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Different forms of copper and kinetin impacted element accumulation and macromolecule contents in kidney bean (*Phaseolus vulgaris*) seeds

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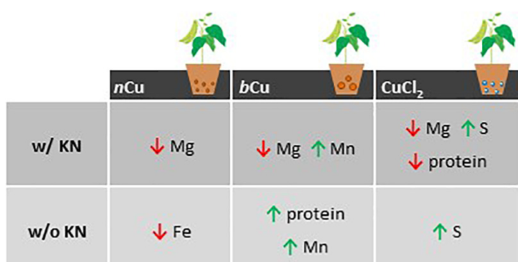
HIGHLIGHTS

- The nutritional value of seeds was altered by Cu × KN interactions.
- Mn and S were increased by *b*Cu and $CuCl_2$, respectively, with/without KN.
- On average, *b*Cu stimulated protein content and $CuCl_2$ + KN diminished it.
- Variations in sugar and starch content were insignificant, compared to controls.
- Seed production was unaffected by Cu × KN.

GRAPHICAL ABSTRACT



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ABSTRACT

The relationship between engineered nanomaterials and plant biostimulants is unclear. In this study, kidney bean (*Phaseolus vulgaris*) plants were grown to maturity (90 days) in soil amended with nano copper (*n*Cu), bulk copper (*b*Cu), or copper chloride ($CuCl_2$) at 0, 50, or 100 mg kg^{-1}, then watered with 0, 10, or 100 μM of kinetin (KN). Seeds were harvested and analyzed via ICP-OES and biochemical assays. While seed production was largely unaffected, nutritional value was significantly impacted. Accumulation of Cu was enhanced by 5–10% from controls by Cu-based treatments. Fe was the only macro/microelement significantly altered by *n*Cu, which was ~29% lower than seeds from untreated plants. All forms of Cu combined with 10 μM KN reduced Mg from 9 to 12%. Application of KN plus *b*Cu or $CuCl_2$ elevated concentrations of Mn (31–41%) and S (19–22%), respectively. Protein content of seeds was stimulated (11–12%) by *b*Cu, on average, and depressed by $CuCl_2$ + KN (up to 22%). Variations in sugar and starch content were insignificant, compared to controls. Our results indicate that the interaction Cu × KN significantly altered the nutritional value of common beans, which has potential implications to agricultural practices incorporating Cu as either a pesticide or fertilizer.

1. Introduction

Metallic engineered nanoparticles (NPs) have been introduced as an emerging solution to address challenges within the agricultural industry (García et al., 2010; Prasad et al., 2017). The size-dependent (<100 nm) physiochemical properties of NPs, which contrast from those of corresponding bulk materials, allow the possibility to maximize

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crop production, while minimizing the use of additives (Cicek and Nadaroglu, 2015; Iavicoli et al., 2017). Smart distribution systems are among the different uses of nanotechnology in agriculture. Nano-enabled delivery of fertilizers, pesticides, and herbicides can improve overall efficiency and effectiveness (Rai and Ingle, 2012). Essential elements in nanostructured form can be directly applied to the growth medium or by foliar spray, although the former method is preferred for a more controlled release of nutrients (Morales-Díaz and Ortega-Ortíz, 2017). These nanoscale carriers function by adhering to plant roots enhancing stability and preventing environmental induced degradation in the process (Cicek and Nadaroglu, 2015). While there have been innovations and improvements in agriculture due to nanotechnology, it also presents new possible hazards to ecological health. The deliberate introduction of NPs induces human and environmental contact, which is only heightened by exposure routes such as bioaccumulation within the food chain (Tolaymat et al., 2017). There is a pressing need to properly manage the potential environmental health and safety risks posed by NPs.

Due to the slower release of ions from Cu nanopesticides, and their recommendation for use on a wide range of crops by manufacturers, the distribution of NPs in the agricultural market is likely to increase (Keller et al., 2017). The effects of Cu NPs on crop plants have been found to vary according to species, particle properties, method of application, and growth media (Zuverza-Mena et al., 2017). While young plants have demonstrated negative symptoms in response to Cu NPs, mature plants generally displayed fewer observable effects and, in some cases, even procured higher amounts of tissue biomass (Keller et al., 2017). Overall, there is still a lack of understanding on the impact of Cu NPs on mature terrestrial plants grown under realistic environmental conditions and comparative analysis on the effects between different sizes of Cu.

Cytokinin is the common name assigned to plant hormones that moderate cellular division and tissue formation in plants (Strnad, 1997; Barciszewski et al., 1999). Serving a practical application in agriculture, the effects of exogenous application of cytokinins on plant physiological processes is well documented throughout literature. The benefits of cytokinins in plants include enhanced production, improved environmental stress tolerance, and delayed senescence (Weaver, 1972; Papadopoulos et al., 2006; Ma, 2008; Barbafieri et al., 2012). The relationship between NPs and plant growth regulators is not well established, despite the capacity for altered phytohormone uptake and toxicity, and potential secondary impacts following the introduction of NPs. In a recent paper by Ochoa et al. (2017), green pea plants were treated with Cu-based compounds and indole-3-acetic acid (IAA). In general, the combination of nano CuO, bulk CuO, and ionic Cu (50 and 100 mg kg^{-1}) with IAA (10 and 100 μM) reduced the number of plants by 23% and 34%, respectively. Treatments containing 10 μM of IAA with 50 mg kg^{-1} of nano CuO and 100 mg kg^{-1} of ionic Cu reduced pod biomass by 50%. In our previous study, it was found that the combination of ionic Cu and kinetin (KN) significantly altered the accumulation of nutritional elements in plant tissues (Apodaca et al., 2017). However, the effect of the Cu × KN interaction on bean seed quality has not been reported yet.

This work aimed to elucidate the effects of different forms of Cu (nano, bulk, and ionic) and KN on kidney bean plants over their full life cycle. The purpose of nano/micro-sized and ionic-based treatments was to: (1) isolate the effects of particle size, and (2) distinguish the effects between ions and particles. As a follow-up to Apodaca et al. (2017), which focused on plant growth factors, nutritional value was the primary focus of this research. Plants were grown to maturity and harvested seeds were analyzed for elemental accumulation (micro and macro), macromolecule composition (protein and carbohydrates), and production (yield and weight), using analytical techniques. To our best knowledge, this is the first study comparing the effects of different forms of Cu and their interaction with KN on the nutritional quality of bean seeds.

2. Materials and methods

2.1. Suspensions/solutions preparation

Nano copper (*n*Cu) was supplied by the University of California Center for Environmental Implications of Nanotechnology (UC-CEIN). Both bulk copper (*b*Cu) and copper chloride ($CuCl_2$) were purchased from Sigma Aldrich. Characterization of these compounds can be found in the Supplementary material (Table S1) (Hong et al., 2015). More information can be found in (http://www.us-nano.com/inc/sdetail/162) stock # US1090. Suspensions/solutions were prepared to have final concentrations of 0, 50, and 100 mg kg^{-1} of soil, which were chosen after Apodaca et al. (2017). Dry compounds were weighed with respect to Cu content, upon which they were immersed in Millipore water (MPW, 18 MΩ) and brought up to a final volume of 100 mL. To ensure homogeneity, suspensions/solutions were sonicated (Crest Ultrasonics, Trenton, NJ) for 30 min at 25 °C with an intensity of 180 watts. Stock solutions of kinetin (KN, Alfa Aesar) were prepared and diluted to concentrations of 0, 10, and 100 μM, according to López et al. (2009). Approximately 2 to 3 mL of 0.1 N sodium hydroxide (NaOH) (Sigma Aldrich) was used to improve the solubility of KN in MPW. To maintain stability and prevent degradation, KN solutions were stored at 0 °C in the dark for up to seven days (López et al., 2009).

2.2. Soil treatment and growth conditions

Red hawk kidney bean (*Phaseolus vulgaris*) seeds were provided by Dr. James Kelly of Michigan State University. Treatments were prepared in plastic pots containing 420 g of Miracle-Gro® organic potting mix amended with *n*Cu, *b*Cu, and $CuCl_2$ at the aforementioned concentrations. Characterization of the potting mix can be found in the Supplementary material (Table S2) (Barrios et al., 2015). Pots with unmodified soil served as controls. Each treatment had 3 replicates and each pot contained 5 seeds. All treatments/pots were kept in a growth chamber (Environmental Growth Chamber, Chagrin Falls, OH) under the following conditions: 25/20 °C temperature, 14/10 h photoperiod, 65 ± 3% relative humidity, and illumination of 340 μmol m^{-2} s^{-1}. They were watered with deionized (DI) water on alternating days until 20 days, after which they were watered daily. On the 15th day, the KN solution was directly applied to the soil of designated treatments. After 90 days, seeds were harvested from mature plants. Further details on seed and soil preparation, soil composition, and greenhouse conditions can be found in Apodaca et al. (2017).

2.3. Elemental analysis

A coffee grinder (Hamilton Beach) was used to ground oven dried tissues (65 °C, 72 h) into a fine powder. Samples weighing 0.2 g were mixed with 1 mL of plasma pure HNO_3 (SPC Science, Champlain, NY), digested in a DigiPREP MS digestion block (SCP Science, NY), and adjusted to a total volume of 50 mL with MPW. Cu and macro/micro elemental (Ca, B, Fe, K, Mg, Mn, Mo, Ni, P, and Zn) concentrations were quantified via inductively coupled plasma – optical emission spectrometry (ICP-OES, Perkin-Elmer Optima 4300 DV). For quality assurance/control (QA/QC) of analytical methods, samples with only MPW were used as blanks and spinach leaves (1507a NIST, Gaithersburg, MD) were used as standard reference material.

2.4. Crude protein and carbohydrate analysis

The Kjeldahl method of nitrogen analysis was used to determine protein content under the following sequence: (1) extraction, (2) distillation, and (3) titration (Labconco, Kansas City, MO) (AOAC2000). Samples of dry powdered seeds weighing 0.1 g were placed in digestion flasks and combined with a tablet of K_2SO_4–catalyst mixture and 6 mL of concentrated sulfuric acid (H_2SO_4). Flasks were heated at 175 °C for

1 h, then again at 375 °C for 2 h (to ensure complete digestion), and left to cool. Digested samples were agitated upon the addition of 20 mL of MPW. Distillated samples were mixed with 40 mL of 10 N NaOH and allocated to a distillation chamber (Labconco, Kansas City, MO). Samples were combined with 10 mL of indicator containing boric acid (BH_3O_3) in a beaker, upon which the 50 mL distillation was collected. Titration was performed using 0.05 N H_2SO_4 (Bremner, 1996). Total Kjeldahl nitrogen (TKN) was used as surrogate for protein in seeds. TKN was expressed as % N and crude protein content was calculated as % N $\times$ 6.25 (Allen, 2002; Bremner, 1996).

Total sugar and starch extractions were performed as per Verma and Dubey (2001), and samples were measured in microplate colorimetric format by a phenol-sulfuric acid method (Masuko et al., 2005). Standard glucose and corn starch were used to generate a calibration curve for QA/QC of lab procedures.

2.5. Experimental design and statistical analysis

Under a completely random design, a factorial treatment arrangement was used: KN at 3 levels (0, 10, and 100 μM) and Cu (*n*Cu, *b*Cu, $CuCl_2$) at 3 levels (0, 50, and 100 mg kg^{-1}). Triplicates for 21 treatments of Cu $\times$ KN gave a final total of 63 samples (Table S3). The Statistical Package for Social Sciences 22.0 (SPSS, Chicago, IL) was used to perform all statistical analyses. Differences among treatment means were evaluated via one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post-hoc test, based on a probability (p value) of 0.05. A factorial multi-way ANOVA was used to examine the interaction between Cu and KN (Cu $\times$ KN).

3. Results and discussion

3.1. Effects of treatments on nutrient element composition

3.1.1. Copper uptake and accumulation

Uptake and accumulation of Cu in bean seeds from plants grown for 90 days in soil amended with *n*Cu, *b*Cu, $CuCl_2$, and/or KN are shown in Fig. 1 and discussed below. As expected, the addition of Cu and Cu-based treatments resulted in a greater uptake and accumulation of Cu. This increase varied from 5% to 10% among all concentrations, which were significantly different compared to respective controls. An exception was found in the highest 100 μM KN concentration, where the Cu content in corresponding control seeds was 3% higher than other controls. As a result, the observed differences among *n*Cu and *b*Cu treatments were not statistically significant. The addition of KN has been found to increase metal uptake in a wide variety of plant species (Tassi et al., 2008; López et al., 2009; Cassina et al., 2012; Bücker-Neto et al., 2017). However, due to the lack of an interaction effect by Cu $\times$ KN (Table S4), this shift is most likely due to the relatively high margin of error seen in the control treatment 100 μM KN. Although an essential trace element for healthy functioning, excess Cu intake can lead to generation of damaging radicals in humans (Khattab et al., 2009). The recommended dietary intake of Cu for adults is 0.7 to 10 mg Cu/day (Trumbo et al., 2001). In our study, the highest Cu content found was 5.9 mg per 100 g of beans, a typical serving size. Consumption of fruits and vegetables exposed to Cu and Cu-based compounds, in addition to other sources of Cu, could conceivably increase Cu intake above the recommended dietary threshold (Keller et al., 2017). According to our findings, the form of Cu (nano, bulk, or ionic) does not perpetuate this risk, and the presence of KN is irrelevant.

3.1.2. Macro and microelement accumulation

Among the macro elements analyzed, only Mg and S showed significant differences (Table 1). Nearly all 10 μM KN treatments decreased Mg content by 9% to 11%. In our previous experiment, kidney bean plants treated with 10 μM and 100 μM KN reduced Mg in leaves (72%) and stems (78%), but accumulation was enhanced in roots (59%) (Apodaca et al., 2017). Given that Mg is taken up by the root system via permeable cation channels, it is possible that KN interfered with this mechanism (Medina-Velo et al., 2017). An excess of KN has been observed to have a profound influence on the formation and growth of roots, possibly blocking the translocation of specific elements (Werner et al., 2001). The alterations in S content were varied. Seeds from plants treated with 50 mg kg^{-1} $CuCl_2$ and 100 $CuCl_2$ + 100 μM KN were 19% and 22%, correspondingly, higher in S than controls. Conversely, S in kidney bean plant stems was reduced from 18% to 58% upon exposure to *n*Cu, *b*Cu, and $CuCl_2$ (without KN) (Apodaca et al., 2017). Cu ions could have up-regulated sulfate transporters by influencing the signal transduction pathway or bonded with sulfate in the soil, translocating to aerial tissues as $CuSO_4$ (Hong et al., 2015; Rawat et al., 2018). Bell pepper plants grown in soil amended with 250 mg kg^{-1} to 500 mg kg^{-1} $CuCl_2$ had significantly increased S in root and leaf tissues (Rawat et al., 2018). The contrast with previous results is unclear, which suggests further work to understand the possible interference of KN with this mechanism and its relationship with ionic Cu.

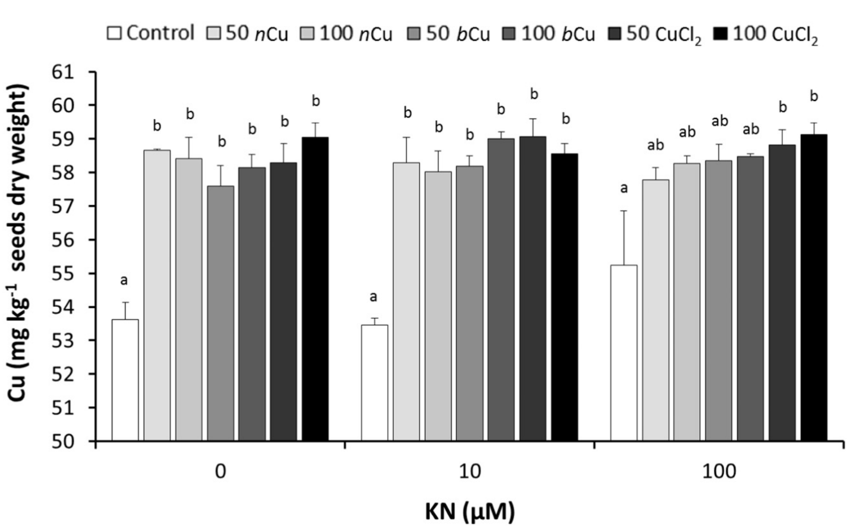

Fig. 1. Cu content in seeds of kidney bean plants grown to full maturity (90 days) in soil rendered with 0, 50, or 100 mg kg^{-1} of *n*Cu, *b*Cu, and $CuCl_2$, and watered with 0, 10, or 100 μM of KN. Data are means of three replicates ± SE ($n = 3$). Different letters represent statistically significant differences between concentrations and treatments within the same KN treatment concentration ($p \leq 0.05$).

Table 1
Macro and microelement concentration in seeds reaped from kidney bean plants (90 days) grown in soil amended with 0, 50, or 100 mg kg^{-1} of *n*Cu, *b*Cu, and $CuCl_2$ and 0, 10, or 100 μM of KN. Data are means of three replicates ± SE ($n = 3$). Only elements within the limit of detection and that had statistically significant differences from respective controls are included ($p \le 0.05$). Symbols + and – stand for percent of increase and decrease in element concentration.

Element	Treatment Cu product + kinetin (mg kg^{-1} soil + μM)	Concentration (mg kg^{-1} d wt tissue)			%
Fe	Control 0 KN	100.3	±	4.3	–
	100 *n*Cu + 0 KN	71.2	±	3.5	−29.1
Mg	Control 10 KN	1955.3	±	41.6	–
	50 *n*Cu + 10 KN	1774.5	±	15.5	−9.3
	100 *n*Cu + 10 KN	1729.0	±	38.3	−11.6
	50 *b*Cu + 10 KN	1762.6	±	15.9	−9.9
	100 *b*Cu + 10 KN	1733.8	±	35.0	−11.3
	50 $CuCl_2$ + 10 KN	1736.1	±	45.4	−11.2
Mn	Control 0 KN	16.6	±	1.1	–
	100 *b*Cu + 0 KN	21.9	±	1.7	+31.5
	Control 10 KN	16.3	±	0.4	–
	50 *b*Cu + 10 KN	23.0	±	0.9	+41.4
	100 *b*Cu + 10 KN	22.1	±	0.5	+35.7
	Control 100 KN	16.4	±	0.4	–
	50 *b*Cu + 100 KN	21.4	±	1.1	+30.6
S	Control 0 KN	2812.8	±	107.6	–
	50 $CuCl_2$ + 0 KN	3348.4	±	31.3	+19.0
	Control 100 KN	2823.6	±	56.4	–
	100 $CuCl_2$ + 100 KN	3431.2	±	171.0	+21.5

As for the microelements, Fe and Mn were found to be significantly altered (Table 1). Accumulation of Fe was decreased (29%) by 100 mg kg^{-1} *n*Cu from the control, representing the only element to be significantly affected by the nano treatment (without KN). In a study by Rico et al. (2013), rice grains collected from 500 mg kg^{-1} $nCeO_2$-treated plants had reduced Fe (69%). While this signifies that Fe accumulation is modified by different types of NPs, suggesting a size-dependent effect, it is challenging to determine the exact cause for this reduction. A clear consensus on the environmental behavior of elements in soil amended with NPs is lacking in current literature, which could clarify these results. There could be competition in the uptake channel between Fe and *n*Cu (Kochian, 1991). Tobacco plants that expressed ferretin, an Fe-storage protein, were found to be more tolerant to oxidative damage (Deák et al., 1999). It is possible that Fe was complexed as ferretin in response to oxidative stress from Cu treatments. Nevertheless, a decrease in Fe is problematic for those who seek beans as a means to combat Fe deficiency, which has been identified as pervasive global threat to public health (Finkelstein et al., 2017). Both 50 and 100 mg kg^{-1} *b*Cu treatments with/without KN increased Mn accumulation from 31% to 41%. Similarly, previous studies with cilantro and wheat reported that low concentrations of Cu (0.5 mg kg^{-1} to 80 mg kg^{-1}) increased the concentration of Mn in various plant tissues (Kumar et al., 2009; Zuverza-Mena et al., 2015). Mn was reduced from 62% to 78% in kidney bean plant leaves by 100 mg kg^{-1} $CuCl_2$ (with/without KN) (Apodaca et al., 2017). This suggests that not only is the concentration of Cu relevant towards Mn uptake and accumulation, but also particle size. Additional experiments are required to elucidate the driving force for this mechanism. Overall, our results indicate that *n*Cu and Cu-based compounds have the capacity to significantly alter the nutrient content of kidney bean plants.

3.2. Effects on macromolecular composition

3.2.1. Effects on protein content

Fig. 2 (A) shows that the main factor *b*Cu, at both concentrations of 50 and 100 mg kg^{-1}, significantly increased protein by 11% to 12% from averaged control treatments ($p \le 0.05$). In contrast, a concentration dependent negative trend was imparted by the main factor $CuCl_2$ and the interaction $CuCl_2 \times$ KN (Fig. 2 B–C), with a reduction as high as 12%, compared to controls. However, there were no statistically significant differences in protein content from controls when comparisons were made among treatment groups (Fig. S1). As a transition metal, Cu forms stable complexes with a variety of molecules, especially proteins,

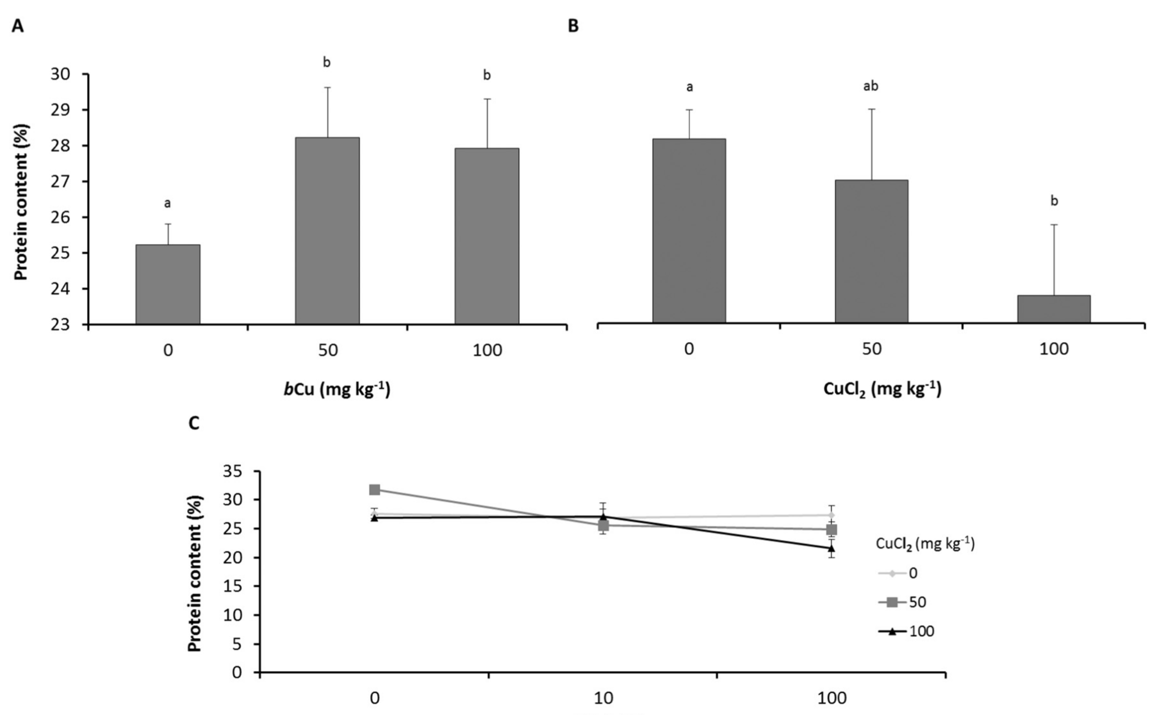

Fig. 2. (A–C). Averages of protein content for factors (A) *b*Cu, (B) $CuCl_2$, and (C) $CuCl_2 \times$ KN in seeds of 90-day old kidney bean plants exposed to 0, 50, or 100 mg kg^{-1} of *b*Cu and $CuCl_2$, and 0, 10, or 100 μM KN. Data are means of three replicates ± SE ($n = 3$). Different letters represent statistically significant differences ($p \le 0.05$).

that either bring stability to structural features or drive biochemical reactions (Printz et al., 2016). A positive correlation between Cu and crude protein was found in four species of legumes grown in Germany and England (Rasheed and Seeley, 1966). Although Cl^- has been found to be toxic to plants (Eaton, 1942; White and Broadley, 2001; Tavakkoli et al., 2011; Flowers et al., 2015). Rapid accumulation of Cl^- was seen in kidney bean plants exposed to 80 meq L^{-1} (2840 mg L^{-1}) of NaCl, ultimately culminating with plant death after 44 days (Hajrasuliha, 1980). Alternatively, exogenous KN application could have facilitated the uptake and translocation of Cu ions from $CuCl_2$, with toxicity manifesting in the form of decreased protein content. Previously, Apodaca et al. (2017) found a positive association between the interaction $CuCl_2$ × KN and accumulation of Cu in kidney bean plant leaves. Opposing effects in protein content by bulk and ionic treatments could be due to differences in chemical properties: *b*Cu, which has a zeta potential of −35.4 mV (Table S1), is far less soluble than $CuCl_2$. The slow release of Cu ions by *b*Cu could have been therapeutic, stimulating protein synthesis (Fig. 2A), and its immediate release by $CuCl_2$ could toxic, retarding protein formation (Fig. B, C). Further, the pH (6.8 to 7.2) and organic matter content (50% to 60%) of the soil likely influenced the dissolution process (Table S2) (Sekine et al., 2017). Several studies have reported mixed results in protein content of plants exposed to KN. In Gangwar et al. (2010), pea seedlings treated with Mn (50 and 100 mg kg^{-1}) plus 100 μM KN had significantly reduced total protein in roots and shoots, compared to untreated plants. While application of 50 mg kg^{-1} Mn and 10 μM KN reversed this decrease. Another study observed that pea seedlings stressed with 25 and 50 μM of Cd were found to have lower amounts of soluble protein in roots and shoots than controls when treated with 10 and 20 μM of KN (Al-Hakimi, 2007). It is possible that the combination of KN with a metal could escalate protein degradation and enhance protease activity (Balestrasse et al., 2003). This interactive effect seems to be dependent on the type of metal. Our findings indicate that the form of metal (bulk or ionic) is also an important factor. Proteins play a critical role in cells by sustaining the structure, function, and regulation of internal tissues and organs (Khattab et al., 2009). As a top plant-based source of protein, beans are widely consumed throughout the world (Messina, 2014). Thus, these findings could have negative implications for human nutrition and there is a need for further research in this area.

3.2.2. Effects on carbohydrate content

Sugar and starch were quantified in seeds harvested from 90-day old kidney bean plants cultivated in soil treated with *n*Cu, *b*Cu, $CuCl_2$, and/or KN. Although there were no statistically significant differences from controls when comparisons were made among similar hormone treatment groups (Fig. S2), multivariate analysis revealed that 50 mg kg^{-1} *n*Cu reduced starch and sugar content from the higher 100 mg kg^{-1} *n*Cu dosage by ~50% and 40%, respectively (Fig. 3 A–B). It has been reported that metal-based nanoparticles reduced sugar and/or starch contents in several plant species (Mirzajani et al., 2013; Rico et al., 2013; Salama, 2012). Although in Alaoui-Sossé et al. (2004), cucumber plants treated with 20 μg g^{-1} of ionic $CuCl_2$ were found to have significantly higher starch and glucose content from controls. It is possible that the low exposure of Cu to cucumber plants may not have been high enough to cause toxicity, hence explaining the controversy with our results. Since the results are not statistically significant when control samples are used as a reference value, we cannot conclude whether the effects of *n*Cu are detrimental or beneficial. However, it seems there is a clear allocation of plant resources that is dependent on concentration. Further testing is needed to determine the extent and significance of this phenomenon. Carbohydrates are integral structural components that provide energy to sustain a myriad of bodily and brain-related functions (Raigond et al., 2015). Given its potential influence on public health, it is critical to direct research efforts towards understanding the effects of NPs on macromolecules in mature crop plants.

3.3. Effects on seed production

The production of seeds reaped from plants exposed to *n*Cu, *b*Cu, $CuCl_2$, and/or KN are shown in Table 2. There were no statistically significant differences in yield (number of produced seeds) of Cu-based treatments when compared among similar hormone concentrations. The results for seed weight resembled those of seed yield. The exception was the 50 mg kg^{-1} *b*Cu + 10 μM KN treatment, which was at least twice that of its respective control. In our previous study, plants treated with 50 mg kg^{-1} *b*Cu + 10 μM KN had significantly higher root and stem biomass (Apodaca et al., 2017). Based on these results, we can conclude that there is a synergistic effect imparted by *b*Cu + KN. The reason for this interaction is not known; perhaps the lower zeta potential of *b*Cu (−35.4 ± 1.27 mV), compared to *n*Cu and $CuCl_2$, made the difference. The optimum dose of Cu for enhanced seed weight could be 50 mg kg^{-1}. The lack of studies on the interaction between exogenous KN and *b*Cu limit our ability to compare our findings with other previous reports. Further work is needed to clarify these results, determine

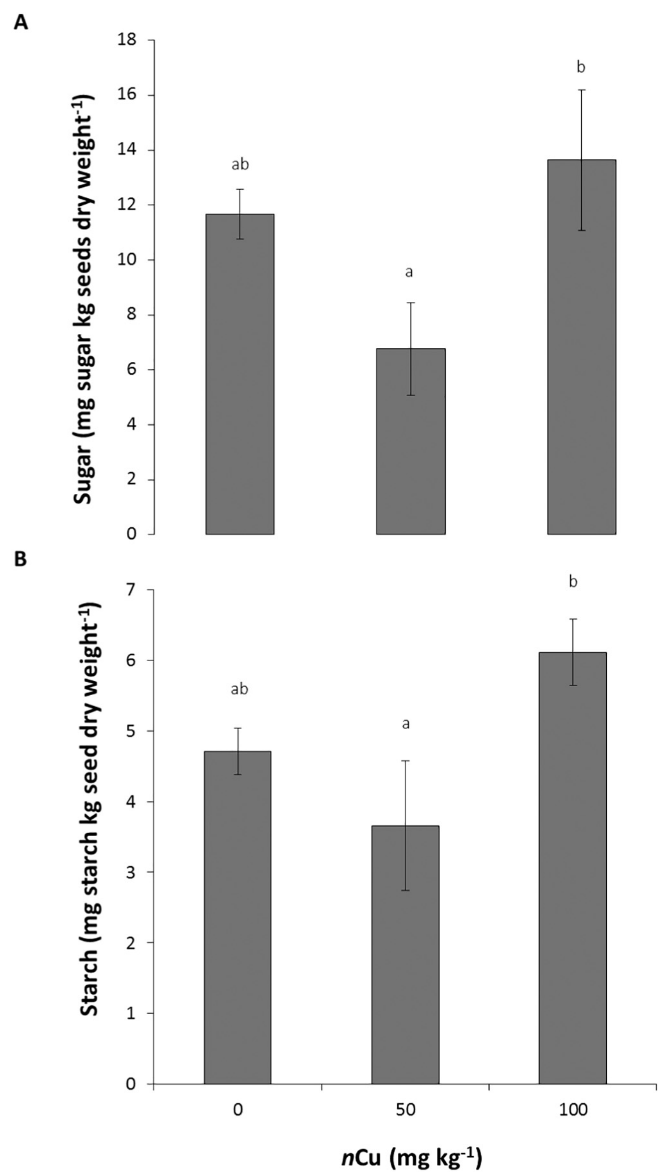

Fig. 3. (A–B). Averages of sugar (A) and starch (B) content for factor nCu in seeds of kidney bean plants (90 days) exposed to 0, 50, or 100 mg kg^{-1} of bCu and $CuCl_2$, and 0, 10, or 100 μM of KN. Data are means of three replicates ± SE (n = 3). Different letters represent statistically significant differences ($p \leq 0.05$).

Table 2
Overall production (yield and weight) of seeds harvested from 90-day old kidney bean plants cultivated in soil amended with 0, 50 or 100 mg kg^{-1} of *n*Cu, *b*Cu, and $CuCl_2$ and 0, 10, or 100 μM of KN. Data are means of three replicates ± SE ($n = 3$). Different letters represent statistically significant differences between concentrations and treatments within the same KN treatment concentration at ($p \leq 0.05$).

Kinetin (μM)	Treatment (mg kg^{-1} soil)	Yield (# seeds)			Weight (g)		
0	Control	9.7	±	0.3	3.0	±	0.1
	50 *n*Cu	13.0	±	1.7	4.2	±	0.7
	100 *n*Cu	11.3	±	0.9	4.3	±	0.8
	50 *b*Cu	12.0	±	0.6	3.8	±	0.3
	100 *b*Cu	13.0	±	1.2	4.5	±	0.4
	50 $CuCl_2$	11.3	±	1.9	3.4	±	0.6
	100 $CuCl_2$	13.0	±	0.6	3.9	±	0.3
10	Control	8.7	±	0.9	2.4	±	0.2 a
	50 *n*Cu	12.7	±	1.7	3.8	±	0.8 ab
	100 *n*Cu	10.7	±	0.3	3.9	±	0.4 ab
	50 *b*Cu	12.7	±	1.2	4.8	±	0.4 b
	100 *b*Cu	11.7	±	0.3	3.9	±	0.4 ab
	50 $CuCl_2$	11.0	±	0.6	3.7	±	0.3 ab
	100 $CuCl_2$	12.7	±	1.9	3.4	±	0.6 ab
100	Control	10.7	±	0.9	3.4	±	0.5
	50 *n*Cu	12.3	±	0.3	3.7	±	0.2
	100 *n*Cu	9.7	±	1.2	4.2	±	0.2
	50 *b*Cu	13.7	±	0.9	5.0	±	0.8
	100 *b*Cu	14.0	±	0.0	4.4	±	0.5
	50 $CuCl_2$	14.0	±	1.2	4.9	±	0.4
	100 $CuCl_2$	10.3	±	1.2	3.5	±	0.3

planting strategies under these conditions, and advocate commercial viability.

4. Conclusion

The aim of this research was to describe the impact of metallic copper nanoparticles, in the absence and presence of external kinetin (KN), on the nutrient profile of common bean seeds. Bulk and ionic Cu were used as counterparts to differentiate between the effects of ions and particle size. Particle size was demonstrated to be an influential aspect given that Mn and protein content were elevated by *b*Cu treatments, and Fe was decreased by 100 mg kg^{-1} *n*Cu. An interaction was observed between $CuCl_2$ and KN that depressed protein synthesis, which was most likely due to the release of toxic chloride ions. Sugar and starch were not found to be significantly affected by any of the Cu treatments, compared to controls. Further analysis is required to understand this mechanism. Our findings suggest that particle size and the combination Cu + KN are principle factors affecting the nutritional value of common beans. This study not only provides a continuing assessment on the effects of NPs to a crop plant, but also suggests the need for future research on the interaction of NPs with phytohormones.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.04.360.

References

Alaoui-Sossé, B., Genet, P., Vinit-Dunand, F., Toussaint, M.L., Epron, D., Badot, P.M., 2004. Effect of copper on growth in cucumber plants (Cucumis sativus) and its relationships with carbohydrate accumulation and changes in ion contents. Plant Sci. 166 (5):1213–1218. https://doi.org/10.1016/j.plantsci.2003.12.032.

Al-Hakimi, A.M.A., 2007. Modification of cadmium toxicity in pea seedlings by kinetin. Plant Soil Environ. 53:129–135. https://doi.org/10.17221/2228-PSE.

Allen, E., 2002. Forage quality interpretations. Oklahoma Cooperative Extension Service http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Document-2557/PSS-2117web.pdf, Accessed date: March 2018.

Apodaca, S.A., Tan, W., Dominguez, O.E., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2017. Physiological and biochemical effects of nanoparticulate copper, bulk copper, copper chloride, and kinetin in kidney bean (Phaseolus vulgaris) plants. Sci. Total Environ. 599–600:2085–2094. https://doi.org/10.1016/j.scitotenv.2017.05.095.

Balestrasse, K.B., Benavides, M.P., Gallego, S.M., Tomaro, M.L., 2003. Effect of cadmium stress on nitrogen metabolism in nodules and roots of soybean plants. Funct. Plant Biol. 30:57–64. https://doi.org/10.1007/s11104-006-0050-z.

Barbafieri, M., Peralta-videa, J.R., Pedron, F., Gardea-torresdey, J.L., 2012. Plant growth regulators and improvements in phytoremediation process efficiency. In: Anjum, N.A., Pereira, M.E., Ahmad, I., Duarte, A.C., Umar, S., Khan, N.A. (Eds.), Phytotechnologies: Remediation of Environmental Contminants. CRC Press, Boca Raton, FL, pp. 377–390.

Barciszewski, J., Rattan, S.I.S., Siboska, G., Clark, B.F.C., 1999. Kinetin — 45 years on. Plant Sci. 148 (1):37–45. https://doi.org/10.1016/S0168-9452(99)00116-8.

Barrios, A.C., Rico, C.M., Trujillo-Reyes, J., Medina-Velo, I. a, Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2015. Effects of uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate, and citric acid on tomato plants. Sci. Total Environ. 563–564:956–964. https://doi.org/10.1016/j.scitotenv.2015.11.143.

Bremner, J.M., 1996. Nitrogen-total. In: Sparks, D.L. (Ed.), Methods of Soil Analysis Part 3–Chemical Methods, Soil Science Society of America Book Series No. 5. American Society of Agronomy, Madison, WI, pp. 1085–1121.

Bücker-Neto, L., Paiva, A.L.S., Machado, R.D., Arenhart, R.A., Margis-Pinheiro, M., 2017. Interactions between plant hormones and heavy metals responses. Genet. Mol. Biol. 40 (1):373–386. https://doi.org/10.1590/1678-4685-gmb-2016-0087.

Cassina, L., Tassi, E., Pedron, F., Petruzzelli, G., Ambrosini, P., Barbafieri, M., 2012. Using a plant hormone and a thioligand to improve phytoremediation of Hg-contaminated soil from a petrochemical plant. J. Hazard. Mater. 231–232:36–42. https://doi.org/10.1016/j.jhazmat.2012.06.031.

Cicek, S., Nadaroglu, H., 2015. The use of nanotechnology in the agriculture. Adv. Nano Res. 3 (4):207–223. https://doi.org/10.12989/anr.2015.3.4.207.

Deák, M., Horváth, G.V., Davletova, S., Török, K., Sass, L., Vass, I., Barna, B., Király, Z., Dudits, D., 1999. Plants ectopically expressing the iron-binding protein, ferritin, are tolerant to oxidative damage and pathogens. Nat. Biotechnol. 17:192–196. https://doi.org/10.1038/6198.

Eaton, F.M., 1942. Toxicity and accumulation of chloride and sulfate salts in plants. J. Agric. Res. 64 (7), 357–399.

Finkelstein, J.L., Haas, J.D., Mehta, S., 2017. Iron-biofortified staple food crops for improving iron status: a review of the current evidence. Curr. Opin. Biotechnol. 44:138–145. https://doi.org/10.1016/j.copbio.2017.01.003.

Flowers, T.J., Munns, R., Colmer, T.D., 2015. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. Ann. Bot. 115 (3):419–431. https://doi.org/10.1093/aob/mcu217.

Gangwar, S., Singh, V.P., Prasad, S.M., Maurya, J.N., 2010. Modulation of manganese toxicity in *Pisum sativum* L. seedlings by kinetin. Sci. Hortic. 128 (4):467–474. https://doi.org/10.1016/j.scienta.2010.08.013.

García, M., Forbe, T., Gonzalez, E., 2010. Potential applications of nanotechnology in the agro-food sector. Cienc. Tecnol. Aliment. 30 (3):573–581. https://doi.org/10.1590/S0101-20612010000300002.

Hajrasuliha, S., 1980. Accumulation and toxicity of chloride in bean plants. Plant Soil 55 (1):133–138. https://doi.org/10.1007/BF02149716.

Hong, J., Rico, C.M., Zhao, L., Adeleye, A.S., Keller, A.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2015. Toxic effects of copper-based nanoparticles or compounds to lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*). Environ. Sci.: Processes Impacts 17 (1):177–185. https://doi.org/10.1039/c4em00551a.

Iavicoli, I., Leso, V., Beezhold, D.H., Shvedova, A.A., 2017. Nanotechnology in agriculture: opportunities, toxicological implications, and occupational risks. Toxicol. Appl. Pharmacol. 329:96–111. https://doi.org/10.1016/j.taap.2017.05.025.

Keller, A.A., Adeleye, A.S., Conway, J.R., Garner, K.L., Zhao, L., Cherr, G.N., Hong, J., Gardea-Torresdey, J.L., Godwin, H.A., Hanna, S., Zhaoxia, J., Chitrada, K., Sijie, L., Lenihan, H.S.,

Miller, R.J., Nel, A.E., Peralta-Videa, J.R., Walker, S.L., Taylor, A.A., Torres-Duarte, C., Zink, J.I., Zuverza-Mena, N., 2017. Comparative environmental fate and toxicity of copper nanomaterials. NanoImpact 7:28–40. https://doi.org/10.1016/j.impact.2017.05.003.
Khattab, R.Y., Arntfield, S.D., Nyachoti, C.M., 2009. Nutritional quality of legume seeds as affected by some physical treatments, Part 1: protein quality evaluation. LWT Food Sci. Technol. 42 (6):1107–1112. https://doi.org/10.1016/j.lwt.2009.02.008.
Kochian, L.V., 1991. Mechanisms of micronutrient uptake and translocation in plants. In: Mortvedt, J.J., Cox, F.R., Shuman, L.M., Welch, R.M. (Eds.), Micronutrients in Agriculture, 2nd Edn Soil Science Society of America, Madison, Wisconsin, pp. 229–296.
Kumar, R., Mehrotra, N.K., Nautiyal, B.D., Kumar, P., Singh, P.K., 2009. Effect of copper on growth, yield and concentration of Fe, Mn, Zn and Cu in wheat plants (*Triticum aestivum* L.) crop. J. Environ. Biol. 30 (4), 485–488.
López, M.L., Peralta-Videa, J.R., Parsons, J.G., Gardea-Torresdey, J.L., Duarte-Gardea, M., 2009. Effect of indole-3-acetic acid, kinetin, and ethylenediaminetetraacetic acid on plant growth and uptake and translocation of lead, micronutrients, and macronutrients in alfalfa plants. Int. J. Phytoremediation 11 (2):131–149. https://doi.org/10.1080/15226510802378434.
Ma, Q.H., 2008. Genetic engineering of cytokinins and their application to agriculture. Crit. Rev. Biotechnol. 28 (3):213–232. https://doi.org/10.1080/07388550802262205.
Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S., Lee, Y.C., 2005. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. Anal. Biochem. 339 (1):69–72. https://doi.org/10.1016/j.ab.2004.12.001.
Medina-Velo, I.A., Dominguez, O.E., Ochoa, L., Barrios, A.C., Hernández-Viezcas, J.A., White, J.C., Gardea-Torresdey, J.L., 2017. Nutritional quality of bean seeds harvested from plants grown in different soils amended with coated and uncoated zinc oxide nanomaterials. Environ. Sci. Nano 4:2336–2347. https://doi.org/10.1039/C7EN00495H.
Messina, V., 2014. Nutritional and health benefits of dried beans. Am. J. Clin. Nutr. 100 (Supplementary S1):437S–442S. https://doi.org/10.3945/ajcn.113.071472.2.
Mirzajani, F., Askari, H., Hamzelou, S., Farzaneh, M., Ghassempour, A., 2013. Effect of silver nanoparticles on *Oryza sativa* L. and its rhizosphere bacteria. Ecotoxicol. Environ. Saf. 88:48–54. https://doi.org/10.1016/j.ecoenv.2012.10.018.
Morales-Díaz, A.B., Ortega-Ortíz, H., 2017. Application of nanoelements in plant nutrition and its impact in ecosystems. Adv. Nat. Sci. Nanosci. Nanotechnol. 8. https://doi.org/10.1088/2043-6254/8/1/013001/meta.
Ochoa, L., Medina-Velo, I.A., Barrios, A.C., Bonilla-Bird, N.J., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2017. Modulation of CuO nanoparticles toxicity to green pea (Pisum sativum Fabaceae) by the phytohormone indole-3-acetic acid. Sci. Total Environ. 598:513–524. https://doi.org/10.1016/j.scitotenv.2017.04.063.
Papadopoulos, A.P., Saha, U., Hao, X., Khosla, S., 2006. Response of rockwool-grown greenhouse cucumber, tomato, and pepper to kinetin foliar sprays. HortTechnology 16 (3), 493–501.
Prasad, R., Bhattacharyya, A., Nguyen, Q.D., 2017. Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives. Front. Microbiol. 8:1–13. https://doi.org/10.3389/fmicb.2017.01014.
Printz, B., Lutts, S., Hausman, J.-F., Sergeant, K., 2016. Copper trafficking in plants and its implication on cell wall dynamics. Front. Plant Sci. 7:1–16. https://doi.org/10.3389/fpls.2016.00601.
Rai, M., Ingle, A., 2012. Role of nanotechnology in agriculture with special reference to management of insect pests. Appl. Microbiol. Biotechnol. 94:287–293. https://doi.org/10.1007/s00253-012-3969-4.
Raigond, P., Ezekiel, R., Raigond, B., 2015. Resistant starch in food: a review. J. Sci. Food Agric. 95 (10):1968–1978. https://doi.org/10.1002/jsfa.6966.
Rasheed, M.A., Seeley, R.C., 1966. Relationship between the protein and copper contents of some plants. Nature 212:644–645. https://doi.org/10.1038/212644a0.
Rawat, S., Pullagurala, V.L.R., Hernandez-Molina, M., Sun, Y., Niu, G., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2018. Impacts of copper oxide nanoparticles in bell pepper (*Capsicum annum* L.) plants: a full life cycle study. Environ. Sci. Nano 5:83–95. https://doi.org/10.1039/c7en00697g.
Rico, C.M., Morales, M.I., Barrios, A.C., Mccreary, R., Hong, J., Lee, W., Nunez, J., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2013. Effect of cerium oxide nanoparticles on the quality of rice (*Oryza sativa* L.) grains. J. Agric. Food Chem. 61 (3):11278–11285. https://doi.org/10.1021/jf404046v.
Salama, H.M.H., 2012. Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). Int. Res. J. Biotechnol. 3 (10), 190–197.
Sekine, R., Marzouk, E.R., Khaksar, M., Scheckel, K.G., Stegemeier, J.P., Lowry, G.V., Lombi, E., 2017. Aging of dissolved copper and copper-based nanoparticles in five different soils: short-term kinetics vs. long-term fate. J. Environ. Qual. 46 (6):1198–1205. https://doi.org/10.2134/jeq2016.12.0485.
Strnad, M., 1997. The aromatic cytokinins. Physiol. Plant. 101 (4):674–688. https://doi.org/10.1111/j.1399-3054.1997.tb01052.x.
Tassi, E., Pouget, J., Petruzzelli, G., Barbafieri, M., 2008. The effects of exogenous plant growth regulators in the phytoextraction of heavy metals. Chemosphere 71 (1): 66–73. https://doi.org/10.1016/j.chemosphere.2007.10.027.
Tavakkoli, E., Fatehi, F., Coventry, S., Rengasamy, P., McDonald, G.K., 2011. Additive effects of Na+ and Cl− ions on barley growth under salinity stress. J. Exp. Bot. 62 (6): 2189–2203. https://doi.org/10.1093/jxb/erq422.
Tolaymat, T., Genaidy, A., Abdelraheem, W., Dionysiou, D., Andersen, C., 2017. The effects of metallic engineered nanoparticles upon plant systems: an analytic examination of scientific evidence. Sci. Total Environ. 579:93–106. https://doi.org/10.1016/j.scitotenv.2016.10.229.
Trumbo, P., Yates, A.P., Schlicker, S., Poos, M., 2001. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J. Am. Diet. Assoc. 101 (3):294–301. https://doi.org/10.1016/S0002-8223(01)00078-5.
Verma, S., Dubey, R.S., 2001. Effect of cadmium on soluble sugars and enzymes of their metabolism in rice. Biol. Plant. 44 (1):117–123. https://doi.org/10.1023/A:1017938809311.
Weaver, R.J., 1972. Plant Growth Substances in Agriculture. W. H. Freeman, San Francisco, CA.
Werner, T., Motyka, V., Strnad, M., Schmulling, T., 2001. Regulation of plant growth by cytokinin. Proc. Natl. Acad. Sci. 98:10487–10492. https://doi.org/10.1073/pnas.171304098.
White, P., Broadley, M.R., 2001. Chloride in soils and its uptake and movement within the plant: a review. Ann. Bot. 88 (6):967–988. https://doi.org/10.1006/anbo.2001.1540.
Zuverza-Mena, N., Medina-Velo, I.A., Barrios, A.C., Tan, W., Peralta-Videa, 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. https://doi.org/10.1039/C5EM00329F.
Zuverza-Mena, N., Martínez-Fernández, D., Du, W., Hernandez-Viezcas, J.A., Bonilla-Bird, N., López-Moreno, M.L., Komárek, M., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2017. Exposure of engineered nanomaterials to plants: insights into the physiological and biochemical responses—a review. Plant Physiol. Biochem. 110:236–264. https://doi.org/10.1016/j.plaphy.2016.05.037.