



# Uptake and Biochemical Effects of Copper Nanoparticle Exposure to Grey Zucchini (*Cucurbita pepo*)

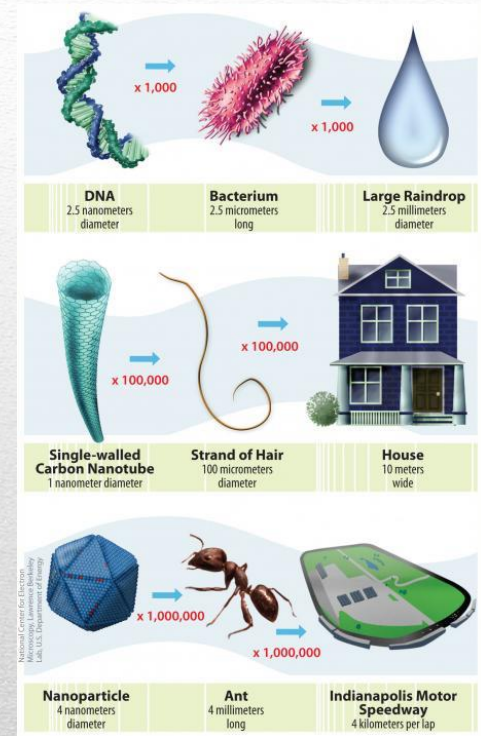
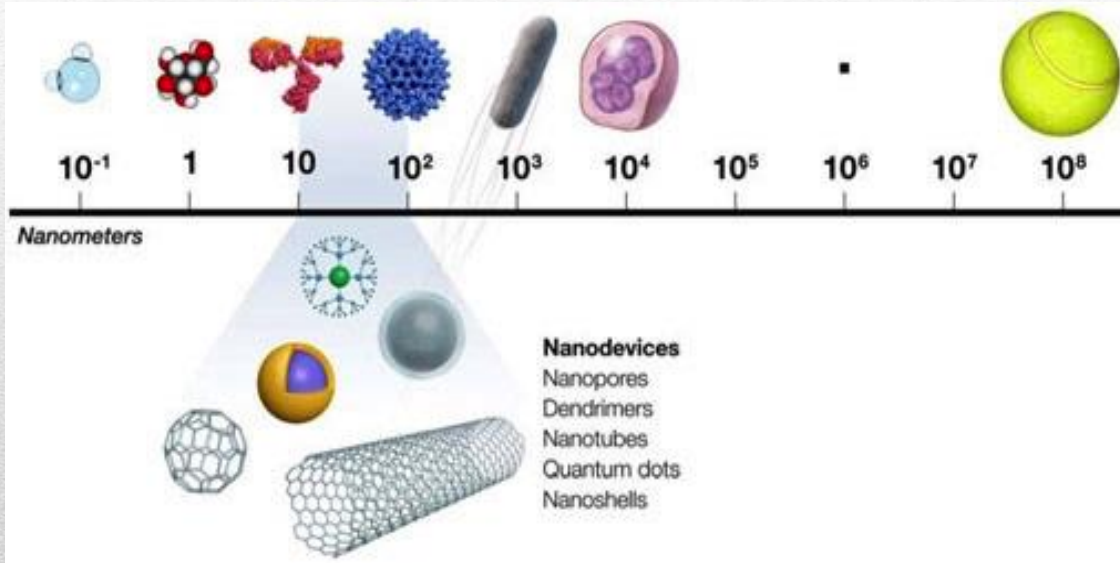
Carlos Tamez, MS

Environmental Science and Engineering PhD Program

University of Texas at El Paso

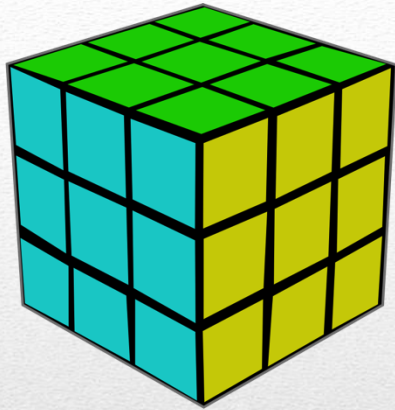
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**Nanoparticle** – materials with at least two dimensions between 1 and 100 nm.



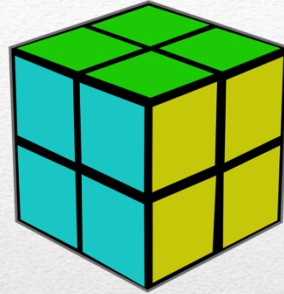
# Introduction

Nano Scale Visualized



sides = 3  
surface =  $3^2 \times 6 = 54$   
volume =  $3^3 = 27$

surface/volume = 2



sides = 2  
surface =  $2^2 \times 6 = 24$   
volume =  $2^3 = 8$

surface/volume = 3

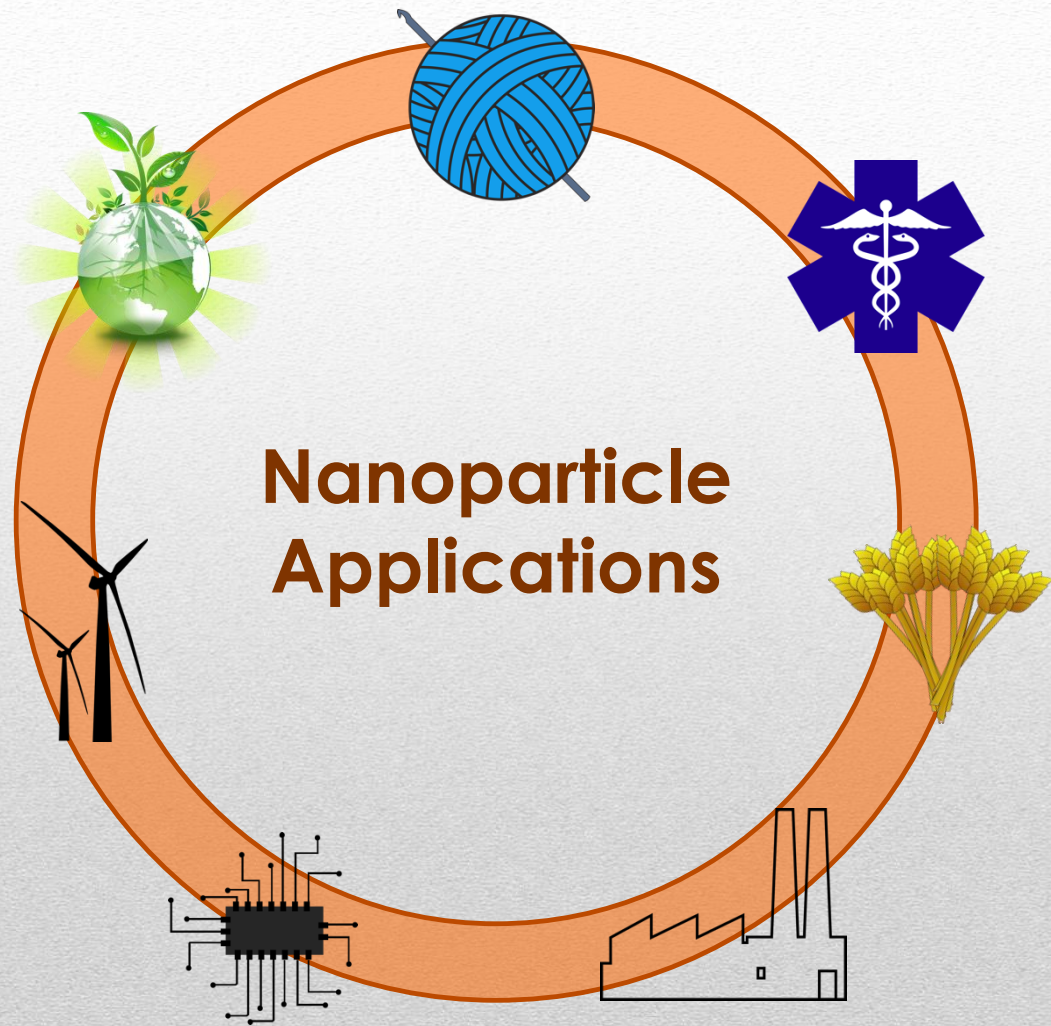


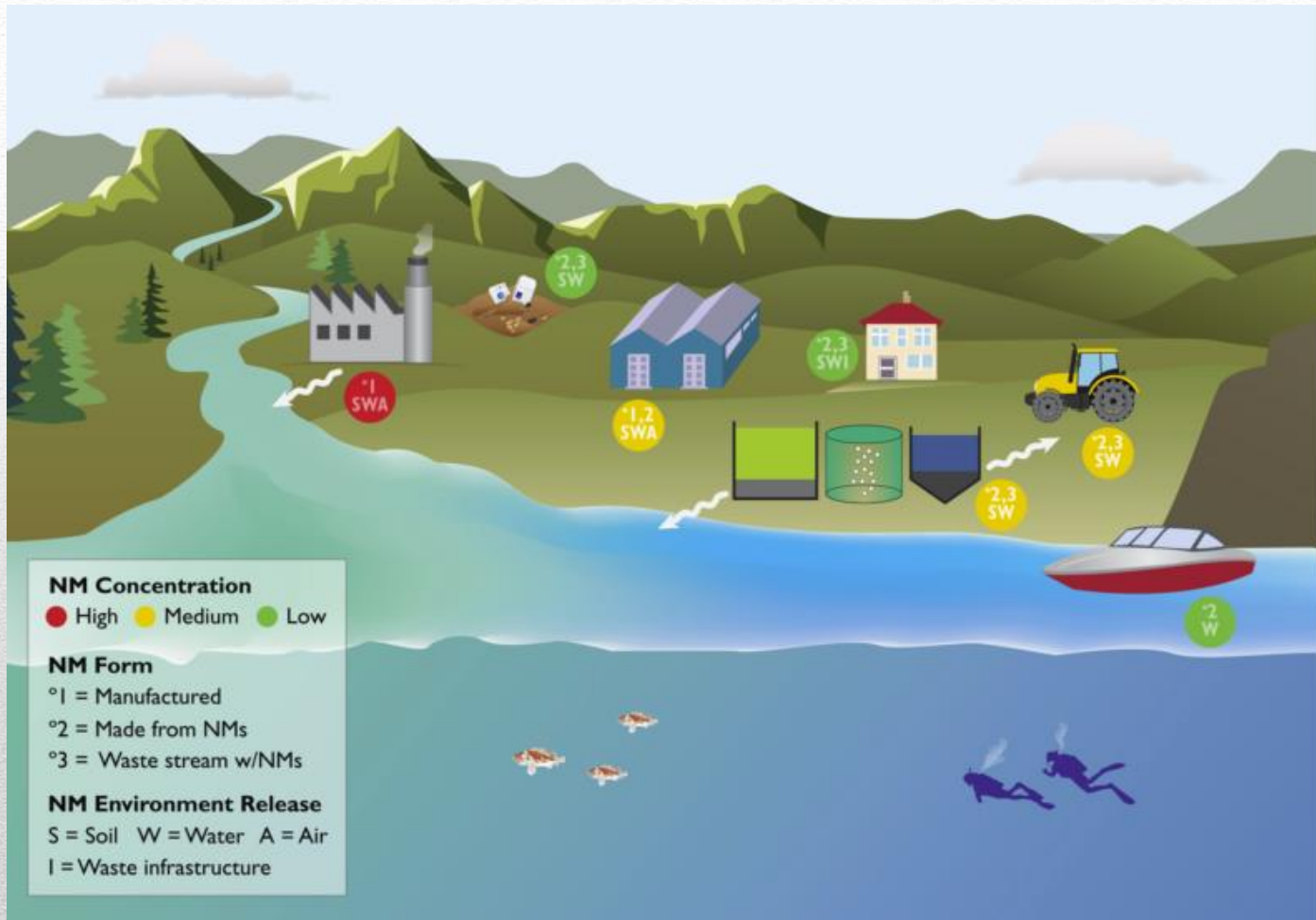
sides = 1  
surface =  $1^2 \times 6 = 6$   
volume =  $1^3 = 1$

surface/volume = 6

# Introduction

Increased Surface Area to Volume







Anti-fouling paints on boats and ships



Copper based inks on printed circuit boards



Antimicrobial coatings on food packaging



Lumber preservative in pressure treated woods



Increasing interest in the uses of nano-sized pesticides and fertilizers

# Introduction

Copper Nanoparticle Applications

NP	Plant	Experiment Type	Results	Reference
CuO	Yellow Squash	hydroponics	root length ↓ biomass ↓	(Stampoulis, Sinha, White 2009)
CuO	Duckweed	solution	plant growth ↓	(Perreault et al. 2010)
CuO	Waterweed	solution	phytotoxicity	(Johnson et al. 2011)
CuO	Waterweed	solution	oxidative stress ↓ photosynthesis ↓	(Nekrasova et al. 2011)
CuO	Cucumber	hydroponic	seedling biomass ↓	(Kim, Lee, Lee 2012)
CuO	Maize	hydroponic	growth ↓	(Wang et al. 2012)
Cu	Yellow Squash	hydroponic	biomass ↓	(Musante and White 2012)
CuO	Wheat	sand	shoot length ↓ root length ↓ biomass ↑	(Dimkpa et al. 2012) (Dimkpa et al. 2013)
CuO	Buckweat	3 day germination	root length ↓ seedling biomass ↓	(Lee et al. 2013)
CuO	Rice	14 day germination	germination ↓ oxidative stress ↑	(Shaw and Hossain 2013)
Cu/Cu O	Lettuce	hydroponic	chlorophyll ↓ root length ↓ biomass ↓	(Trujillo-Reyes et al. 2014)
CuO	Lettuce/Alfalfa	hydroponic	growth ↓ stress ↓	(Hong et al. 2015)
CuO	Carrots	sand	no observable effects	(Ebbs et al. 2016)
CuO	Sweet Potato	Soil, maturity	biomass ↓	(Bradfield et al. 2017)



- Grey Zucchini
  - Variety of summer squash
  - Consumed worldwide
  - Domestic production,  $2.6 \times 10^{11}$  kg (all varieties)



*Cucurbita pepo*

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<http://bit.ly/1kXOpQu>



<http://bit.ly/1QFoDwP>



<http://bit.ly/2yP0Zwe>



<http://bit.ly/2y4byHs>

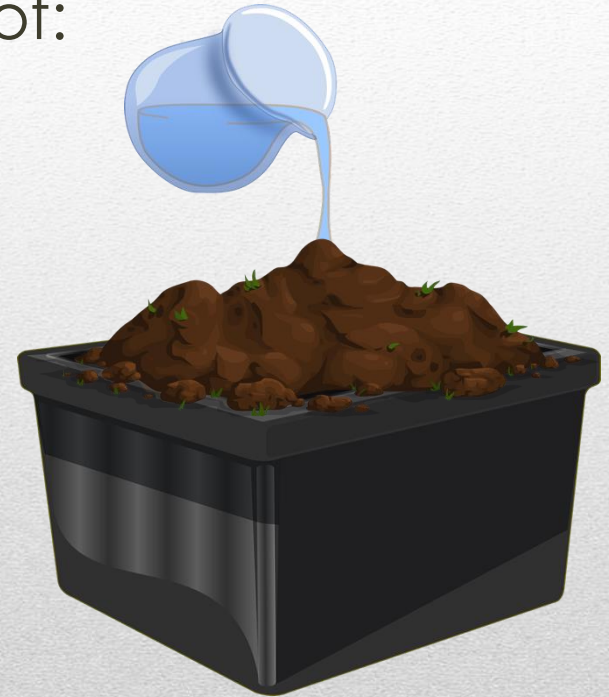
Potential for human exposure through edible tissues



# METHODS

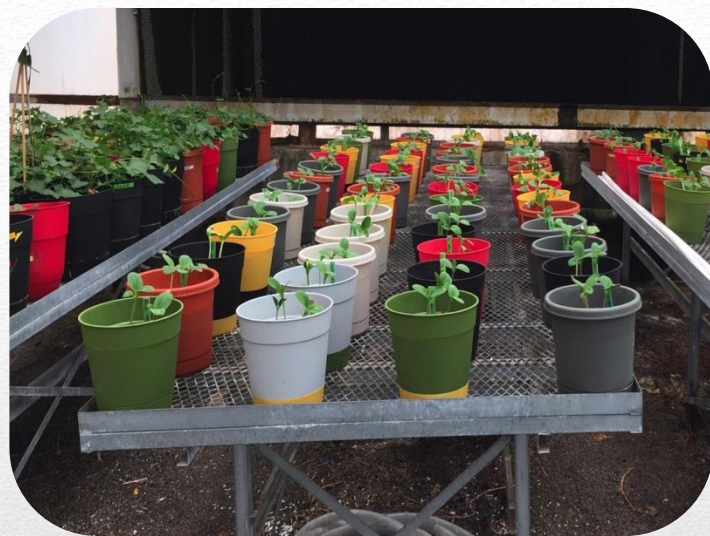
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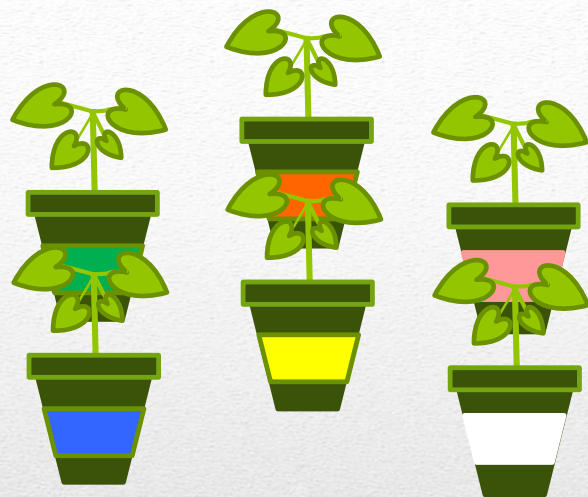
- Native soil amended with Miracle-Gro
- Soil treated with a suspension of:
  - Kocide 3000 (46.1%  $\text{Cu}(\text{OH})_2$ )
  - $n\text{CuO}$
  - bulk  $\text{CuO}$
  - $\text{Cu NP}$
  - $\text{CuCl}_2$



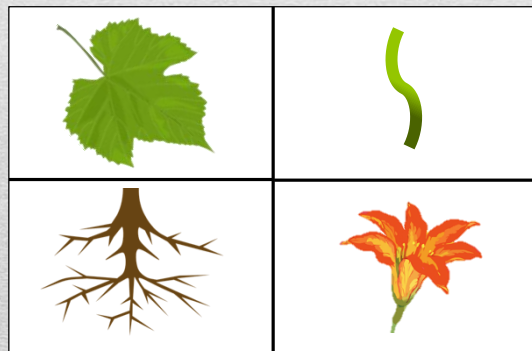
## Mesocosm Experiments

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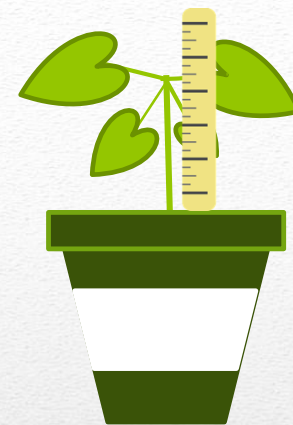




Tissue Harvest



Tissue Separation



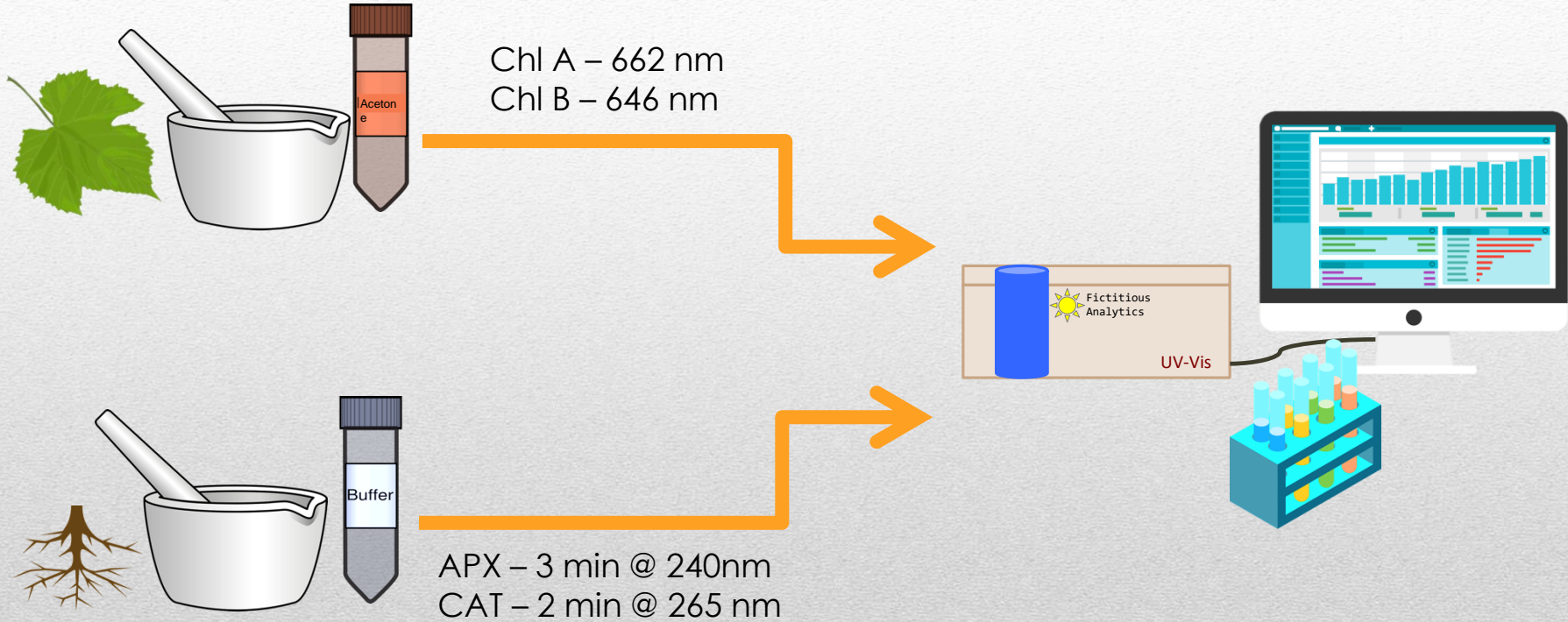
Collect Measurements

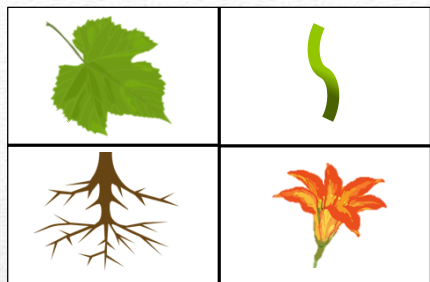


Tissue Washing

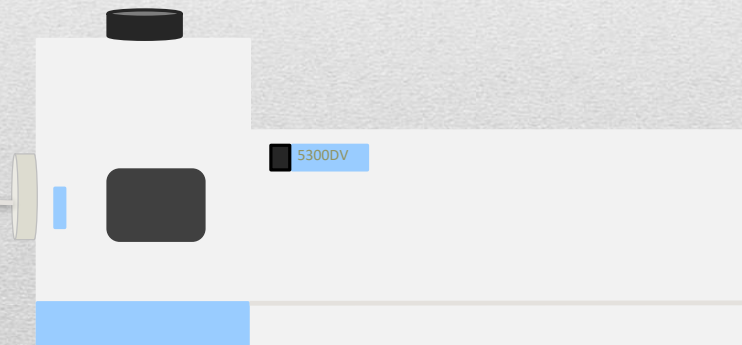
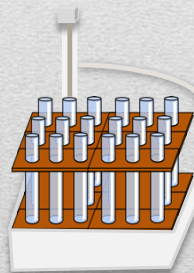
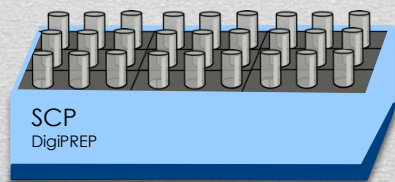
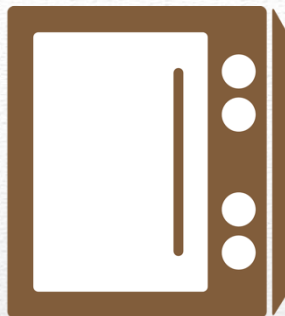


# Fresh Tissue Analysis





70°C  
72 h



45 min @ 115°C

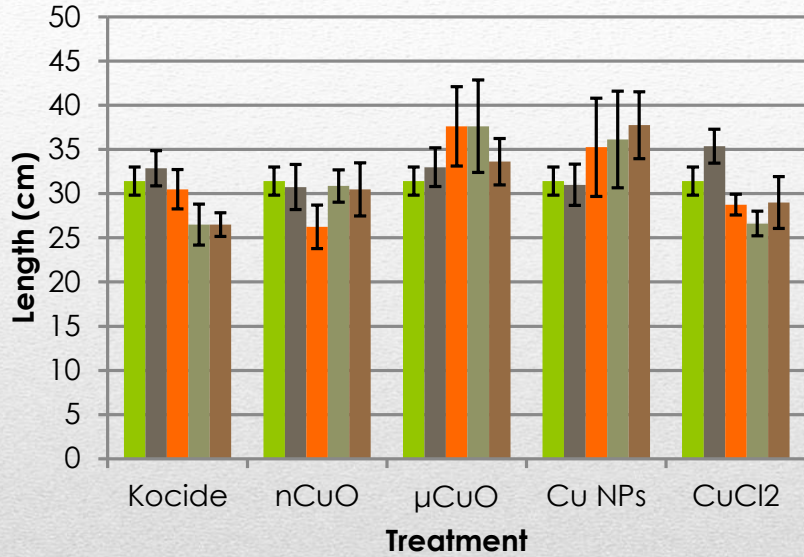


# RESULTS

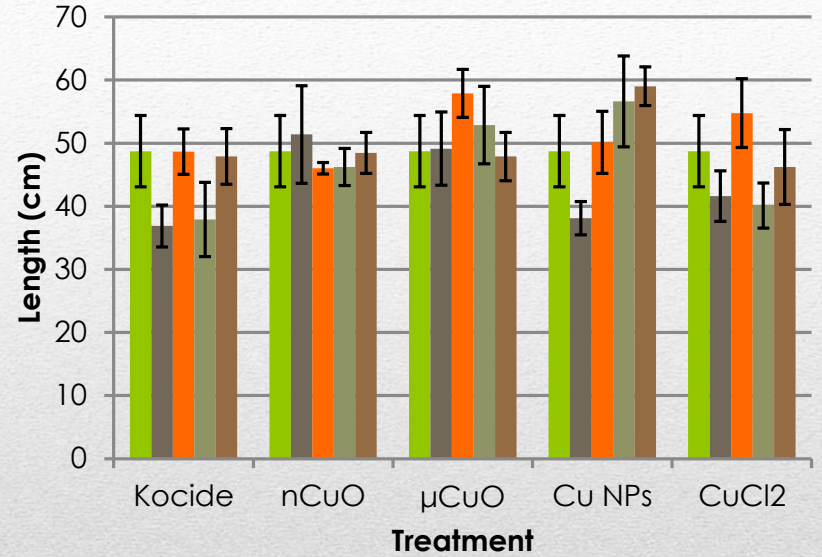
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### Plant Length

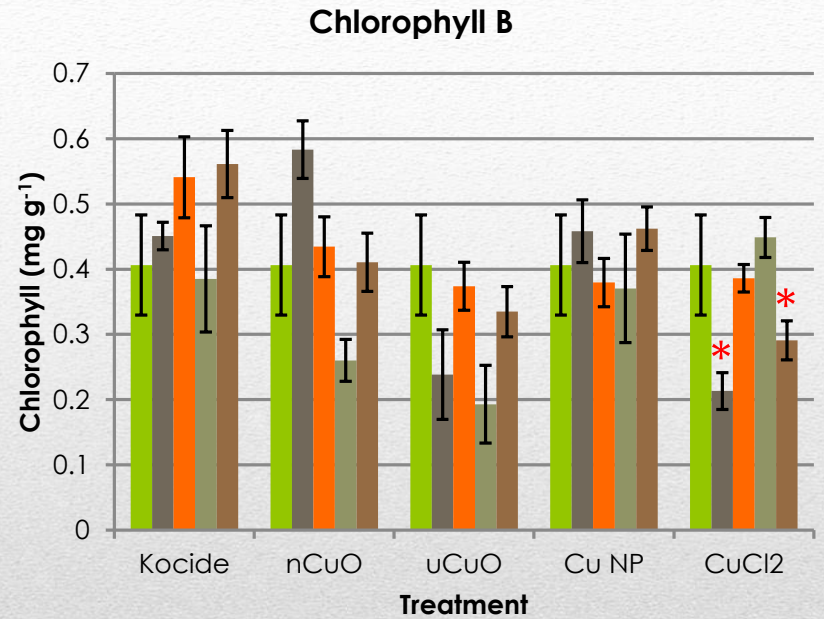
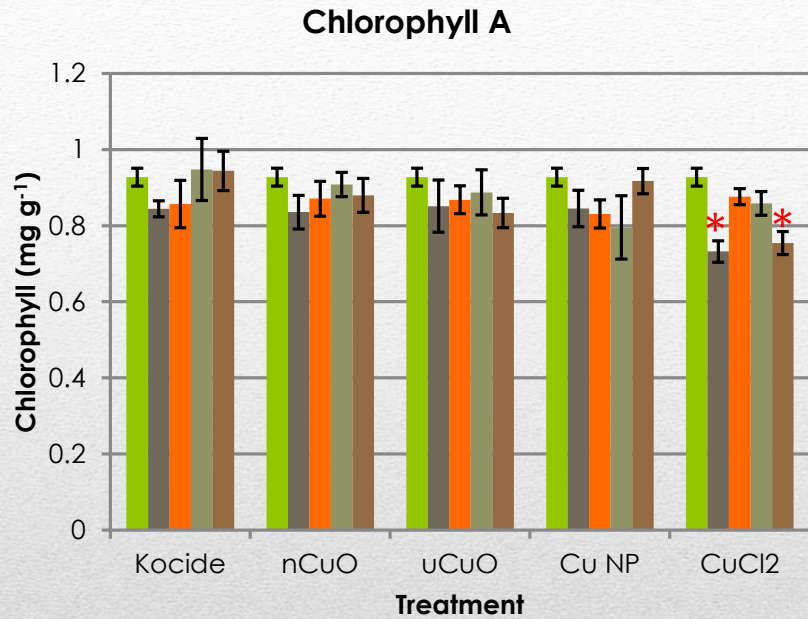


### Root Length



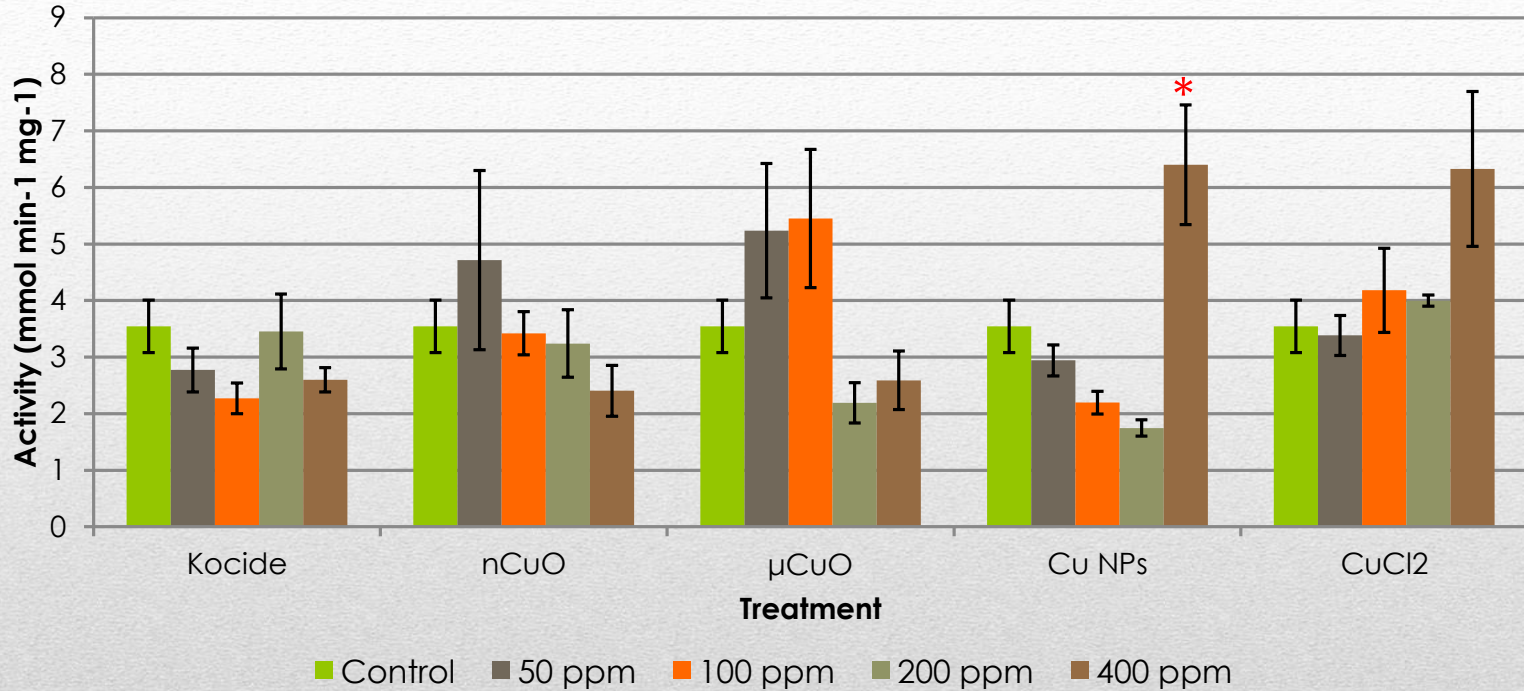
■ Control ■ 50 ppm ■ 100 ppm ■ 200 ppm ■ 400 ppm

# Agronomics

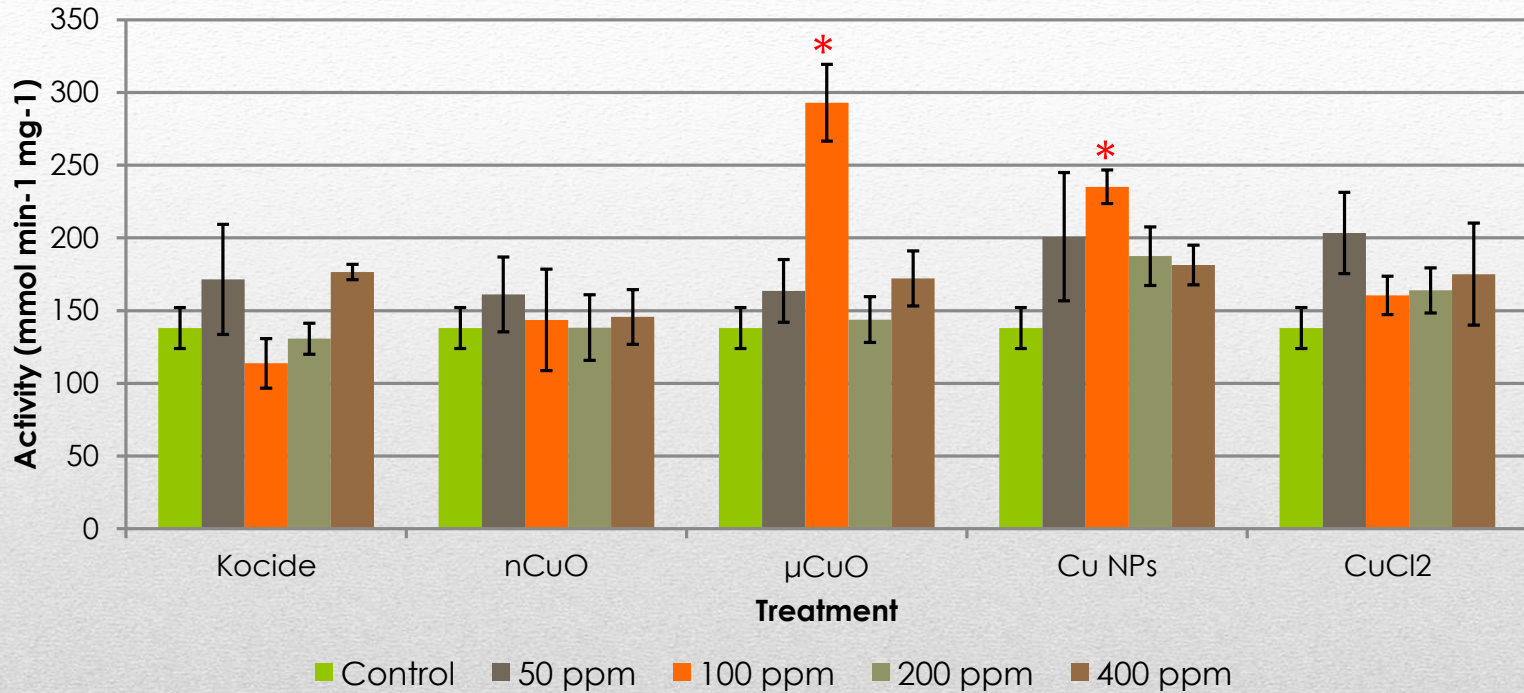


■ Control 
 ■ 50 ppm 
 ■ 100 ppm 
 ■ 200 ppm 
 ■ 400 ppm

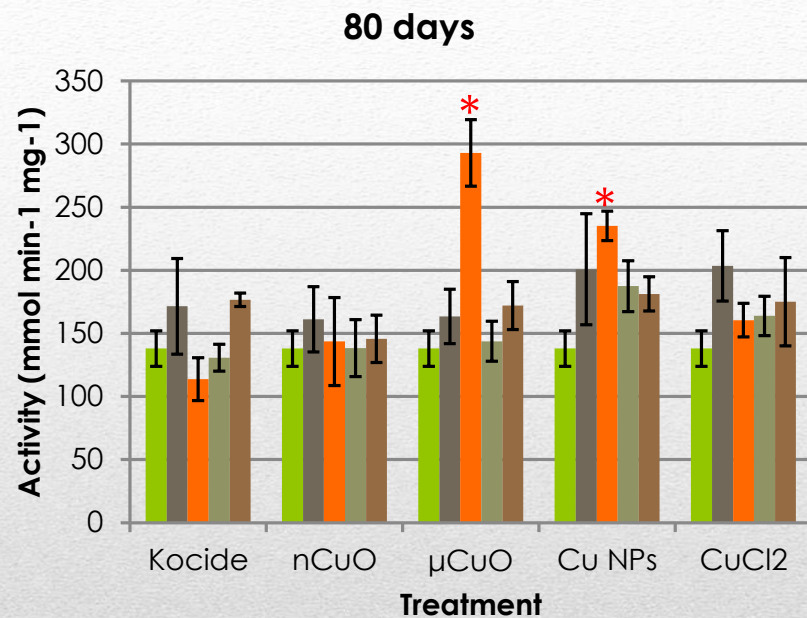
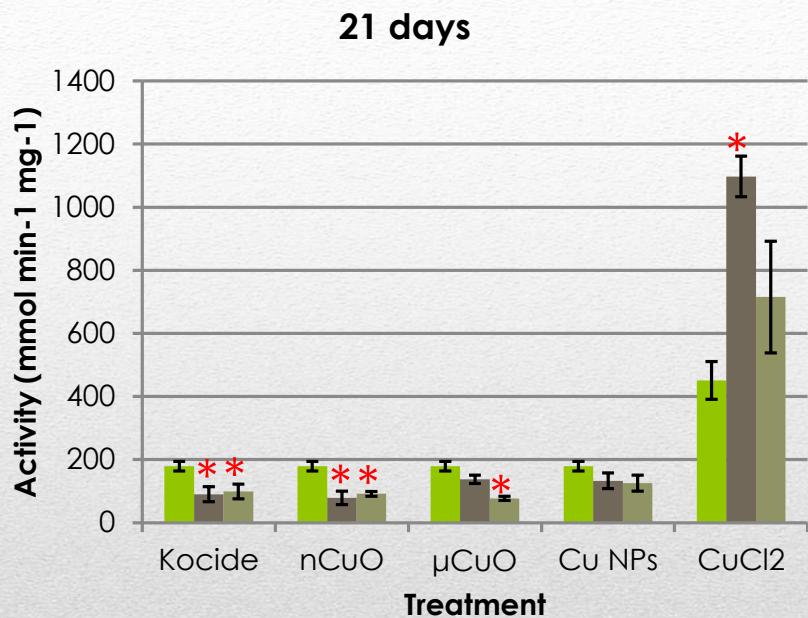
# Chlorophyll Content



# Catalase Activity



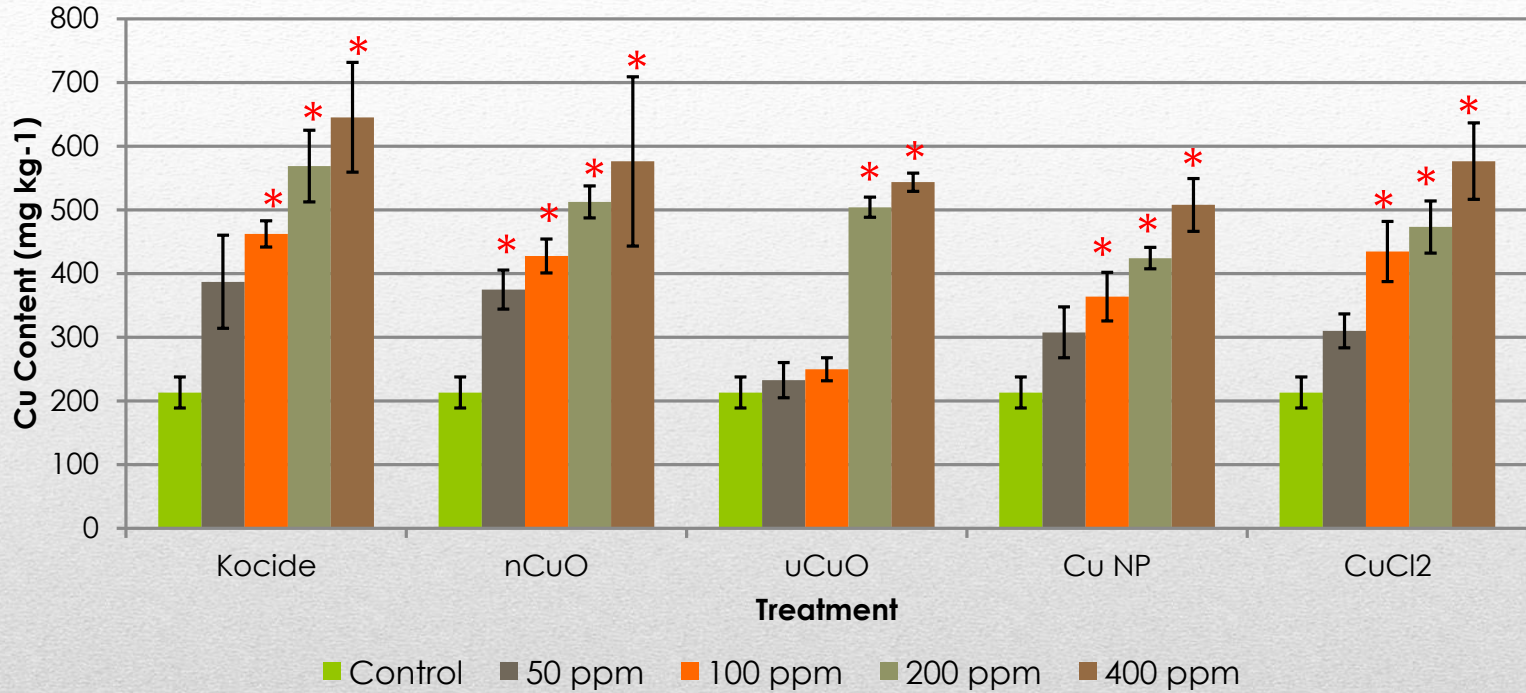
# Ascorbate Peroxidase Activity



■ Control 
 ■ 50 ppm 
 ■ 100 ppm 
 ■ 200 ppm 
 ■ 400 ppm

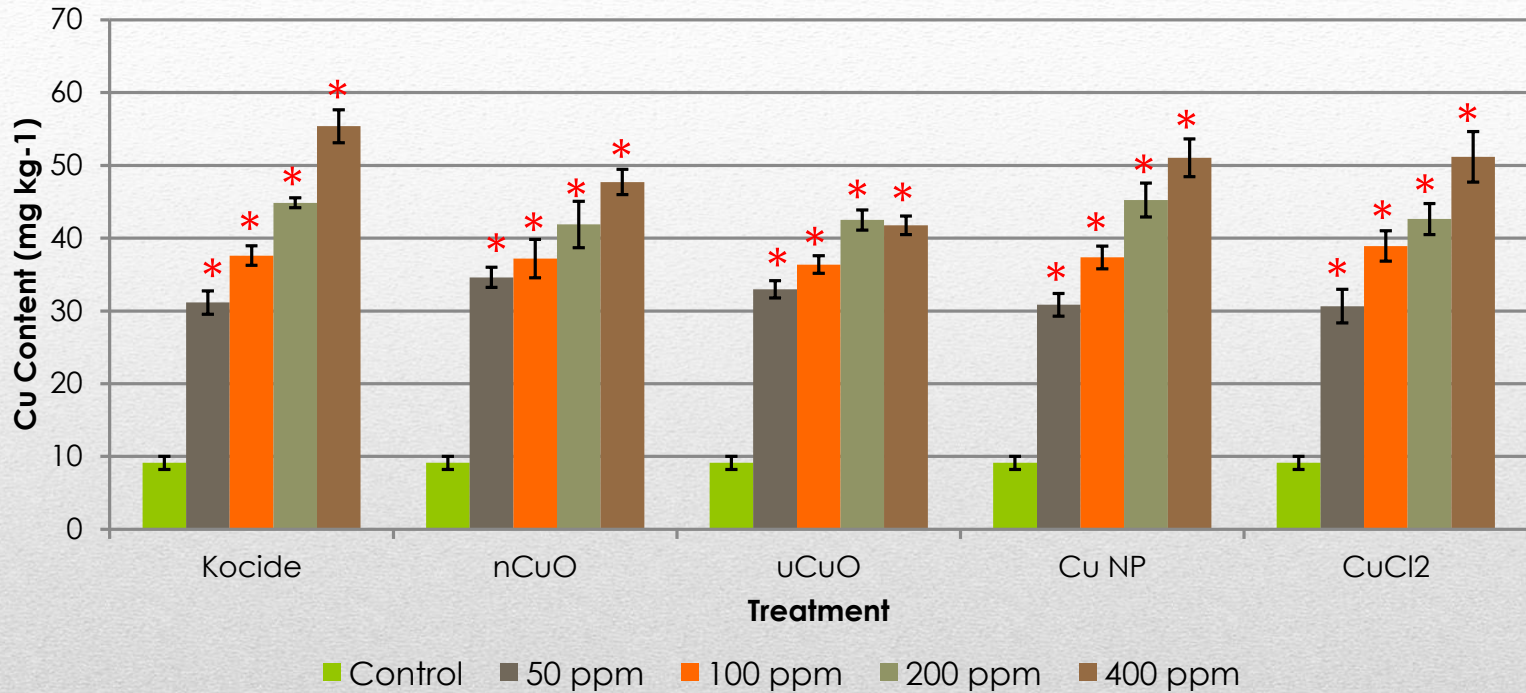
# Ascorbate Peroxidase

21 days vs. 80 days



# Copper Uptake

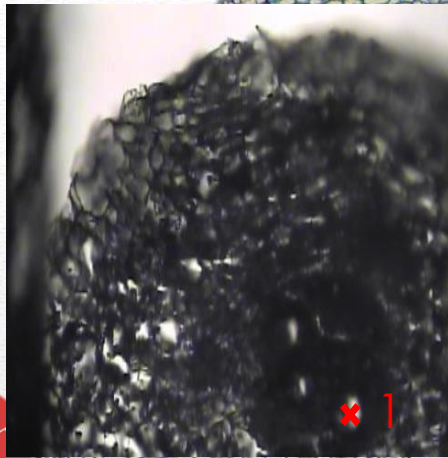
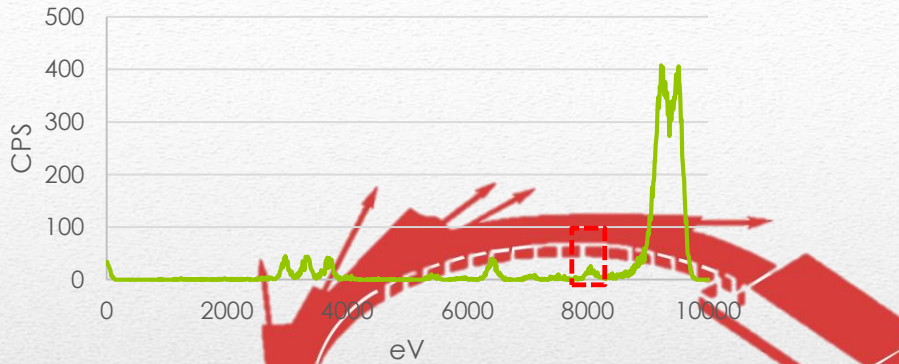
Roots



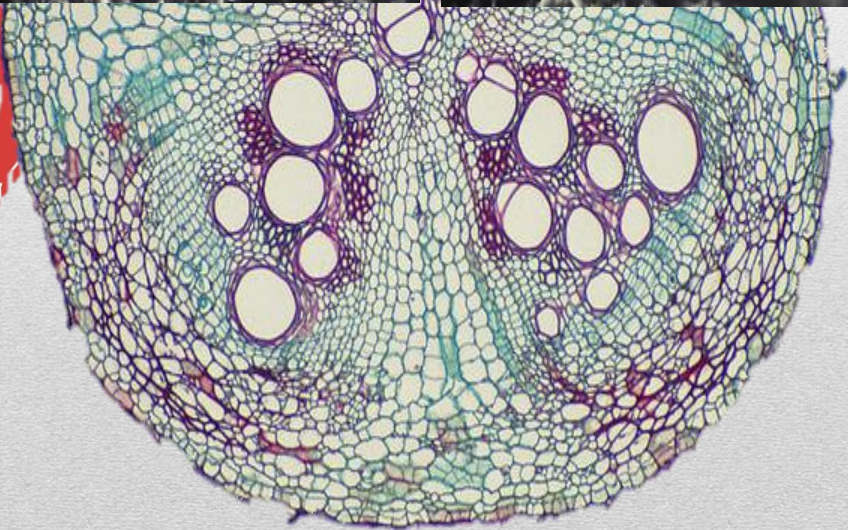
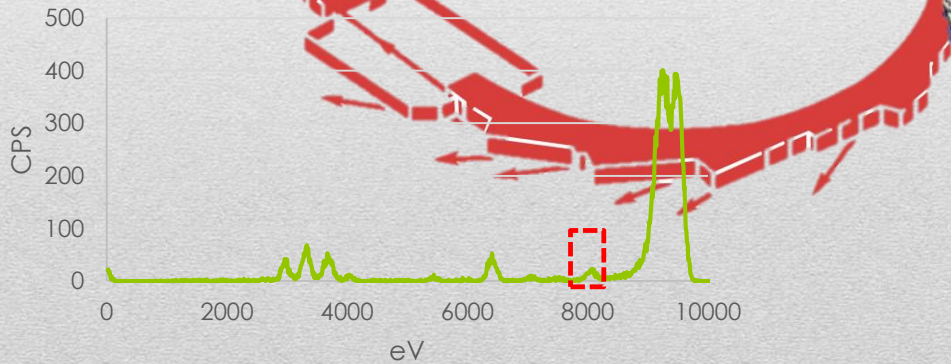
# Copper Uptake

Flowers

# 1 X-Ray Fluorescence-Vascular

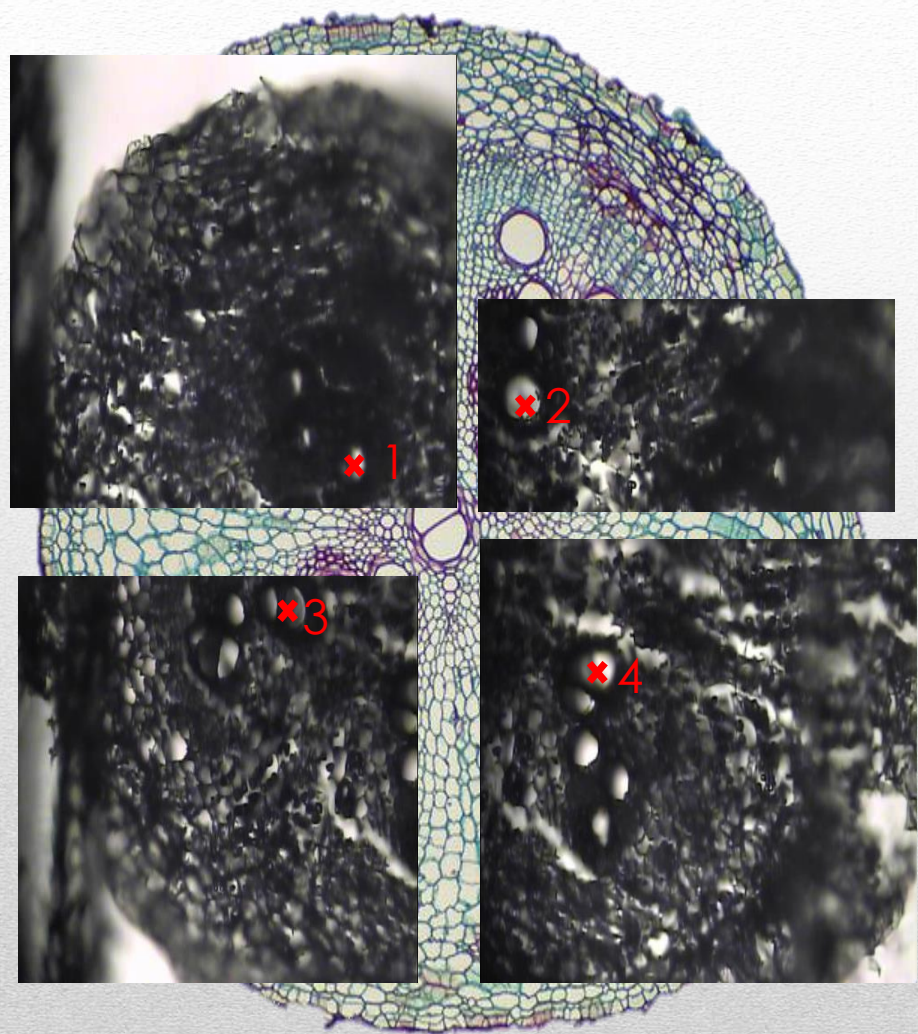
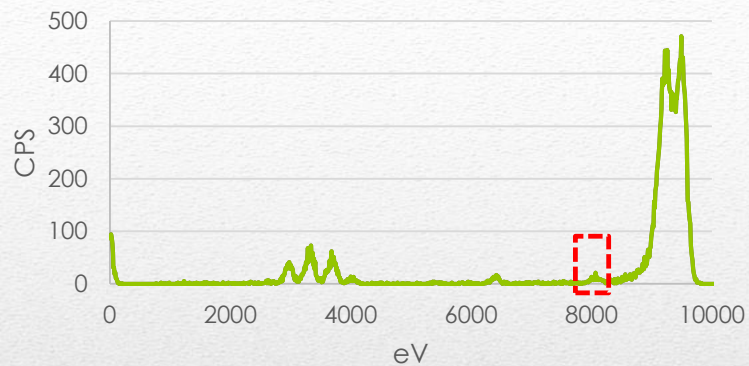


# 2 X-Ray Fluorescence-Vascular

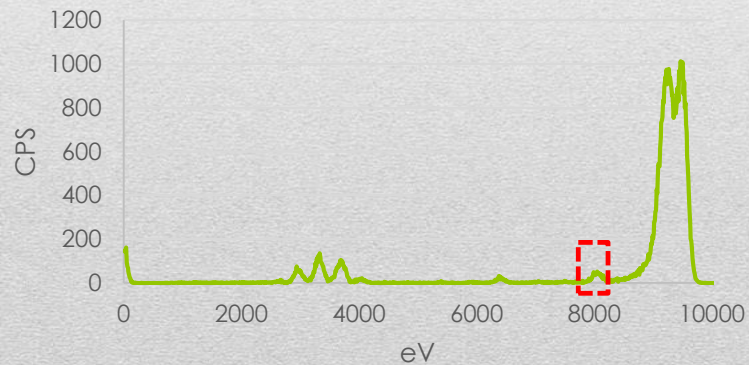




### 3 X-Ray Fluorescence-Vascular



### 4 X-Ray Fluorescence-Vascular



- No significant differences in plant height or root length.
- Only  $\text{CuCl}_2$  showed a significant decrease in chlorophyll content.
- Catalase activity remained comparable to control levels, except for Cu NPs.

## Conclusions

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- Ascorbate peroxidase activity increase only in plants treated with  $\mu\text{CuO}$  at 100 ppm and with  $\text{CuCl}_2$  at 100 ppm.
- Compared to a 3 week study, APX activity remained similar to control for nearly all treatments.

## Conclusions

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- Copper concentrations in the roots increased with all treatments.
- Copper was transfer to edible tissues, with flowers showing increases at every treatment level.

## Conclusions

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# Acknowledgements