

Physiological and biochemical effects of nano, bulk, and ionic copper in kidney bean (*Phaseolus vulgaris*) plants treated with kinetin

Suzanne Apodaca

Dr. Jorge Gardea's Research Group

November 6th, 2017 | Los Angeles, CA



**Sustainable
Nanotechnology
Organization**

Research | Education | Responsibility



OVERVIEW

1

Background

2

Methodology

3

Results

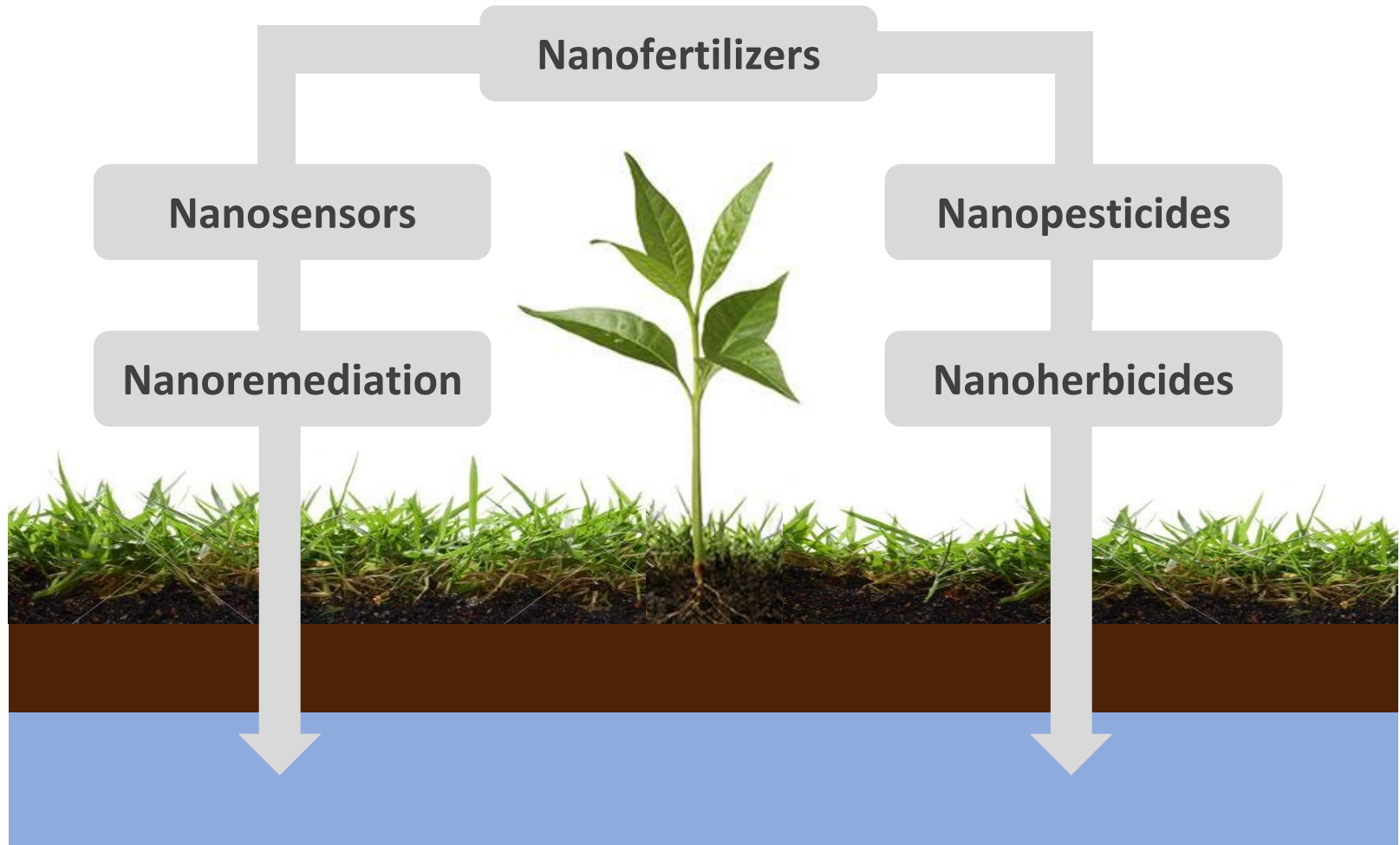
4

Conclusions

BACKGROUND



NANOTECHNOLOGY IN AGRICULTURE



NANOTECHNOLOGY IN AGRICULTURE

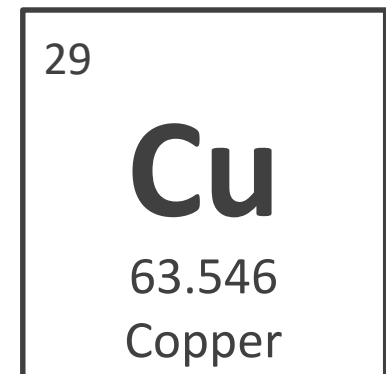
In 2016:



Essential trace mineral

Imbalance can result in toxicity or deficiency

Antimicrobial properties



PLANT GROWTH REGULATORS

Naturally and synthetically derived

Govern developmental processes within plants

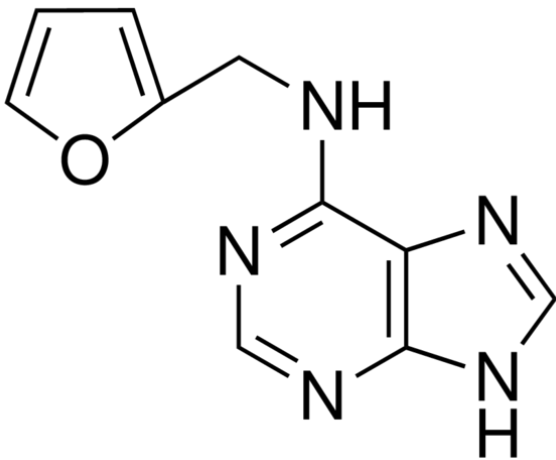


Bio-based fertilizer alternative

Stimulates cell division and enlargement

Delays senescence and acts as anti-stress agent

Phytoextraction potential



KIDNEY BEAN

Nutrient-rich and affordable

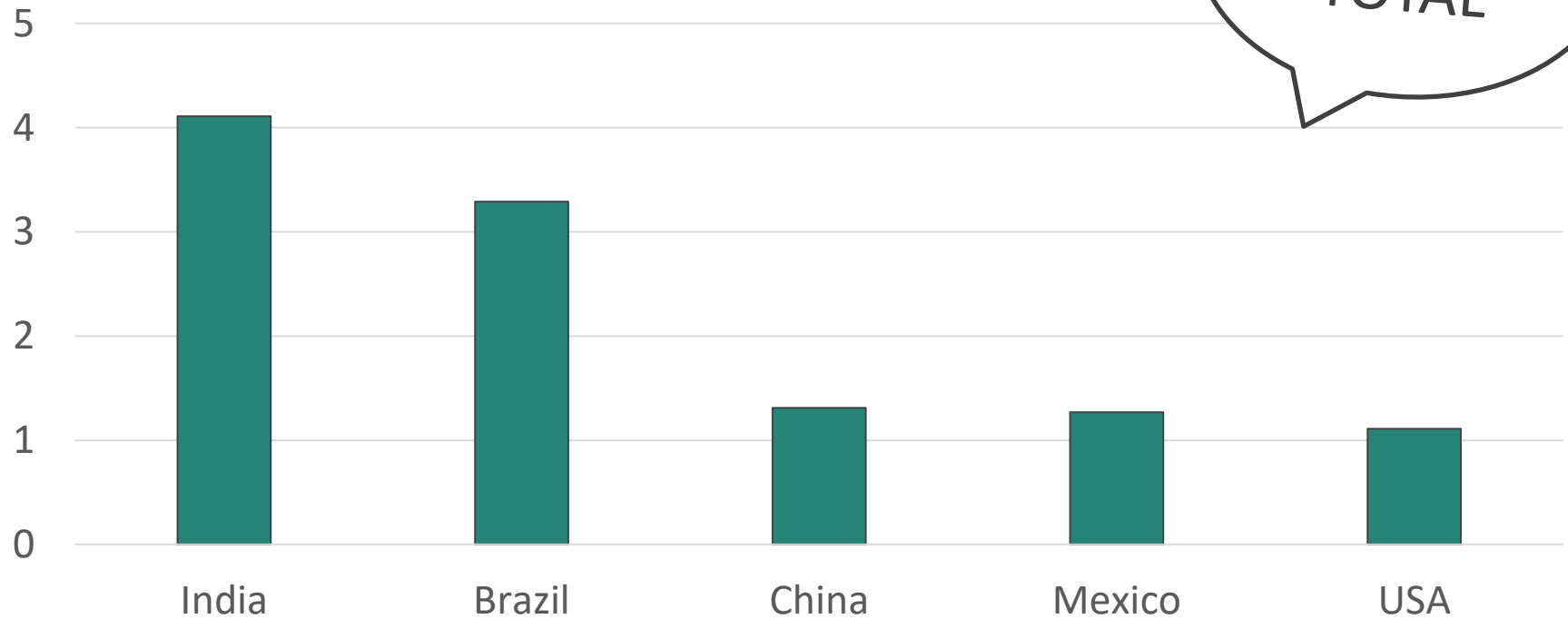
Universal staple food



Serving Size 130 g	
Servings Per Container 2	
Amount Per Serving	
Calories 110	Calories from Fat 0
% Daily Value*	
Total Fat 0g	0%
Saturated Fat 0g	0%
Trans Fat 0g	
Cholesterol 0mg	0%
Sodium 130mg	5%
Total Carbohydrate 19g	6%
Dietary Fiber 8g	32%
Sugars 1g	
Protein 8g	
Vitamin A 0%	• Vitamin C 2%
Calcium 15%	• Iron 15%

KIDNEY BEAN

Dry bean production in 2014
(million metric tons)



LITERATURE REVIEW

1

Humic acid increased ionic Cu content of nano Cu suspension (Musante et al., 2012)

2

Nano Cu influenced nutrient uptake and detoxification mechanisms in cucumber plants (Zhao et al., 2016)

3

Cu-based NPs decreased elemental accumulation of phosphorus in cilantro (Zuverza et al., 2015)

4

KN increased growth characters and chemical constituents in black cumin (Ahmed et al., 2017)

5

KN improved quality, yield and nutrient uptake of rice seedlings (Nazir et al., 2016)

Examine the effects of different Cu compounds and KN on kidney bean plant physiology and biochemistry

- ✓ Determine the elemental uptake in plant tissues
- ✓ Analyze metabolic changes
- ✓ Evaluate seed macromolecular composition

METHODOLOGY



EXPERIMENTAL DESIGN

Factors

Nano copper – *nCu*

Bulk copper – *bCu*

Copper chloride – CuCl_2

Kinetin – KN

Statistics

One-way ANOVA

Multi-way ANOVA

		Cu (mg/kg)		
		0	50	100
KN (μM)	0	3	3	3
	10	3	3	3
	100	3	3	3

EXPERIMENTAL DESIGN

Factors

Nano copper – *nCu*

Bulk copper – *bCu*

Copper chloride – CuCl_2

Kinetin – KN

Statistics

One-way ANOVA

Multi-way ANOVA

		Cu (mg/kg)		
		0	50	100
KN (μM)	0	3	3	3
	10	3	3	3
	100	3	3	3

EXPERIMENTAL DESIGN

Factors

Nano copper – *nCu*

Bulk copper – *bCu*

Copper chloride – CuCl_2

Kinetin – KN

Statistics

One-way ANOVA

Multi-way ANOVA

		Cu (mg/kg)		
		0	50	100
KN (μM)	0	3	3	3
	10	3	3	3
	100	3	3	3

EXPERIMENTAL DESIGN

Factors

Nano copper – *nCu*

Bulk copper – *bCu*

Copper chloride – CuCl_2

Kinetin – KN

Statistics

One-way ANOVA

Multi-way ANOVA

Cu (mg/kg)

	0	50	100
0	3	3	3
10	3	3	3
100	3	3	3

KN (μM)

EXPERIMENTAL DESIGN

Factors

Nano copper – *nCu*

Bulk copper – *bCu*

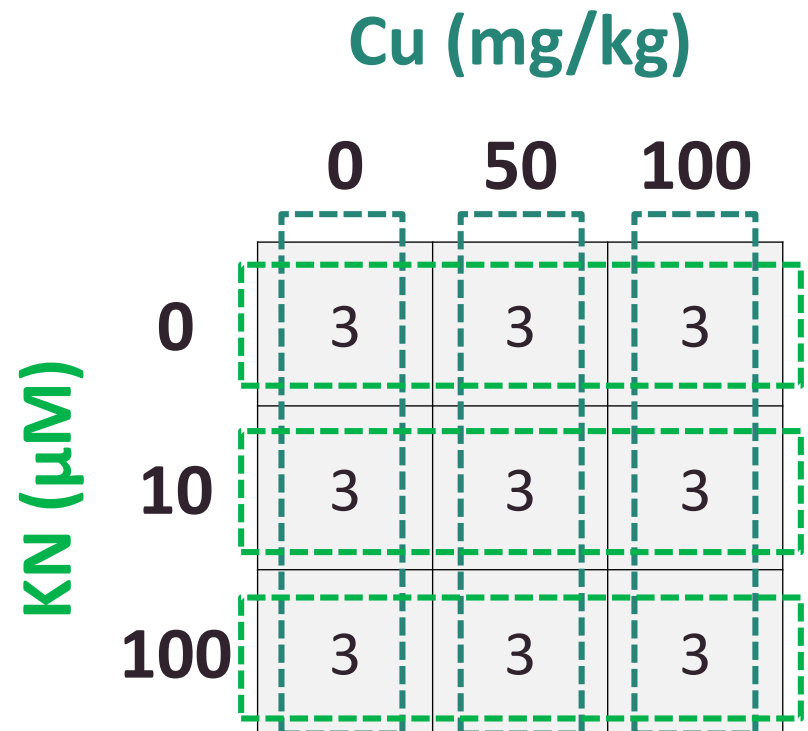
Copper chloride – CuCl_2

Kinetin – KN

Statistics

One-way ANOVA

Multi-way ANOVA



EXPERIMENTAL PROCEDURE

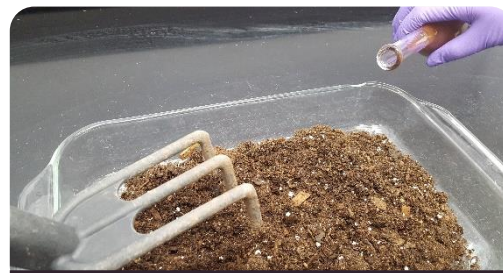
Day(s)

0

1



Suspension preparation



Soil amendment



Seeds planting



Germination



Hormone application



Plant growth

4 - 6

15

16+

11

EXPERIMENTAL PROCEDURE



Plant growth



Day 55



Harvesting

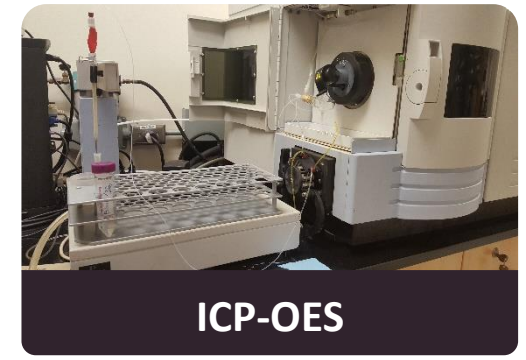
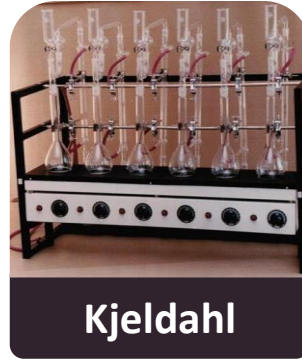


Day 90



Harvesting

QUANTITATIVE ANALYSIS



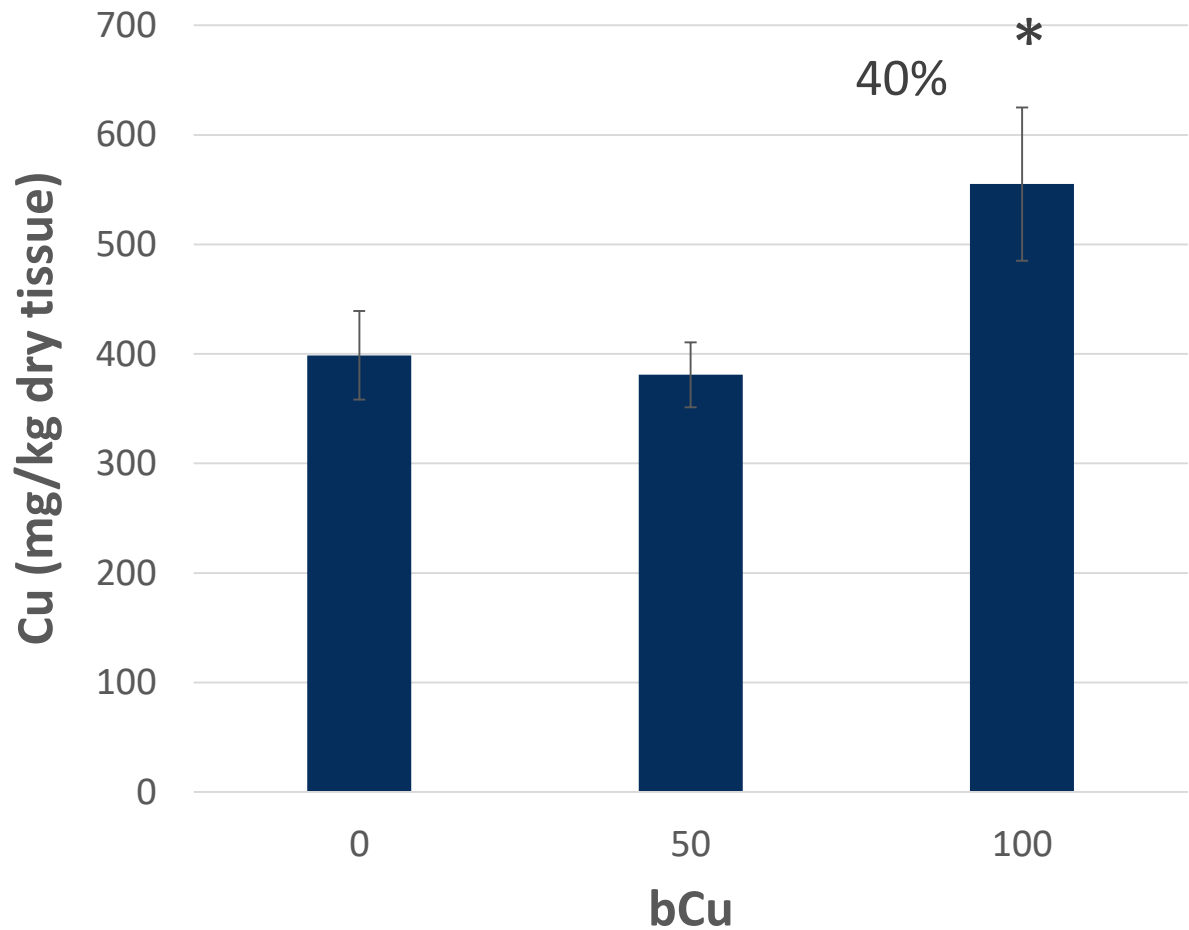
A decorative graphic consisting of a thin horizontal line extending across the width of the page. Two vertical lines are positioned on either side of the word 'RESULTS', extending from the horizontal line down to a thick, multi-colored bar. The bar is composed of several segments in shades of blue, teal, green, and yellow.

RESULTS



COPPER CONCENTRATION

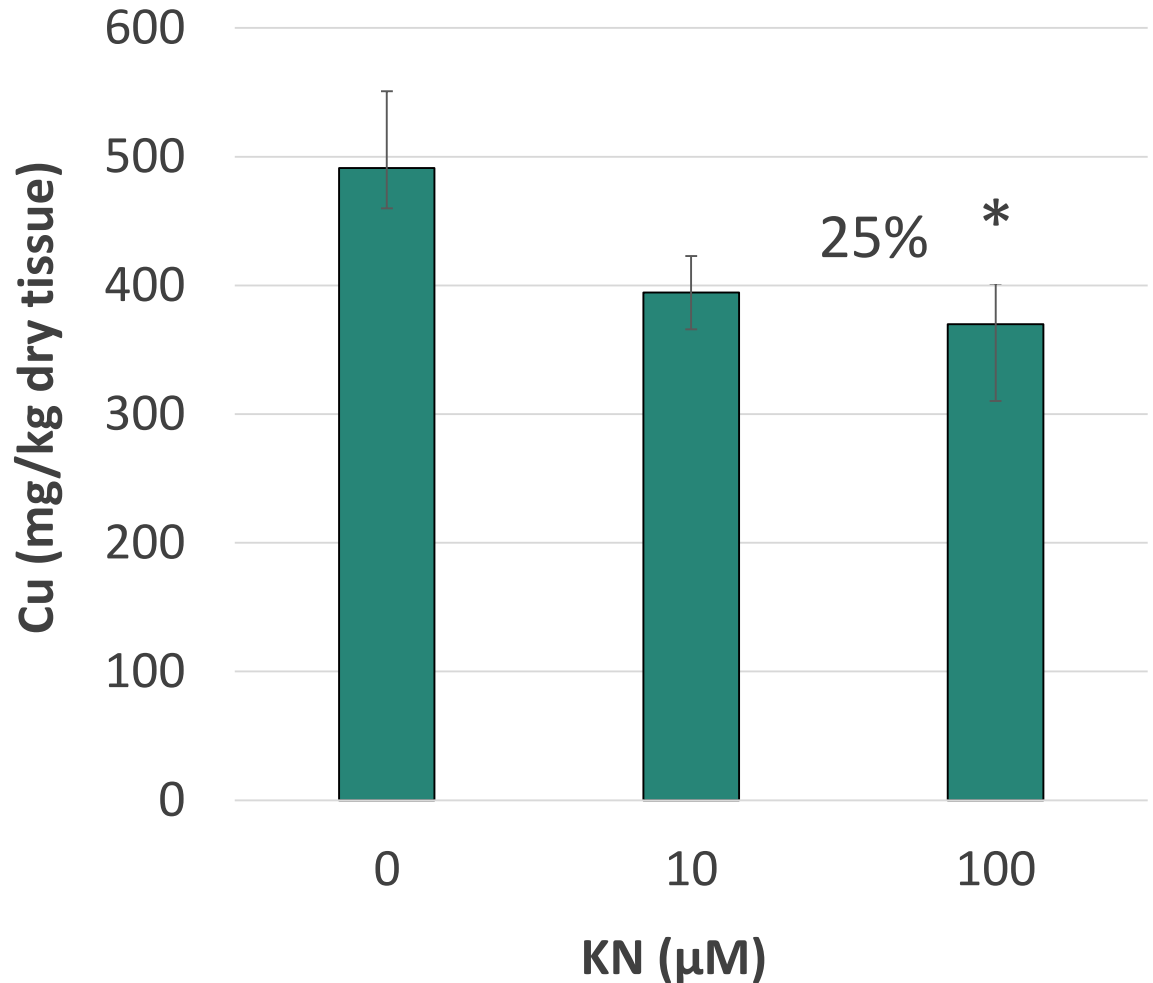
Enlarged *bCu* particles could have adhered to root tissues





COPPER CONCENTRATION

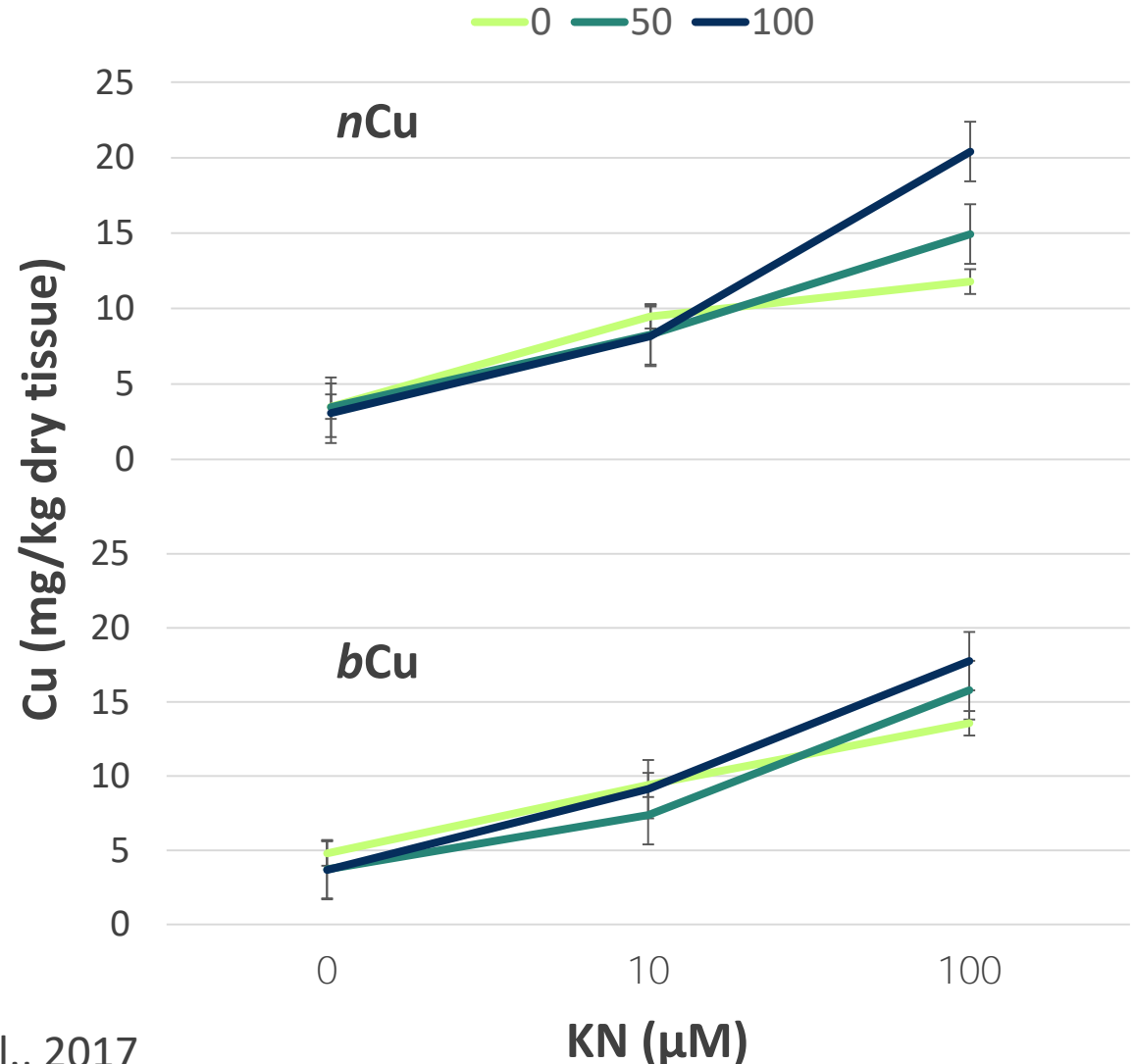
Interference of KN with root growth system could have lowered the amount of cells available for accumulation





COPPER CONCENTRATION

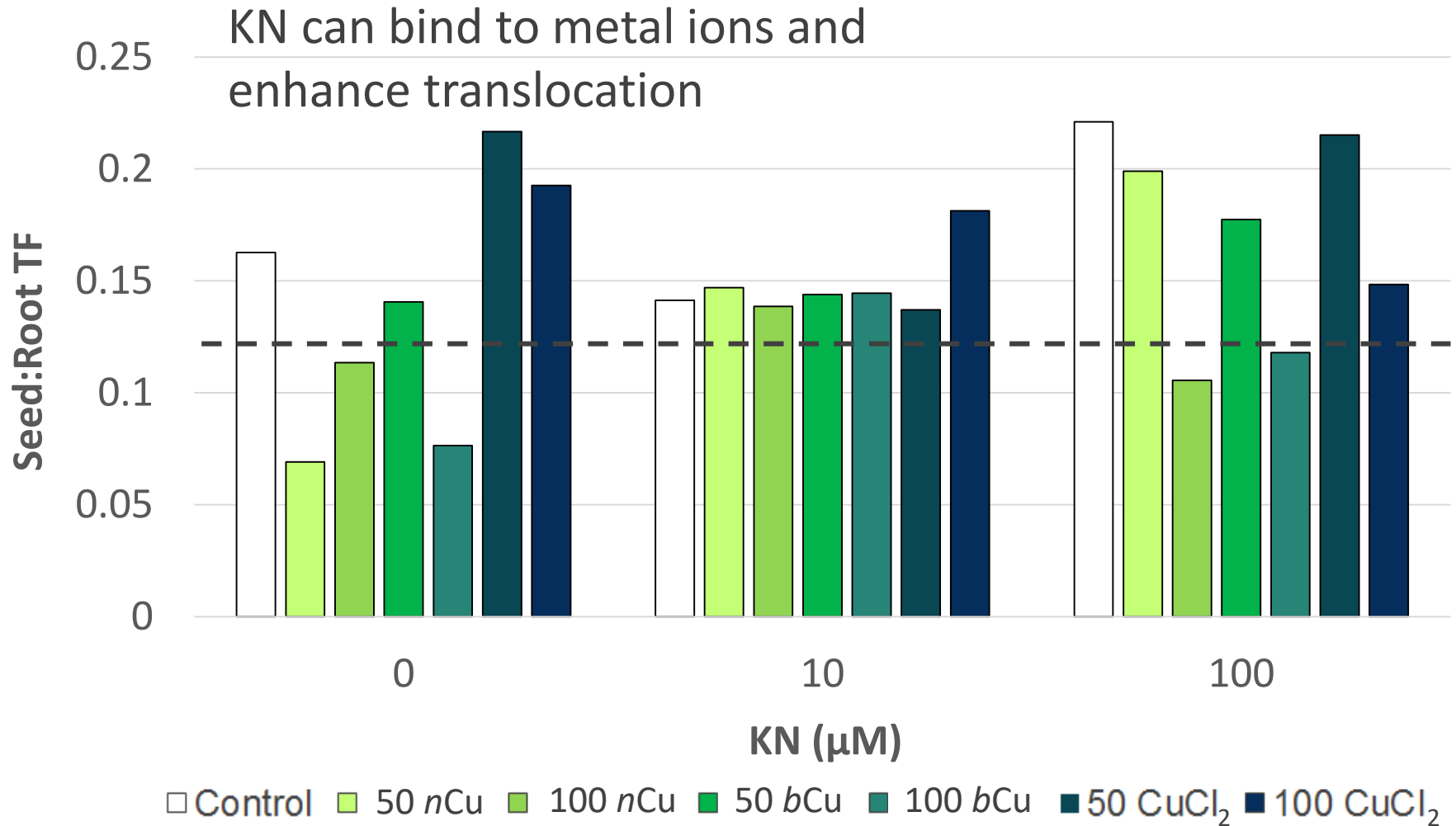
Negative surface charge of *nCu* and *bCu* could have preferentially bound to the N of the KN molecule



TRANSLOCATION FACTOR



KN can bind to metal ions and enhance translocation



NUTRITIONAL ELEMENTS



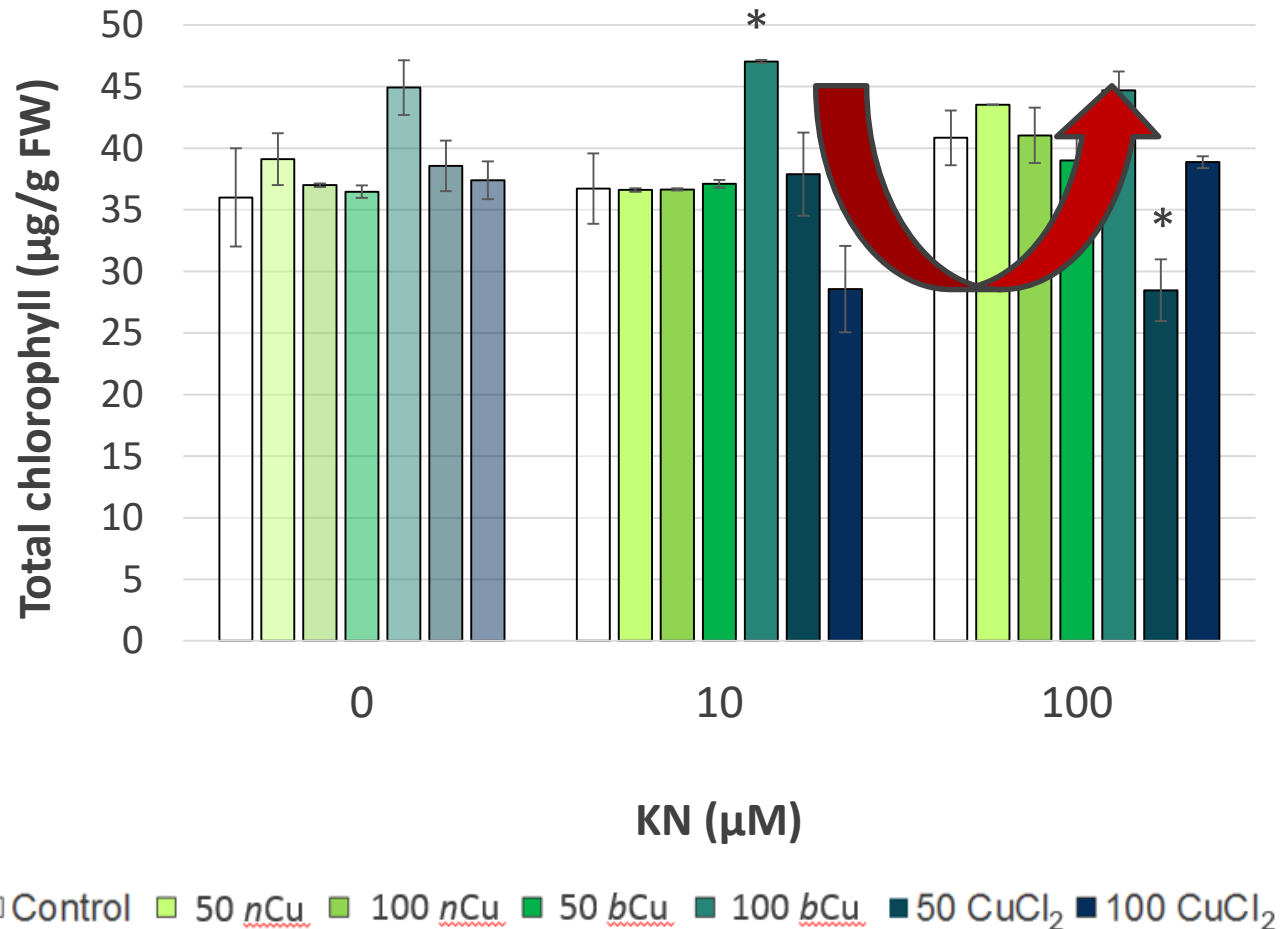
KN (μM)	Trmt (mg/kg)	Mg	S	Fe	Mn
0	50 CuCl_2		↑ 19%	30%	
	100 <i>nCu</i>			↓	
	100 <i>bCu</i>				↑
10	50 <i>nCu</i>	↓			
	50 <i>bCu</i>	↓			↑ $\geq 31\%$
	50 CuCl_2	↓ $\leq 12\%$			
	100 <i>nCu</i>	↓			
	100 <i>bCu</i>	↓			↑
100	50 <i>bCu</i>				↑

CHLOROPHYLL CONTENT



Mn influences chlorophyllase activity

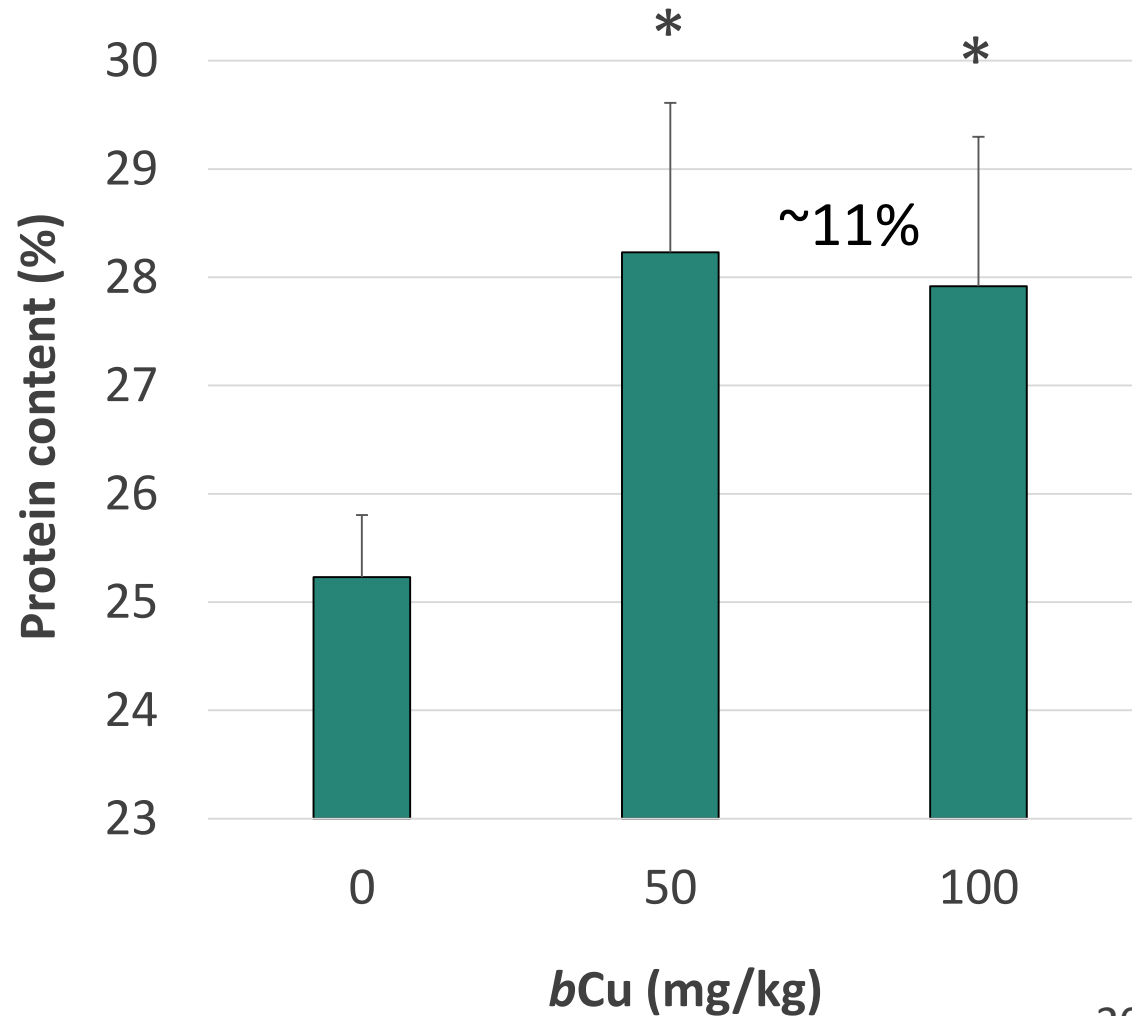
CuCl₂ could have inhibited protein import into chloroplasts





PROTEIN CONTENT

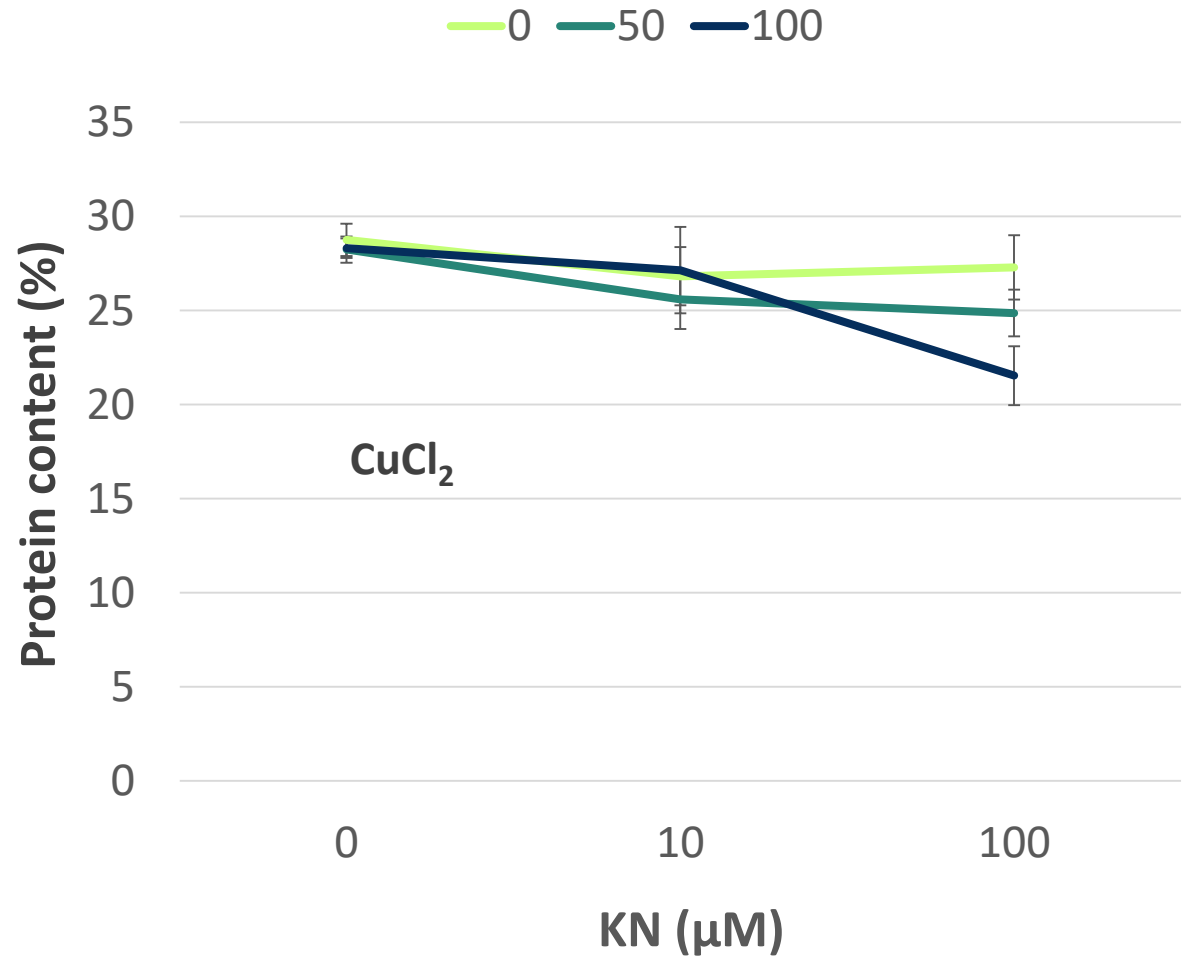
The slow release of
Cu ions could have
been beneficial





PROTEIN CONTENT

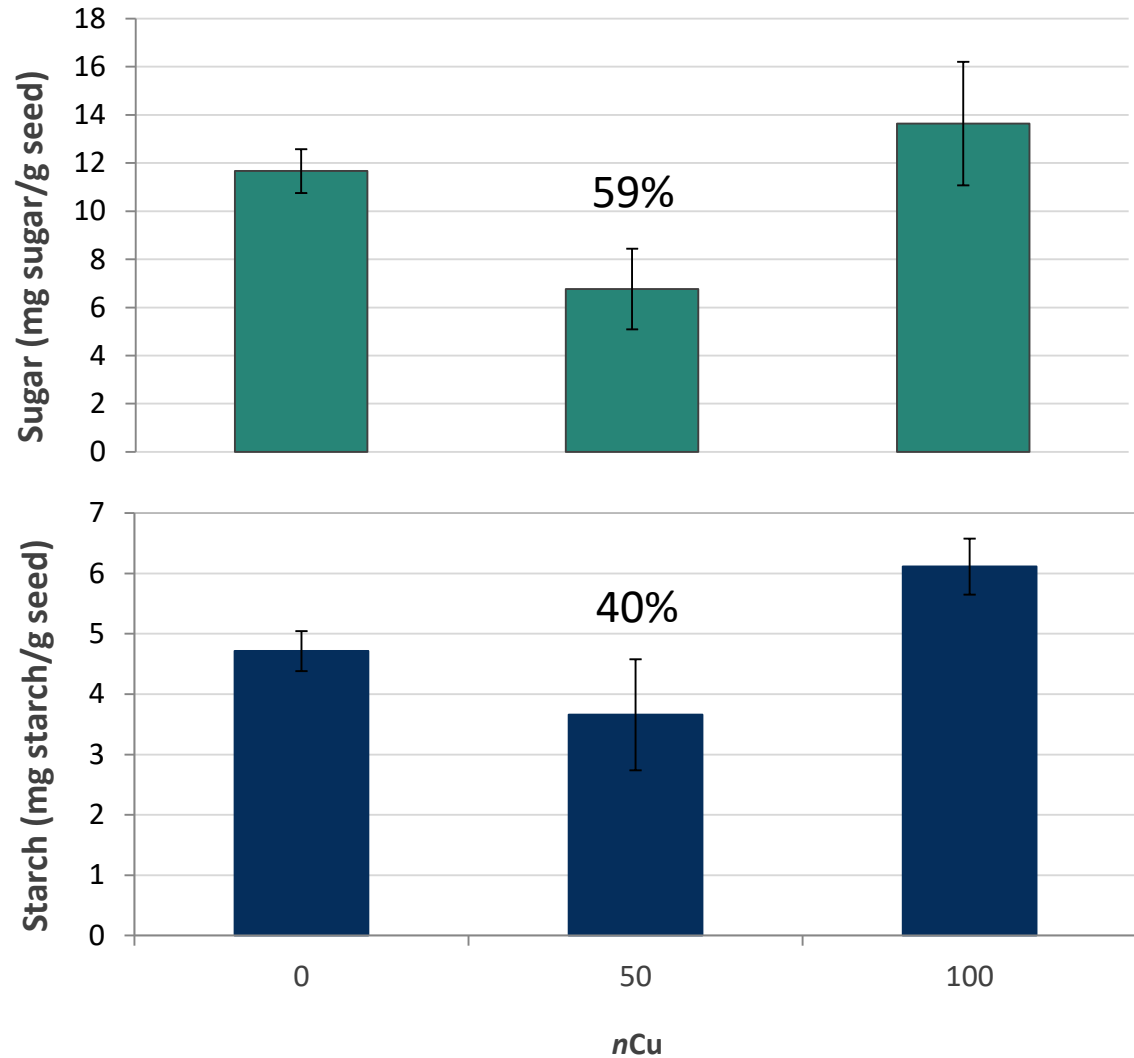
Toxicity due to immediate release of Cu ions



SUGAR & STARCH CONTENT



Further study will be done to fully understand this mechanism



CONCLUSION



SUMMARY & CONCLUSIONS

- ✓ Translocation of Cu was enhanced by KN
- ✓ As a main factor, *b*Cu increased the elemental uptake and accumulation of Mn
- ✓ The combination of *n*Cu + KN is neither toxic nor beneficial
- ✓ Protein was decreased by the interaction between CuCl_2 + KN

ACKNOWLEDGEMENTS

Dr. Jorge Gardea-Torresdey

Dr. Jose R. Peralta-Videa

Dr. Jose A. Hernandez-Viezcas

Dr. Juan P. Flores-Margez



U N I V E R S I T Y O F C A L I F O R N I A

UC  CEIN

Center for Environmental
Implications of Nanotechnology

Musante, C., & White, J. C. (2012). Toxicity of silver and copper to Cucurbita pepo: Differential effects of nano and bulk-size particles. *Environmental Toxicology*, 27(9), 510–517. <http://doi.org/10.1002/tox.20667>

Zuverza-Mena, N., Medina-Velo, I. a., Barrios, A. C., Tan, W., Peralta-Video, J. R., & Gardea-Torresdey, J. L. (2015). Copper nanoparticles/compounds impact agronomic and physiological parameters in cilantro (*Coriandrum sativum*). *Environ. Sci.: Processes Impacts*, 17(10), 1783–1793. <http://doi.org/10.1039/C5EM00329F>

Nazir, H., Asghar, H. N., Zahir, Z. A., Javed, M., & Saleem, M. (2015). Judicious use of kinetin to improve growth and yield of rice in nickel contaminated soil, 6514(March 2016), 0–19. <http://doi.org/10.1080/15226514.2015.1094444>

Zhao, L., Huang, H., Zhou, H., Adeleye, A. S., Wang, H., Ortiz, C., Mazereand, S. J, Keller, A. A. (2016). GC-TOF-MS based metabolomics and ICP-MSbased metallomics of cucumber (*Cucumis sativus*)fruits reveal alteration of metabolites profile andbiological pathway disruption induced by nanocopper. *Environmental Science: Nano*, 5, 1082–1087. <http://doi.org/10.1021/es102647w>

Prasad, R., Bhattacharyya, A., Ngyuyen, Q. D. (2017). Nanotechnology in Sustainable Agriculture: Recent Developments, Challenges, and Perspectives. *Frontiers in Microbiology*, 8, 1-13. <https://doi.org/10.3389/fmicb.2017.01014>

Apodaca, S.A. Tan, W., Dominguez, O.E., Hernandez-Viezcas, J.A., Peralta-Video, J.R., Gardea-Torresdey, J.L. 2017. Physiological and biochemical effects of nanoparticulate copper, bulk copper, copper chloride, and kinetin in bush bean (*Phaseolus vulgaris*) plants. *Science of the Total Environment*. 599-600, 2085-2094.

Werner, T., Motyka, V., Strnad, M., Schmulling, T. (2001). Regulation of plant growth by cytokinin. *Proceedings of the National Academy of Sciences*. 98, 10487-10492. <https://doi.org/10.1073/pnas.171304098>

Novotná, R., Herchel, R., Travnicek, Z. (2012). Structurally varied Cu(II) complexes involving kinetin and its derivatives: Synthesis, characterization and evaluation of SOD-mimic activity. *Polyhedron*. 34, 56–66. <https://doi.org/10.1016/j.poly.2011.12.016>

Olsen, A., Siboska, G. E., Clark, B. F., & Rattan, S. I. S. (1999). N(6)-furfuryladenine, kinetin, protects against fenton reaction-mediated oxidative damage to DNA. *Biochemical and Biophysical Research Communications*, 265(2), 499–502. <https://doi.org/10.1006/bbrc.1999.1669>

thank
you