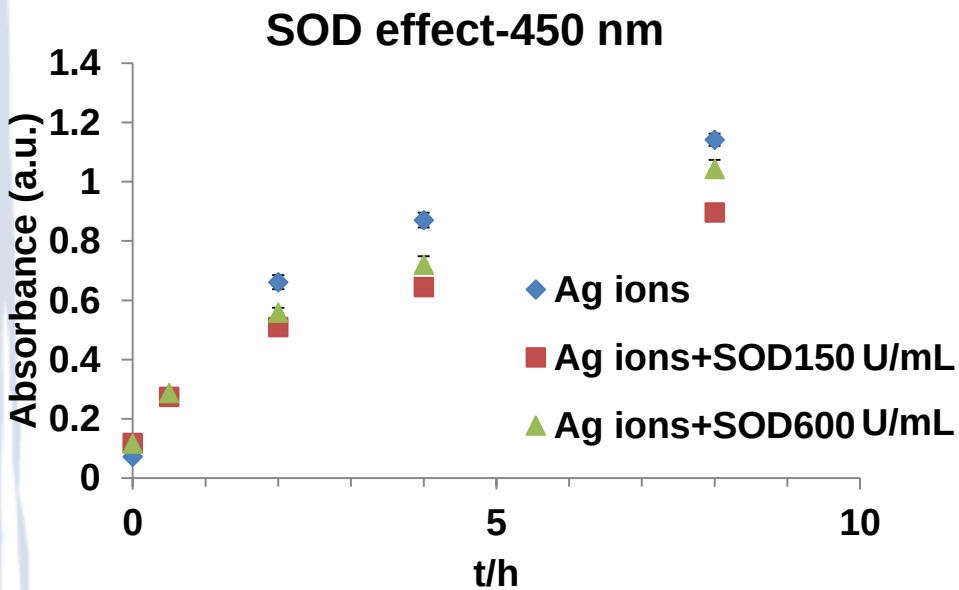
Potential mechanisms

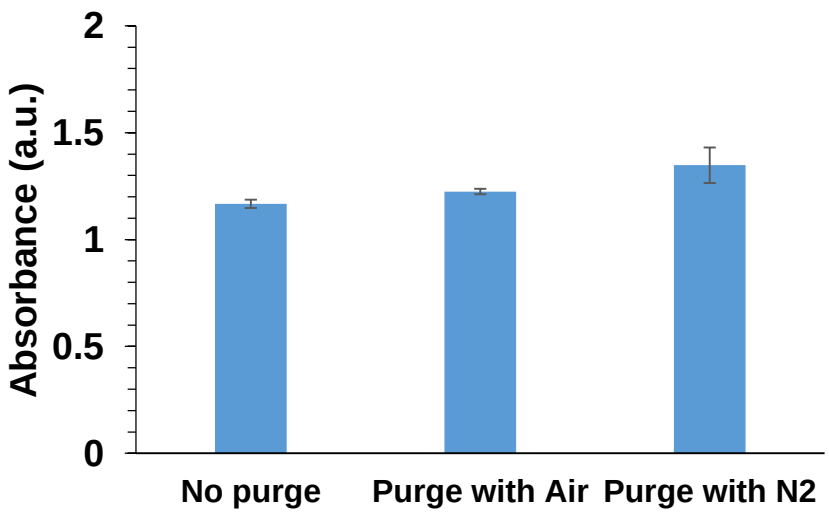
- Superoxide anion radical ($O_2^{\bullet -}$)
- Electron shuttling within Ag^+-organic complexes
- Enzymes or other proteins
- Reducing sugars

1. Superoxide anion radical ($O_2^{\bullet-}$)



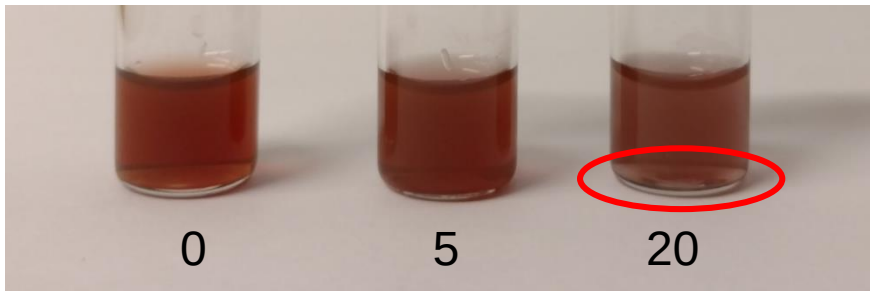


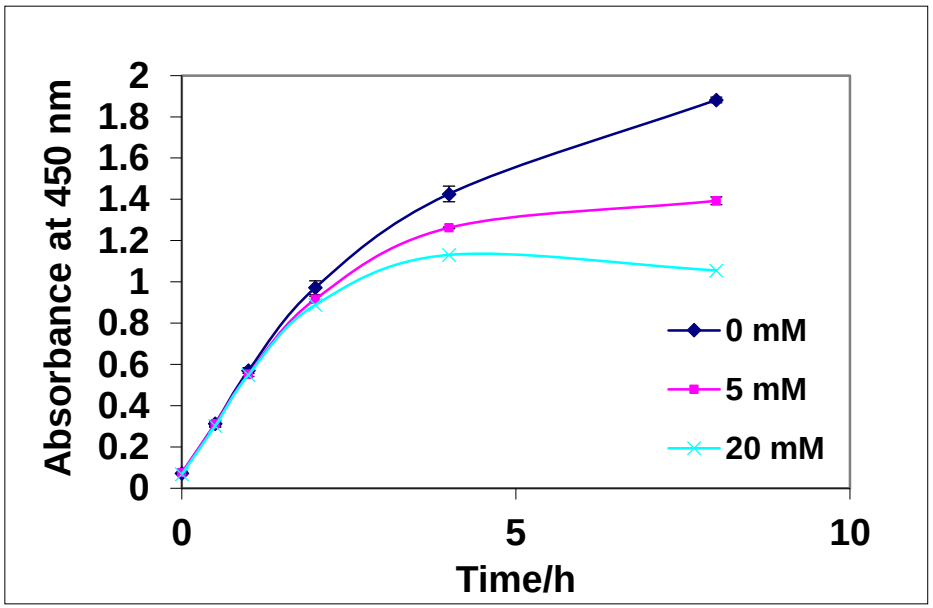




❑ **Superoxide anion radical is not responsible for reduction of Ag^+.**

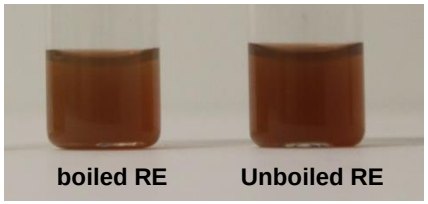
2. Electron shuttling within Ag^+-organic complexes

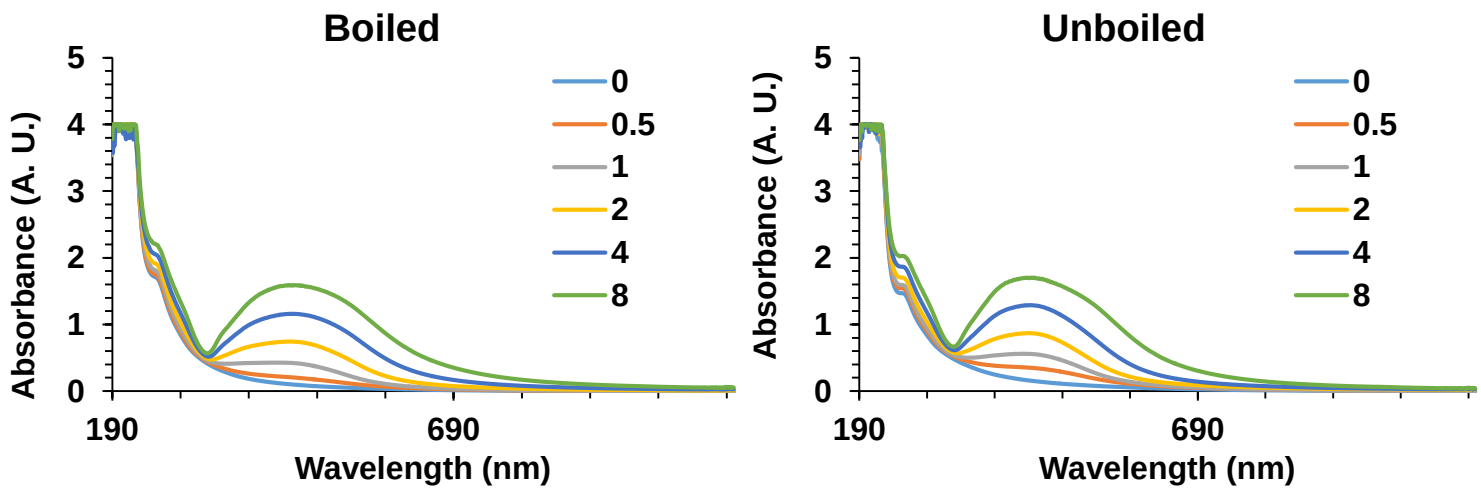




- **Competing cation didn't affect the initial reaction rate but rather resulted in the particle aggregation because of the high ionic strength.**
- **The reduction of Ag^+ is not attributed to the ligand-to-metal charge transfer.**

3. Enzymes or other proteins





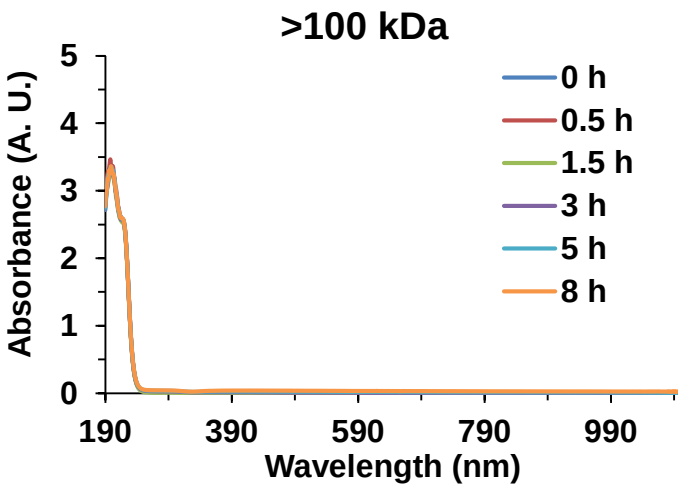
- **Enzymes or other proteins in root exudates didn't contribute to the transformation of Ag^{+} to nAg.**

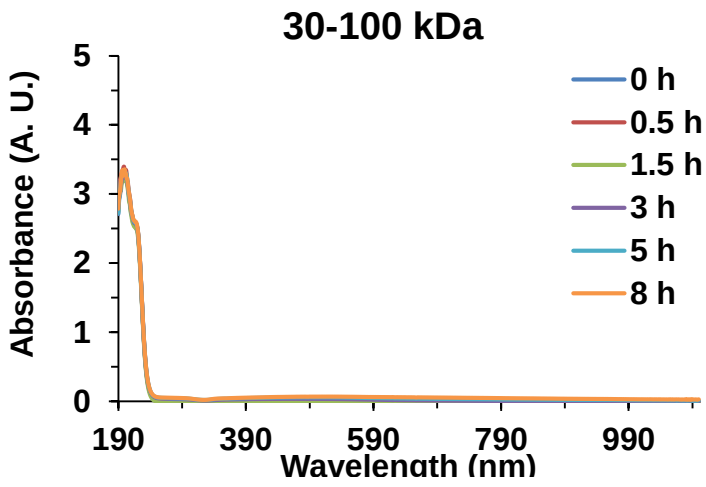
4. Reducing sugars

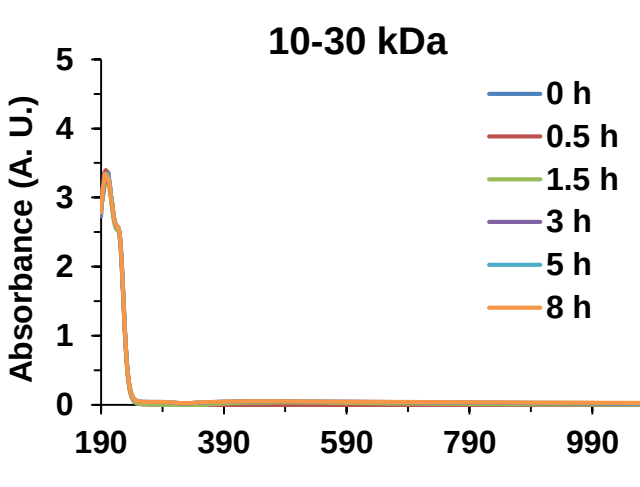
Reducing sugars	Relative abundance	Reduction of Ag^{+}
D-mannose	34.2%	No
D-fructose	13.6%	No
α-D-lactose	22.5%	No
D-cellobiose	8.9%	No
Mixture of 4 sugars	79.2%	No

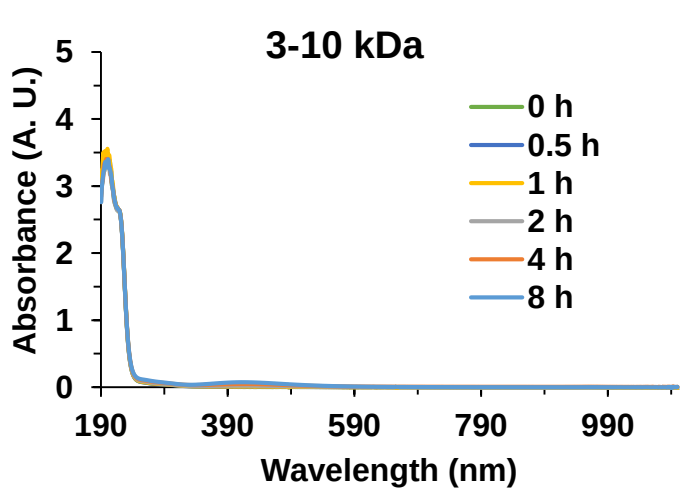
(Based on GC-MS data)

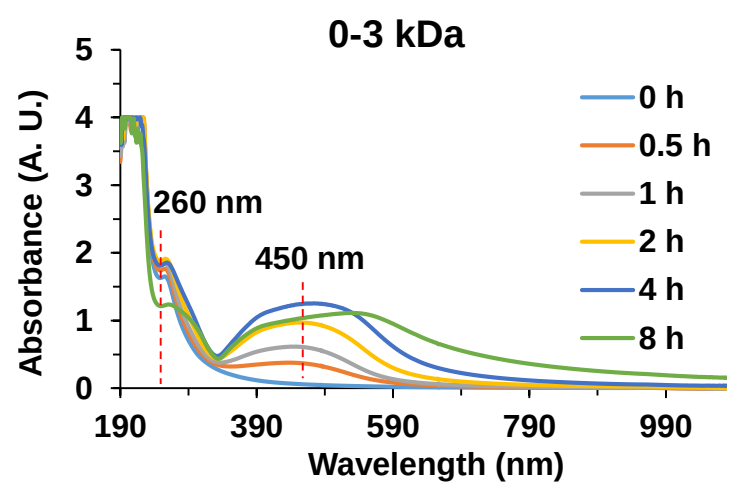
RE fractions based on molecular weight

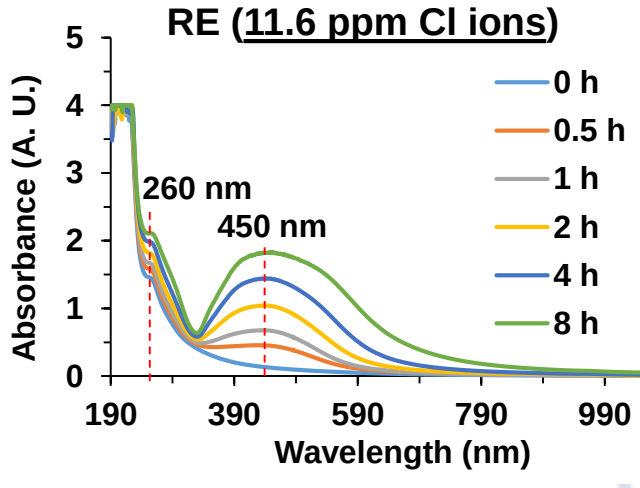






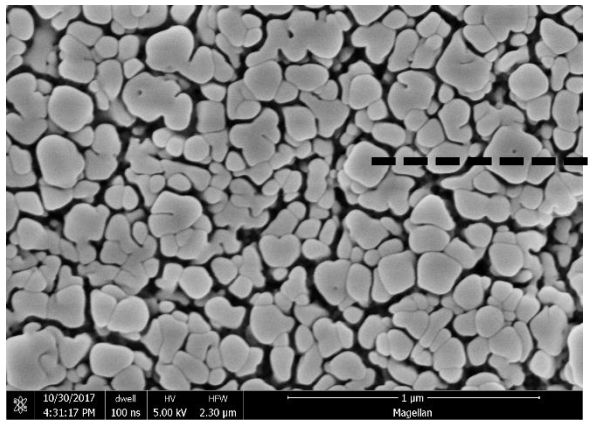




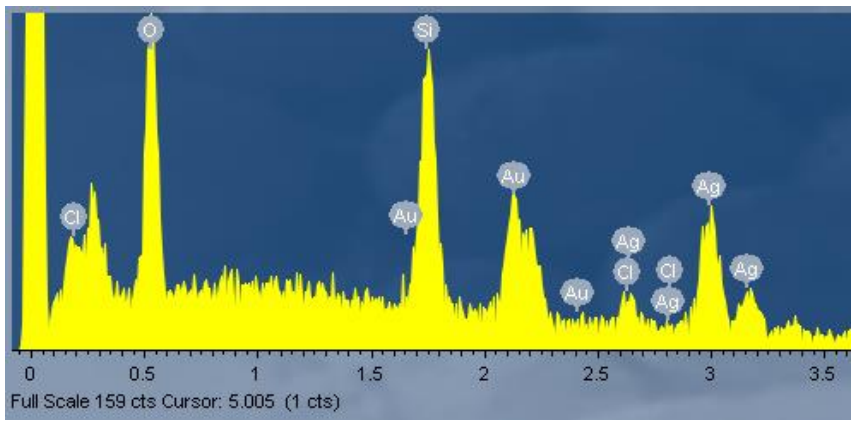


- **The fraction of 0-3 kDa in root exudates plays a critical role in the reduction of silver ions, likely due to the presence of Cl ions in the 0-3 kDa.**
- **Without the >3 kDa fraction, the formed particles are less stable.**

SEM

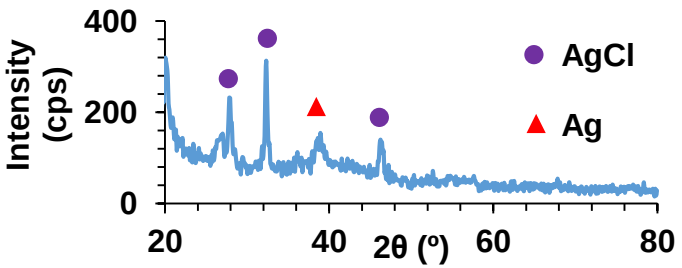


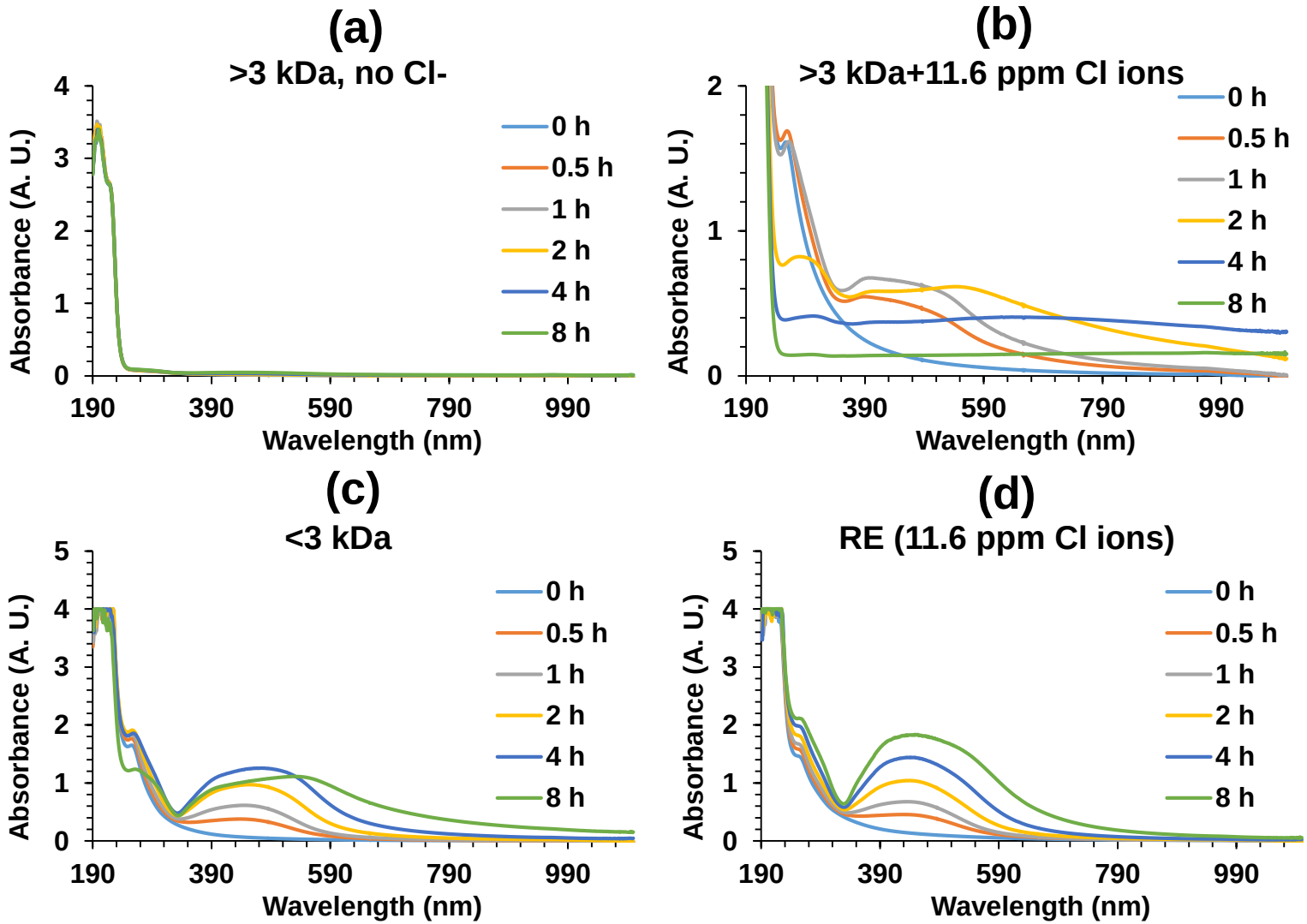
EDS

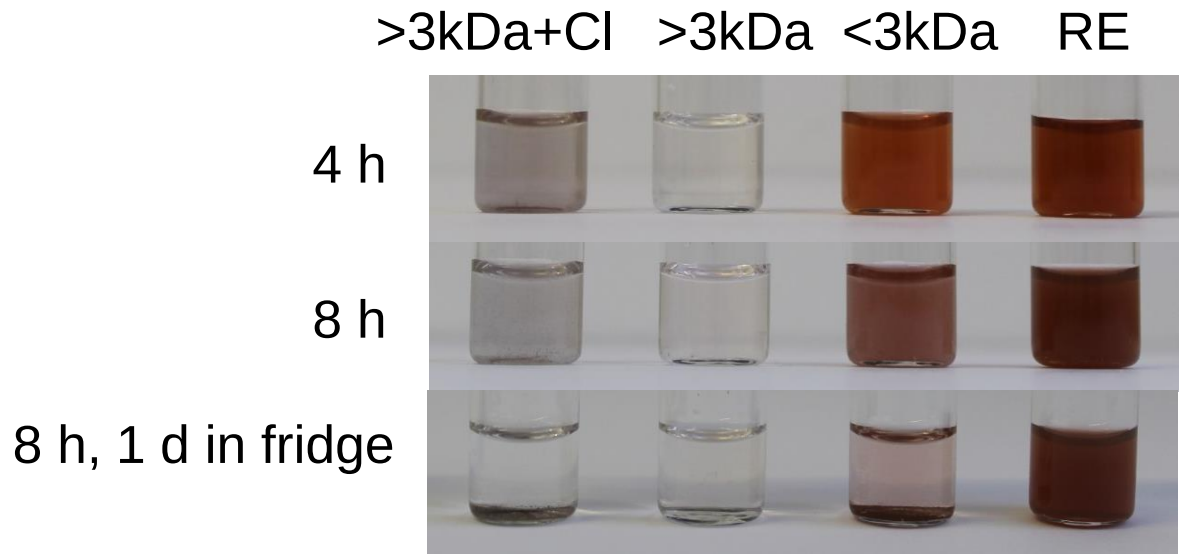


- **EDS and XRD data demonstrate that both nAg(0) and nAgCl are present in the formed particles.**

XRD

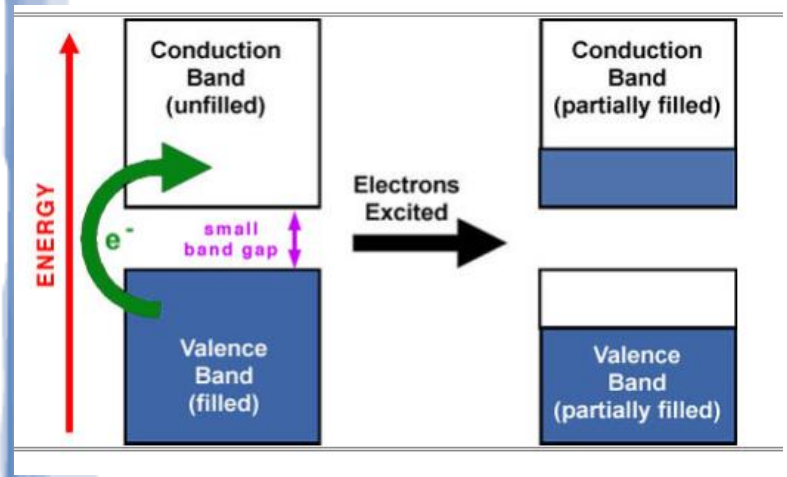


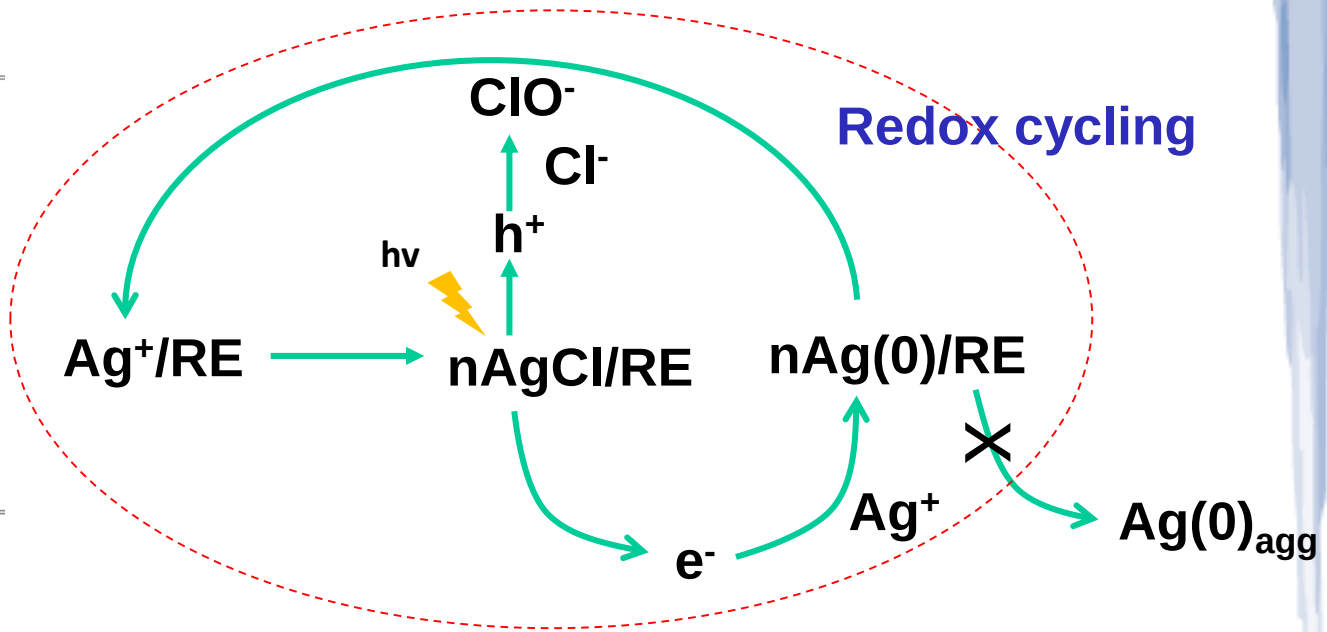

(a)
>3 kDa, no Cl-
Absorbance (A. U.)
Wavelength (nm)
0 h
0.5 h
1 h
2 h
4 h
8 h
(b)
>3 kDa+11.6 ppm Cl ions
Absorbance (A. U.)
Wavelength (nm)
0 h
0.5 h
1 h
2 h
4 h
8 h
(c)
<3 kDa
Absorbance (A. U.)
Wavelength (nm)
0 h
0.5 h
1 h
2 h
4 h
8 h
(d)
RE (11.6 ppm Cl ions)
Absorbance (A. U.)
Wavelength (nm)
0 h
0.5 h
1 h
2 h
4 h
8 h



- **Adding Cl ions into the fraction of >3 kDa leads to the formation of Ag-containing particles.**
- **Both >3 kDa and 0-3 kDa are responsible for the stabilization of the formed Ag-containing particles.**

Proposed Mechanism





Future work

1. **Influence of the Ag/Cl ratio in the system on the reduction pattern of Ag^+.**
2. **Characterize the structure difference using scanning electron microscopy at different ratios of Ag/Cl.**
3. **Quantify the fraction of AgCl and Ag(0) in the formed particles.**

Conclusions

1. **Root exudates possess the ability to reduce Ag^+.**
2. **The reducing strength doesn't derive from superoxide radicals, Ag^+-organic complexes, enzymes or other proteins, or reducing sugars.**
3. **Cl^- in root exudates plays an important function in converting Ag^+ because of the electrons generated by AgCl upon light exposure.**
4. **Both fractions of 0-3 kDa and > 3 kDa are responsible for the stabilization of the formed nAgCl and nAg particles.**

Significance:

- A new pathway leading to natural formation of silver-containing nanoparticles.
- Impact of environmental cycling on the fate and toxicity of silver ions and silver-containing nanoparticles.

Acknowledgements

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Committee members:

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Dr. Lili He
Dr. Wesley R. Autio

Lab members:

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- My Huynh
- Lauren Thistle

- **USDA NIFA Hatch Program (MAS00475)**
  USDA NIFA (2015-67017-23070)
- **BARD (IS-4964-16R)**

Thanks for your attention!

SI

Background

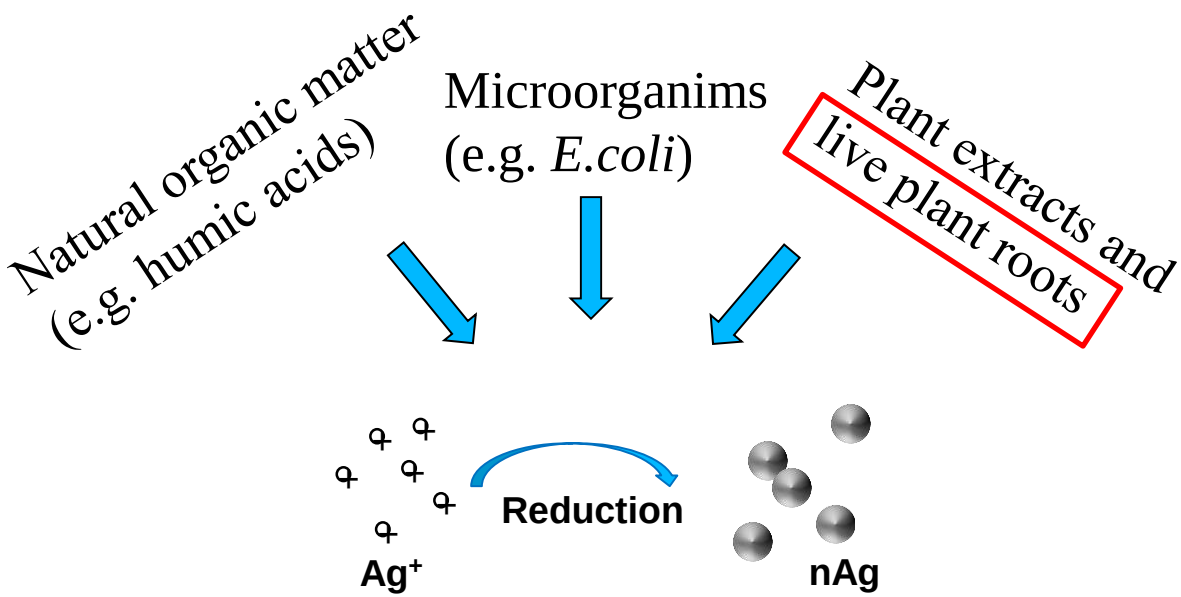
- **Environmental contamination of silver ions:**

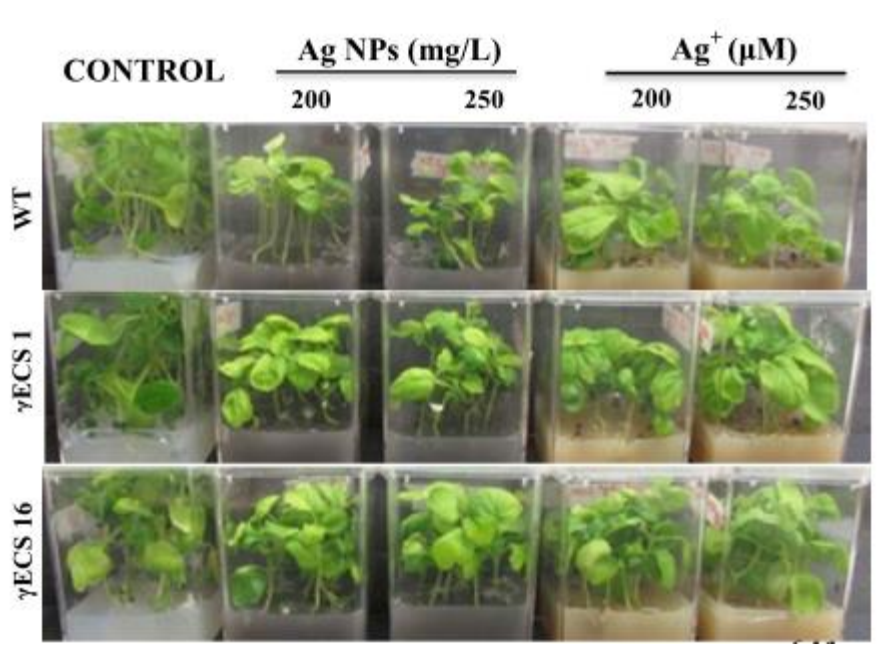
260 μg/L near photographic manufacturing waste discharges; 300 μg/L in treated photoprocessing wastewaters; 31 mg/kg in certain soils; and 150 mg/kg in river sediments.

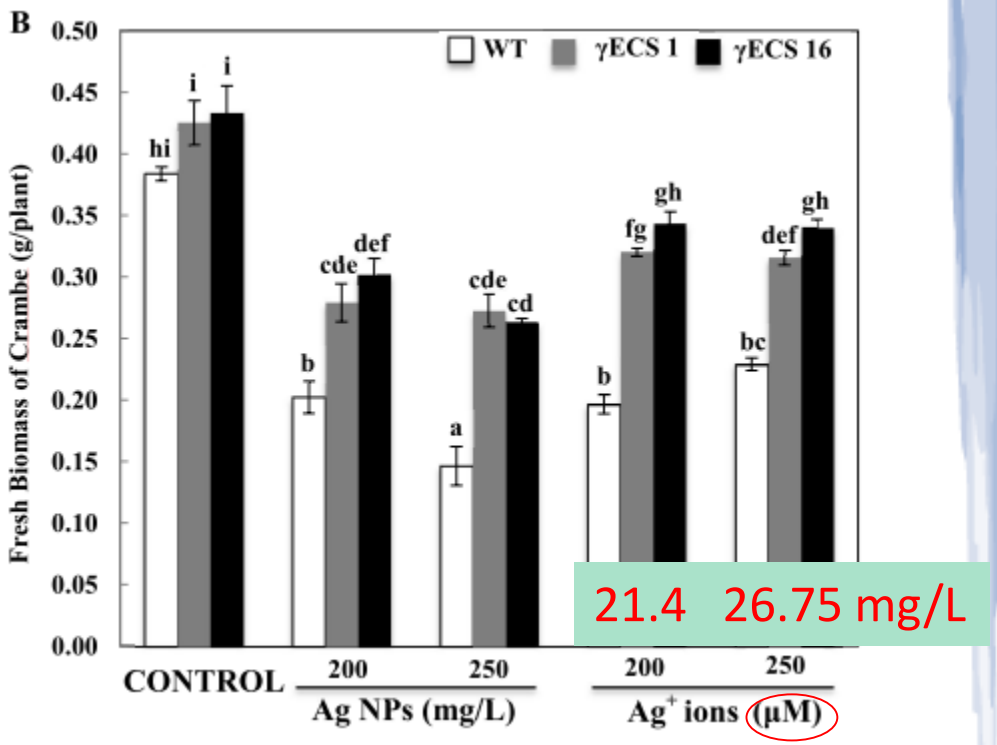
- **Environmental toxicity of silver ions：**

Lethal concentrations for sensitive aquatic plants, invertebrates, and teleosts: 1-5 μg/L. Adverse effects occur on development of trout at concentrations as low as 0.17 μg/L and on phytoplankton species composition and succession at 0.3-0.6 μg/L.
--WHO: Silver and Silver Compounds: Environmental Aspects

- **Reduction of silver ions：**





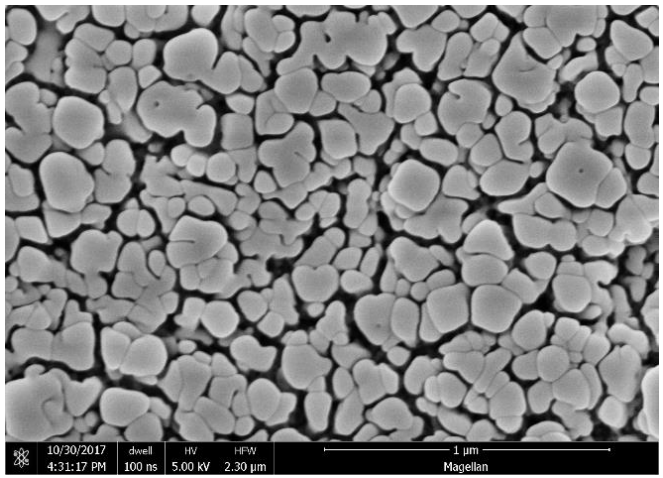


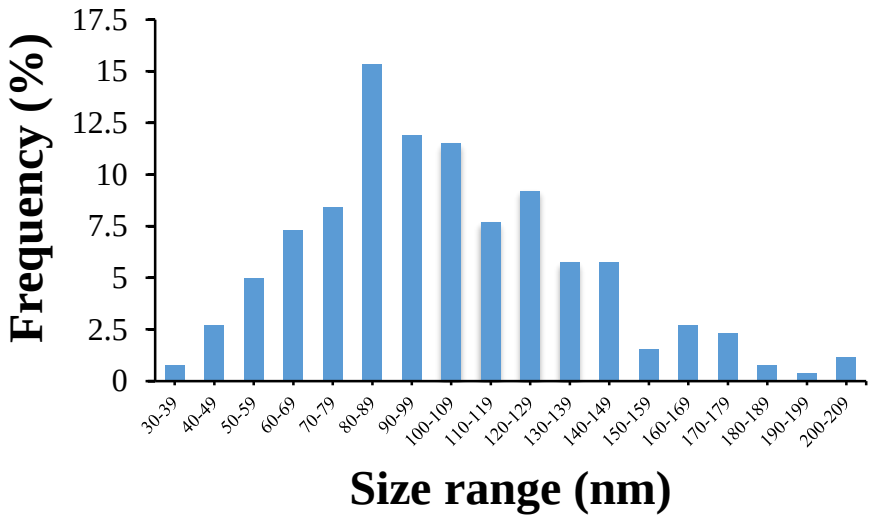
(Ma *et al*. 2015)

- **Ag^+ are more toxic than the same levels of AgNPs**.

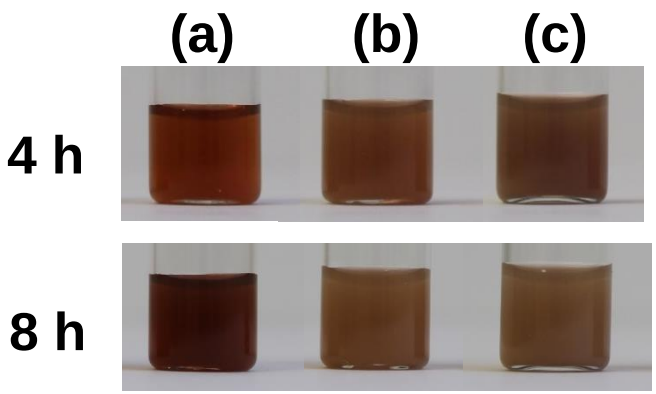
- The ability of plant root exudates to **transform more toxic Ag^+ to less toxic AgNPs** provides one possible pathway for plants **defending the stress caused by heavy metal ions**. Meanwhile, the alteration of silver species by plants may change their fate and toxicity of heavy metal ions in other living organisms.

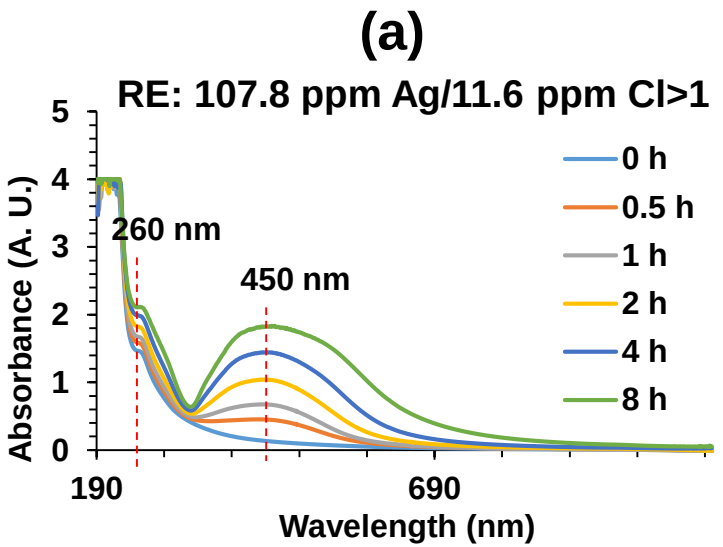
SEM

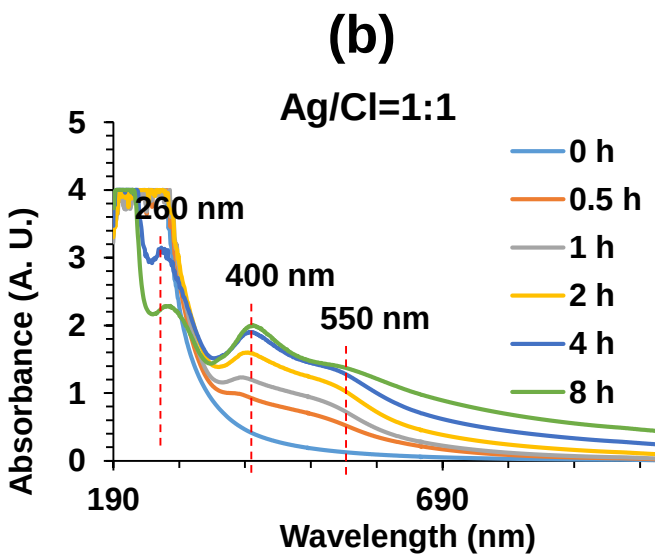


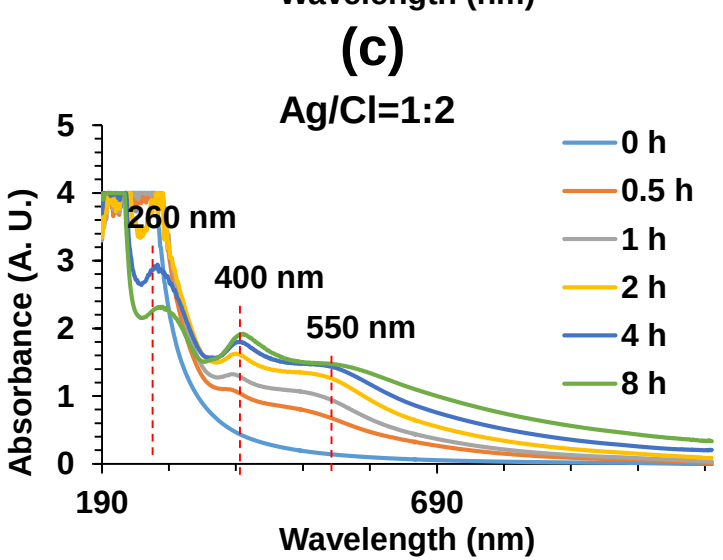


Size characterization	
Average diameter	103 nm
<100 nm	51%
>100 nm	49%

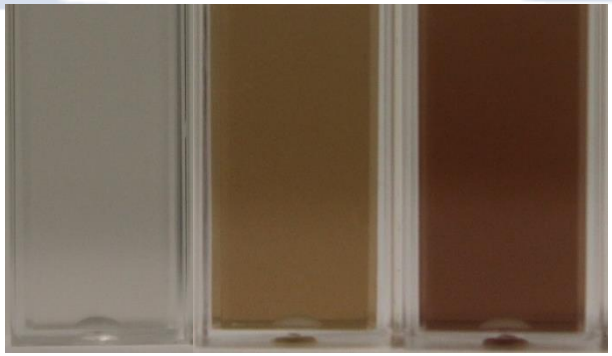


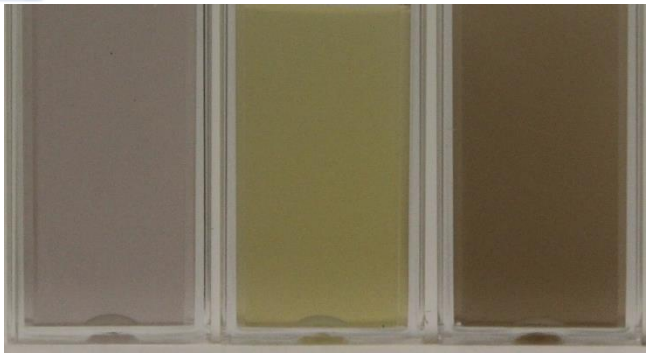


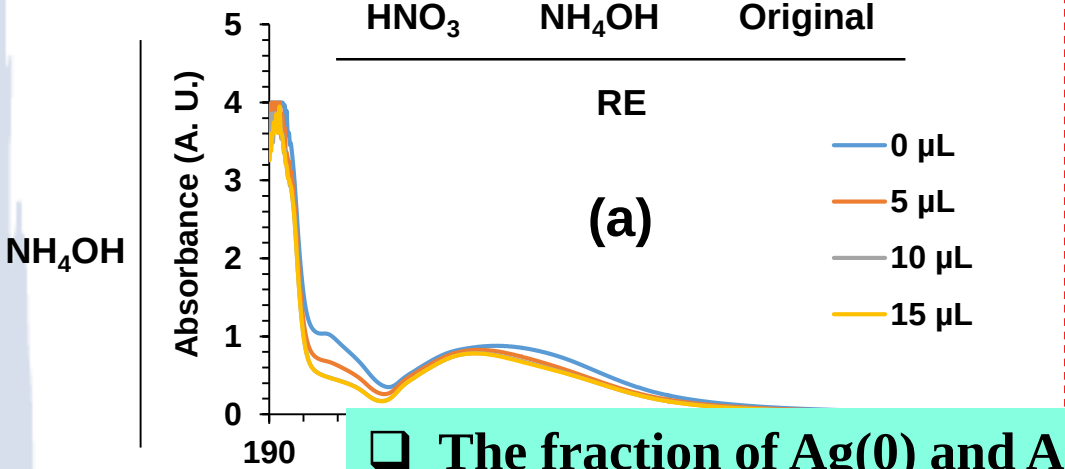


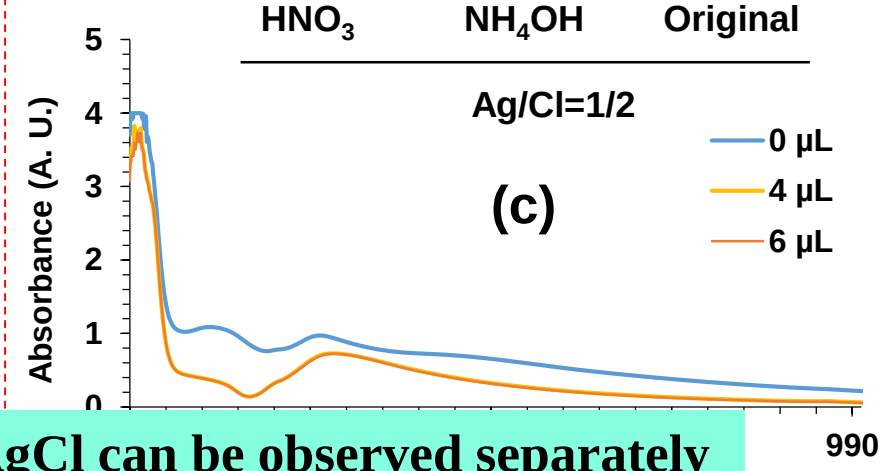


- **The ratio of Ag/Cl in the system can modify the reduction pattern of Ag^{+}.**









❑ **The fraction of Ag(0) and AgCl can be observed separately by using NH_4OH and HNO_3.**