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Research article

Effect of copper oxide nanoparticles on two varieties of sweetpotato plants

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A B S T R A C T

Little information is available on the interaction of CuO nanoparticles (nCuO) with tuberous roots. In this study, Beauregard-14 (B-14, low lignin) and Covington (COV, high lignin) sweetpotato varieties were cultivated until maturity in soil amended with nCuO, bulk copper oxide (bCuO) and CuCl2 at 25–125 mg/kg. The Cu treatments had no significant influence on chlorophyll content. Gas exchange parameters were not affected in B-14. In COV, however, at 125 mg/kg treatments, bCuO reduced the intercellular CO2 (11%), while CuCl2 increased it by 7%, compared with control ($p \leq 0.035$). At 25 mg/kg nCuO increased the length of COV roots (20.7 ± 2.0 cm vs. 14.6 ± 0.8 cm, $p \leq 0.05$). In periderm of B-14, nCuO, at 125 mg/kg, increased Mg by 232%, while the equivalent concentration of CuCl2 reduced P by 410%, compared with control ($p \leq 0.05$). The data suggest the potential application of nCuO as nanofertilizer for sweetpotato storage root production.

1. Introduction

Nanotechnology promises to be a key element in advancing our agricultural practices, specifically by the improvement of pesticides and fertilizers (Kah et al., 2019). Currently, about 40–90% of the applied fertilizers and 10–75% of used pesticides do not reach the desired target (Duhan et al., 2017; Pimentel and Burgess, 2014); nano-enabled delivery systems could bring crop nutrition or protection in an efficient and timely manner (Servin et al., 2015). Nevertheless, and due to their novel properties, it is paramount to prove that nanoformulations are environmentally safe before they are used (Kah et al., 2019). Copper-based compounds have been historically used in agriculture as pesticides (e.g., fungicides) and recently as fertilizers (Lamichhane et al., 2018; Priyanka et al., 2019). Copper oxide nanoparticles (nCuO) is one of the strategies being explored to deliver the antifungal copper to crops in a more efficient way (Borgatta et al., 2018). However, the literature shows mixed results (both positive and negative) about the use of nCuO in plants, which may suggest a plant-dependent behavior or a shortfall in the standardization of experimental parameters (e.g., concentration of CuO, duration of the study, measured variables, etc.). For instance, in a germination study, Adhikari et al. reported that nCuO reduced root elongation by 50% in rice and maize at exposure concentrations of 30 and 60 mg/L, and by 96 and 97% at 2000 mg/L, respectively (Adhikari et al., 2012). In the same study, a concentration as low as 10 mg nCuO/L damaged roots, reduced stomatal density, and changed the cell wall integrity of epidermis and endodermis in spring barley (Adhikari et al., 2012). On the other hand, a recent long-term study (i.e., 11 months) exposed sugarcane to nCuO without any adverse effect on the mature crop (Tamez et al., 2020). Furthermore, it was found that nCuO reduced the impact of drought stress in soybean (Dimkpa et al., 2017). Lin et al. (2005) reported that Cu ions increased lignin concentration in soybean roots, which could impart benefits to some plants like sweetpotato (Ipomoea batatas) Lam. Sweetpotato is one of the most important staple food crops in the world (Sun et al., 2014). The world production is around 105 million tons/year, 23% of which is produced in the United States (FAO, 2016). As a tuberous root, sweetpotato is in direct contact with soil-applied fertilizers and pesticides. Sweetpotato develop lignin, a polymer that gives protection against excoriation and loss of skin, as well as protection against the penetration of pathogens (Villavicencio et al., 2007). Interestingly, the proteins involved in the polymerization of lignin are prone to bind to Cu (Printz et al., 2016).

To the authors' knowledge, only two studies have shown the effects of nanoparticles on sweetpotato. Bradfield et al. (2017) evaluated the element accumulation in sweetpotato roots exposed to nanoparticulate Zn, Cu, or Ce, while Bonilla-Bird et al. evaluated the accumulation of Cu in storage roots exposed to different nCuO, bCuO, and CuCl2

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concentrations (Bonilla-Bird et al., 2018).

The current study shows the effects of nCuO, bCuO, and CuCl2 on plant development, root production, and root composition of two varieties of sweetpotato, with different lignin content. The varieties used were Beauregard-14 (B-14) with a lignin content of 142.5 g/kg and Covington (COV) with 156.1 g/kg (Bird, 2015). The plants were cultivated under full sun exposure and evaluated for differences in chlorophyll, gas exchange, root production, and nutritional components of storage roots. A portable photosynthesis system, UV/Vis, inductively coupled plasma-optical emission spectrometry, scanning electron microscopy, and Fourier transform infrared spectroscopy were used to collect the data and measure the treatments effects.

2. Materials and methods

2.1. Plant material

Sweetpotato fresh slips (± 30 cm length) of Beauregard-14 and Covington varieties were procured from Mississippi State University, Pontotoc Ridge-Flatwoods Branch Experiment Station. The slips management before planting is described in Supporting Information (SI). Slips were planted in plastic pots (27 × 23 cm) containing 3 kg of Miracle-Gro® soil, leaving 2/3 of the distal end of the scion upward. A total of 60 plants (30 per variety) were used. A summary description of the soil is shown in the SI (Table S1) (Barrios et al., 2016)

2.2. Characteristics of the Cu products and suspension/solution preparations

Commercial nCuO, bCuO, and $CuCl_2$ (Sigma Aldrich, St. Louis, MO) were used in this research. These materials were supplied by the UC Center for Environmental Implications of Nanotechnology. The physicochemical properties of both the nCuO and bCuO were previously determined (Hong and. R, 2015). Table S2 (SI) shows major physicochemical properties of the particles used in this study.

Suspensions/solutions of nCuO, bCuO, and $CuCl_2$ were prepared in Millipore water (MPW) and homogenized by sonication in a water bath (Crest Ultrasonics, Trenton, N) at 25 °C for 30 min and 180 W. Enough volume of the sonicated suspension/solution was mixed with 3 kg of commercial potting mix (MiracleGro® with micromax, Marysville, OH, USA) to have 0 (control), 25 (low), 75 (medium), and 125 (high) mg Cu per kg of soil. The 25 mg/kg treatment was selected because the average Cu concentration in soil of the United States is 20 mg/kg (Shacklette and Boerngen, 1984), while 75 mg/kg was selected to mimic the highest Cu concentration reported in agricultural soils amended with sewage sludge (75 mg/kg) (EPA (US Environmental Protection Agency), 1994). The 125 mg/kg treatment represents 83% of the CuO NPs used to control fungi at field levels (Giannousi et al., 2013).

Plants were cultivated under full sun on the “green roof” facility of the Biology building at the University of Texas at El Paso in the span 05/07/16 to 10/26/16. Plants were irrigated four days a week to maintain 80% of the field capacity moisture regime. The water requirements for sweet potato plants are around 40–45 mm/week, depending on the soil type and external temperature. Approximately 640 mm of water were used during the entire growth cycle. No fertilizer was added; no pest control was needed (Ekanayake and Collins, 2004).

2.3. Gas exchange and chlorophyll measurement

Gas exchange measurements were conducted during the middle phase of the growth cycle, at the peak of leaf development using a portable photosynthesis system (LI- 6400XT, LI-COR, Lincoln, Nebraska) with light levels set to 1500 μmol m^{-2} with an LED light source, reference CO_2 set to 400 μmol mol^{-1}, and a flow rate of 500 μmol s^{-1}. Throughout the experiment, the average day temperature was recorded as 27.2 ± 1.6 °C and the average night temperature as 25 ± 2.1 °C. The average daylight was recorded as 10.1 ± 3.1 mol $m^{-2}{\cdot}d^{-1}$.

The ratio of photosynthesis and transpiration rates were further used to calculate the Water Use Efficiency (WUE) (Von Caemmerer et al., 2004). In addition, the relative chlorophyll content was determined by a hand held single photon avalanche diode (SPAD-502, Minolta Camera, Japan) (Spectrum Technologies and I., 2009). Data from SPAD-502 were closely correlated with direct photometric measurements from the extracted chlorophyll (Ling et al., 2011).

2.4. Measurements at harvest

The final measurements were done at the end of the life cycle, 150 days after planting. Three plants per concentration/compound plus control (30 plants per variety) were harvested. Plants with their respective storage, pencil, and adsorption roots were harvested gently to avoid damage to the periderm of storage roots. To determine the above ground plant biomass, stems and leaves were oven-dried at 70 °C for 72 h. Dried samples were weighed and separated for further analyses. Roots were gently washed with MPW to remove soil particles from periderm. Storage and pencil roots were counted, measured longitudinally and transversally, and weighed. The US Department of Agriculture standard grades were used to categorize the storage roots (Table S3) (Clark et al., 2010)

2.5. Sample preparation for ICP-OES

For ICP-OES analyses, root tissues were separated with a scalpel in the following order: periderm, cortex, perimedulla, and medulla. Each tissue was set in a paper envelope, oven dried at 70 °C for 72 h, and mechanically ground using a blender. All sample preparation for the ICP-OES was previously described in Bonilla-Bird et al. (2018)

2.6. Crude protein analysis

Total nitrogen was used to determine crude protein content. Initially, samples were oven dried for 5 day at 60 °C until constant weight was achieved. Dried samples were then powdered, sieved in a nylon mesh, and stored in paper envelopes until assayed. Nitrogen content was determined by the Total Kjeldahl Nitrogen (TKN) using an extraction and distillation unit (Labconco, Kansas City, MO; AOAC2000) and expressed as % N. The protocol was previously published by Bremner (1996), The crude protein content was calculated as % N x 6.25 (Rocateli and Zhang, 2117).

2.7. Sugar and starch determination

Total sugar was determined after Dubois el al (Dubois et al., 1956). Samples of 100 mg of oven-dried sweetpotato storage roots were homogenized in 10 mL 80% ethanol, boiled in a water bath at 80 °C for 30 min, and centrifuged at 5000 rpm for 20 min (Eppendorf AG bench centrifuge 5417 R, Hamburg, Germany). Glucose standard (Sigma-Aldrich, 99.9% pure) and water (blank) were treated with the same protocol to obtain the calibration curve. The absorbance of the samples was recorded using a UV–Vis spectrometer (PerkinElmer Lambda 14 UV/Vis Spectrometer, Uberlinger, Germany) at 490 nm, and total sugar was quantified from the standard calibration curve. Samples were analyzed in a 96 well microplate by a phenol-sulfuric acid method (Masuko et al., 2005). The starch content was estimated in the same

Table 1
Copper concentration in root tissues, stems, and leaves of Beauregard-14 and Covington varieties exposed for 150 days to nCuO, bCuO, and $CuCl_2$ at 0, 25, 75, and 125 mg/kg ($p \leq 0.05$, n = 3).

Varieties	B-14 Cu (mg/kg)	Covington Cu (mg/kg)	B-14 Cu (mg/kg)	Covington Cu (mg/kg)
Treatments	**Periderm**		**Perimedulla**	
Control	18.5 ± 1.3	43.4 ± 9.1	6.2 ± 2.4	1 ± 0.2
nCuO25	52.6 ± 8.3	45.2 ± 10	2.6 ± 0.1	2 ± 0.2
nCuO75	100.6 ± 20	74.8 ± 15.2	4 ± 1.9	3.6 ± 0.2
nCuO125	54.4 ± 14.3	42.3 ± 16.3	6.4 ± 0.7	4.3 ± 0.5
bCuO25	193.5 ± 22.5	91.5 ± 25.9	4.2 ± 0.2	2.2 ± 0.4
bCuO75	70.2 ± 12	65.2 ± 14.9	5 ± 0.3	2.4 ± 0.4
bCuO125	27 ± 4.6	136.8 ± 35.8	6.1 ± 0.6	11.9 ± 9.4
$CuCl_2$ 25	53.3 ± 17.6	22.5 ± 4.3	2.1 ± 0.5	2 ± 1.1
$CuCl_2$ 75	66.6 ± 41.3	73.8 ± 45.2	3.8 ± 1.2	5 ± 1.4
$CuCl_2$ 125	18.5 ± 2.2	60.6 ± 15	5.4 ± 0.5	7.2 ± 0.4
	Cortex		**Medulla**	
Control	5.1 ± 0.2	1 ± 0.1	1.8 ± 0.5b	2 ± 0.4
nCuO25	3.6 ± 0.7	2.5 ± 0.47	2.3 ± 0.6 ab	2.6 ± 0.58
nCuO75	4.2 ± 1.5	4.5 ± 0.9	5.5 ± 3.2 ab	3.5 ± 0.3
nCuO125	30.9 ± 3.5	4.5 ± 0.2	10.1 ± 3.6 ab	3.9 ± 1.3
bCuO25	4 ± 0.2	2.4 ± 0.1	3.7 ± 0.7 ab	2.9 ± 1.2
bCuO75	6 ± 1.4	3.3 ± 0.6	3.4 ± 0.7 ab	3.5 ± 0.7
bCuO125	6.2 ± 0.5	6.9 ± 2	3.5 ± 0.5 ab	5.3 ± 1.6
$CuCl_2$ 25	2.8 ± 0.5	1.4 ± 0.1	1.4 ± 0.3b	1.4 ± 0.1
$CuCl_2$ 75	2.8 ± 0.5	3.7 ± 1.4	9.1 ± 0.6 ab	6 ± 2.2
$CuCl_2$ 125	6.4 ± 0.6	3.8 ± 0.3	13.0 ± 4.5a	5.9 ± 0.3
	Stem		**Leaves**	
Control	1.6 ± 0.3b	2.3 ± 0.6	7.8 ± 1	5.6 ± 2.9b
nCuO25	3.5 ± 0.2 ab	3.1 ± 0.1	13.8 ± 5.1	22.9 ± 11.3 ab
nCuO75	5.9 ± 1a	12.9 ± 9.1	16.9 ± 6.5	9.5 ± 1.3b
nCuO125	4.2 ± 1.7 ab	4 ± 1	7.1 ± 0.3	10.2 ± 2.5 ab
bCuO25	4.3 ± 0.3 ab	3.1 ± 0.7	9.2 ± 2.6	6.7 ± 1.3b
bCuO75	4.4 ± 0.4 ab	4.6 ± 0.2	8.3 ± 0.6	25.6 ± 6.9 ab
bCuO125	6.3 ± 0.3a	4.6 ± 0.9	14.8 ± 0.4	23 ± 6.5 ab
$CuCl_2$ 25	2.8 ± 0.2 ab	1.7 ± 0.3	18.6 ± 5.2	12.1 ± 7.6 ab
$CuCl_2$ 75	3.7 ± 0.4 ab	4.2 ± 2	10 ± 2.8	70.9 ± 24.7 ab
$CuCl_2$ 125	4.8 ± 0.6 ab	5.3 ± 0.2	11.1 ± 2.1	93 ± 26a

way as total sugars (Dubois et al., 1956).

2.8. Fourier transform infrared spectroscopy (FTIR)

Spectra of skin and root of sweetpotato samples were collected using a FT-IR/ATR spectrometer 100 (PerkinElmer, Shelton, CT, USA) at 2 cm^{-1} resolution, using air as background. Three samples per treatment were used for the analysis. The graphics were drawn in Origin Pro 8.5.1© (OriginLab Corporation Wellesley Hills, MA, US).

2.9. Microscopy study of sweetpotato samples

Dry samples from the medulla of storage roots were ground, coated with gold for a minute (*ca.* 1 nm gold layer) by using a Sputter Coater (SPI-MODULE); subsequently, they were observed by scanning electron microscopy (SEM; Hitachi S-4800). All samples used for SEM were prepared following standard procedures (Kopek et al., 2017).

2.10. Statistics analyses

Multivariate Data analysis was performed, followed by One-way ANOVA to determine significant differences among the average of each factor/treatment. Coefficient of Variance (CV) was conducted for chlorophyll content. After ANOVA, Tukey Honestly Significant Difference and Waller-Duncan test were used to perform multiple comparison (SPSS 19.0 package, Chicago, IL, and SAS/STAT 14.2. SAS Institute, Cary, North Carolina, U.S.). For the gas exchange measurements, n = 10, [Control, plus three compounds (3) at three concentrations (3)].

3. Results and discussions

3.1. Gas exchange and chlorophyll measurement

Table S4 shows the multivariate analysis for gas exchange components of photosynthesis in sweetpotato plants. Most of the factors had different significance including $p \leq 0.05$; 0.01; 0.001 for photosynthesis rate, intercellular CO_2, transpiration rate, and water use efficiency (WUE). The Partial Eta-squared statistics showed that the higher variance was for the main component variety, [24%, 19%, 37%, and 12% for photosynthesis, transpiration rate, and WUE, respectively ($p \leq 0.001$)]. However, the copper treatments significantly affected all parameters related to photosynthesis, where the higher variances were 21.5% for conductance ($p \leq 0.001$). Furthermore, the interaction between the varieties × treatments was significant for conductance (12%) and WUE (17%). Overall, conductance and photosynthesis had the

lowest variation associated with these interactions, with a variance of 5.4% and 5.7%, respectively ($p \leq 0.001$). Detailed discussion of the data is presented in the following sections.

3.2. Chlorophyll content

The chlorophyll measurements were made in mature plants, before finishing the experiment. As seen in Fig. S1, SPAD measurements showed differences only COV variety. However, although the ANOVA presented statistical significance between some treatment concentrations, there was not a clear trend, indicating that the treatments had no significant influence on the chlorophyll content in the leaves of sweetpotato plants.

3.3. Effects of treatments on gas exchange parameters

The effects of Cu treatments on gas exchange parameters are shown in Fig. S2 (A-D). As shown in this figure, there was not a clear effect of the treatments on intercellular CO_2 (Fig. S2 -A). However, it is worth noting that at 125 mg/kg, bCuO and ionic Cu showed contrasting effects in COV. While bCuO reduced CO_2 by 11.8%, ionic Cu increased it by 7%, compared with control ($p \leq 0.05$). Though, the results could be influenced by the small sample size. Results were unexpected since excess Cu has been found to reduce Rubisco enzyme activity in rice leaves, which is fundamental for CO_2 assimilation (Demirevska-Kepova et al., 2004). It is possible that sweetpotato tolerates well such Cu concentrations. In other plants, such as cucumber, 50 μM of $CuCl_2$ did not affect intercellular CO_2 (Burzyński and Zurek, 2007). A similar effect was observed in bell pepper plants, where 500 mg/kg of Cu caused minimal effects on gas exchange parameters (Rawat et al., 2018). In addition, the Cu concentration in COV leaves from 125 mg $CuCl_2$/kg treatment was significantly higher, compared with control (Table 1). Concerning the response to bCuO exposure, B-14 plants have shown to absorb bCuO particles (Bonilla-Bird et al., 2018), which dissolve less that nCuO (Pimentel and Burgess, 2014), and translocateto the leaves, affecting the concentration of intercellular CO_2. The results suggest an interaction compound × variety, which is supported by Burzynski and Zurek (Kopek et al., 2017), who reported that 50 μM of $CuCl_2$ did not affect intercellular CO_2 in cucumber. More studies are needed for a better understanding of the response of sweetpotato to CuO and $CuCl_2$ exposure.

Furthermore, there was no clear evidence of changes in transpiration due to the Cu treatments (Figs. S2–C). Copper chloride at 25 mg/kg seemed to reduce the transpiration rate in COV, compared with control. However, the data overlapped with nCuO at 25 mg/kg, bCuO at 125 mg/kg and $CuCl_2$ at 75 mg/kg. Data on water use efficiency (WUE, photosynthesis/transpiration) are shown in Figs. S2B–D. As seen in this figure, none of the varieties showed reduction in WUE due to the Cu treatments. This suggests that sweetpotato tolerate well the concentrations of nCuO, bCuO, and $CuCl_2$ used in this study.

The effects of the Cu treatments on photosynthesis in sweetpotato plants are shown in Figs. S2–B. As seen in this figure, neither B-14 nor COV had a reduction in photosynthesis due to the Cu treatments, except for the 25 mg $CuCl_2$/kg treatment that showed a reduction of 10% in COV, compared with control ($p \leq 0.05$). Although previous studies indicated that Cu reduces photosynthesis by depleting PSII action centers (Conway et al., 2015; Zhao et al., 2016), the results suggest that, at the concentration tested, the three Cu compounds had low to no effect on the photosynthesis of sweetpotato plants. Several factors may be responsible for these results including the plant tolerance to Cu, or the size of n, as the trend in $CuCl_2$ showed no differences due to the higher error bar in the intermediate concentration.

3.4. Copper uptake

The Cu uptake by sweetpotato plants is shown in Table 1. As seen in this table, there were low to no effects of the treatments on Cu accumulation in tissues. At root level, there were no changes in the Cu concertation in periderm, perimedulla, and cortex of both varieties; however, the medulla of B-14 showed increments in Cu concentration. The medulla of B-14 plants exposed to 125 mg $CuCl_2$/kg had 622% more Cu, compared with control ($p \leq 0.05$). Data for B-14 plants correlate with previous results from an experiment where the roots of B-14 and COV were exposed to the same concentrations of the Cu-based treatments (Bonilla-Bird et al., 2018). In such experiments, B-14 showed significant Cu concentration in medulla, which was attributed to the lower lignin content, compared with COV. Lignin is a phenolic polymer that augments plant cell rigidity and it is an important barrier that protects roots against pathogens and pests. Furthermore, the biomolecule promotes mineral transport and has been known to bind to Cu (Bonilla-Bird et al., 2018).

The statistical analysis for the Cu concentration in stems and leaves showed variety × compound interactions. In B-14 exposed to bCuO at 125 mg/k, there was more Cu in stems, compared with control (6.3 ± 0.3 *vs.* 1.6 ± 0.3 kg d wt tissues), the leaves of COV plants exposed to $CuCl_2$ at 125 mg/kg had significantly more Cu than control (93 ± 26 *vs.* 5.96 ± 2.9, $p \leq 0.05$). At this time, there is not enough information to explain the results. It seems that the effects were due to a variety × compound interaction. The bCuO, which dissolve less than nCuO (Rawat et al., 2018), was retained in stems of B-14, while ionic Cu, which is rapidly absorbed by the plants, was translocated easily to the leaves of COV. However, the fact that the multiple comparison test showed overlapping between the treatments means suggest that the sample size could have an effect. Increasing the number of replicates would give a better definition of the statistical differences.

Concerning the Cu translocation to stems, there are very few studies covering the full life cycle of plants exposed to nCuO and bCuO, where the Cu in aboveground tissues was measured. Ochoa reported no differences in Cu translocation to stems and leaves of *Pisum sativum* exposed to 100 mg nCuO/kg for the full life cycle of the plants (Ochoa et al., 2018). On the other hand, Rawat et al. reported that in bell pepper, bCuO at 125 and 250 mg/kg increased Cu in leaves, significantly, compared with control (Rawat et al., 2018). It is important to point out that neither green pea nor bell pepper are root crops and lignin was not a marker in such studies. However, it would be a speculation to assure that only the root lignin modulated the translocation of Cu to aboveground plant part; more studies are needed in order to clarify the responses of sweetpotato plants to CuO exposure.

3.5. Biomass and storage root production

Table 2 shows the effect of Cu treatments on root production. As shown in this table, independent of the variety, none of the treatments affected the number and weight of pencil root, the number and diameter of storage roots, or the ratio number of pencil roots: number of storage roots, compared with control. However, there was an interaction compounds × variety in the fresh weight and root length. In B-14 variety, plants exposed to 125 mg nCuO/kg had statistically lighter storage roots, compared with roots from plants exposed to bCuO at 75 mg/kg (Table 2). On the other hand, storage roots of COV plants exposed to 25 mg nCuO/kg were statistically longer (20.7 ± 2.0 cm), compared with control (14.6 ± 0.8 cm) ($p \leq 0.05$). Table S3 shows the U.S. Standard Classification for commercialization of sweetpotato roots. A comparison of data on Table 2 *vs.* Table S3 suggests that none of the treatments reduced the U.S. commercial classification. The literature has shown that nCuO at 10–300 mg/kg changed the root

Table 2
Root production in sweetpotato plants exposed for 140 days to nCuO, bCuO, and $CuCl_2$ at 0, 25, 75, and 125 mg/kg, $p \leq 0.05$ (n = 3).

Treatment (mg/kg)	Beauregard-14							
	Root fresh weight (g)		Total weight (g)	No. Storage roots	No. Pencil roots	Length of storage roots (cm)	Diameter of storage roots (cm)	Ratio no.pencil/ no.storage
	Storage Roots	Pencil Roots						
Control	457.3 ± 16.4abc	28 ± 3.1	485.3	9.7 ± 1.8	7 ± 1.2	14.7 ± 1.3	4.6 ± 0.7	0.72
nCuO25	414.5 ± 11.5abc	29 ± 12.1	443.5	9.5 ± 4.5	13.5 ± 1.5	12 ± 0.8	4.3 ± 0.4	1.42
nCuO75	514 ± 23.4abc	36.7 ± 10.1	550.7	7.7 ± 2	8 ± 2.1	13.4 ± 1.3	3.6 ± 0.3	1.04
nCuO125	265 ± 8c	50.3 ± 6.7	315.3	4.5 ± 0.5	11.3 ± 1.8	7.4 ± 0.9	2.8 ± 0.3	2.51
bCuO25	269 ± 64.3bc	27.5 ± 2.9	333.3	8.7 ± 1.2	6.3 ± 2	10 ± 0.8	6.6 ± 2.3	0.72
bCuO75	637.3 ± 30.5a[a]	24.6 ± 8.4	661.9	6.3 ± 0.3	7.3 ± 1.2	15 ± 1.3	4.9 ± 0.2	1.16
bCuO125	619 ± 78.5 ab[a]	37.2 ± 2.4	656.2	11.7 ± 5.6	7.7 ± 0.3	14.6 ± 1.2	5.3 ± 0.4	0.66
$CuCl_2$ 25	280.6 ± 5.3bc	26.3 ± 8.7	306.9	8.3 ± 0.8	6.7 ± 1.2	12.8 ± 1.2	3.3 ± 0.3	8.1
$CuCl_2$ 75	407.3 ± 76.3abc	46.2 ± 3.8	453.5	4.3 ± 1	8.3 ± 0.9	13.5 ± 2.2	4.3 ± 0.2	1.93
$CuCl_2$ 125	507 ± 49.7abc	30 ± 9.6	537	6.6 ± 1.4	8.3 ± 1.5	12.3 ± 0.8	3.9 ± 0.3	1.26
				Covington				
Control	436 ± 41.9	42.5 ± 8.1	478.7	5.3 ± 0.3	7.5 ± 2.3	14.6 ± 0.8b	4.4 ± 0.5	1.42
nCuO25	464.7 ± 54	61.5 ± 31	526.2	2.5 ± 0.5	10.5 ± 1.5	20.7 ± 2a	4.6 ± 0.2	4.2
nCuO75	472.1 ± 75	33 ± 9.1	505.1	5.7 ± 1.3	8 ± 3.5	16 ± 2.5b	4.6 ± 0.3	1.4
nCuO125	428 ± 69.5	15.7 ± 0.9	497.5	5 ± 0.5	6.3 ± 1.3	15 ± 1.8b	4.1 ± 0.2	1.26
bCuO25	424.3 ± 77.6	26 ± 7.9	450.3	4 ± 1.1	6 ± 2.1	17.7 ± 2.8b	4.7 ± 0.3	1.5
bCuO75	563.5 ± 60.3[a]	66.3 ± 12.1	629.8	4.7 ± 1.2	9.3 ± 3	15.2 ± 1.3b	4.6 ± 0.2	1.98
bCuO125	565.5 ± 47.3[a]	54 ± 15.3	619.5	2.3 ± 0.6	11.3 ± 1.5	15.1 ± 2.5b	5.4 ± 0.8	4.91
$CuCl_2$ 25	289.8 ± 53.3	43.6 ± 14.2	333.4	6.3 ± 4.3	7 ± 4	12 ± 1b	3.8 ± 0.3	1.11
$CuCl_2$ 75	366.8 ± 47.6	66 ± 11.3	465.8	3 ± 1.5	10 ± 4	15.3 ± 2.1b	3.9 ± 0.4	3.33
$CuCl_2$ 125	429.5 ± 44.7	61 ± 13.4	490.5	2.6 ± 0.6	6 ± 1	13.4 ± 1.7b	3.8 ± 0.4	2.31

[a] U.S. No.1 according to commercial U.S. classification (Clark et al., 2010 Table S3).

architecture in wheat (*Triticum aestivum* L.), due to imbalances in auxins. The same studies showed that nCuO caused the activation of some defense and detoxification mechanisms in the wheat plants, which affected the growth of the principal root and promoted the lateral roots growth (Adams et al., 2017; Zhang et al., 2018). At this point, the data does not clarify if the effects on sweetpotato roots were due to lignin content or hormonal imbalances caused by CuO. Concerning plant growth, none of the treatments affected the aboveground biomass production, compared with control. However, there was an apparent difference in B-14, where $CuCl_2$ at 25 mg/kg produced lower stem biomass, compared with nCuO at 75 mg/kg (Fig. S3). However, the differences were not clear as the Tukey test showed overlapping with other treatment means.

3.6. Essential elements' accumulation

Macroelements- Among all macroelements analyzed, only P and Mg showed changes in storage roots of B-14 and COV exposed to nCuO and their analogues during the whole life cycle (Table 3). However, only two treatments showed clear statistical differences. At 125 mg/kg, $CuCl_2$/kg reduced P in periderm of B-14 roots, compared with control (7101.4 ± 314.9 *vs*. 1393.15 ± 201.0) and the other treatments, except bCuO at 25 mg/kg ($p \leq 0.05$). Conversely, Mg was significantly increased in the periderm of B-14 plants exposed to nCuO at 125 mg/kg, compared with control (77.3 ± 12.7 *vs*. 54.0 ± 3.6) and the other treatments ($p \leq 0.05$). It has been reported that Cl^- reduces the uptake of some ions due to a competitive effect or antagonism with P and other cations (Chen et al., 2010). This could be the reason why $CuCl_2$, at 125 mg/kg, reduced P accumulation in the periderm of B-14 roots. Previous studies have shown a reduced P uptake by alfalfa roots exposed to 5–20 mg/L of $CuCl_2$ (Hong and. R, 2015). In addition, Zuverza-Mena et al. (2015) reported that 20 and 80 mg of $CuCl_2$ reduced P accumulation in cilantro. Likewise, Rawat et al. reported that in bell pepper, 500 mg bCuO/kg reduced the root absorption of P by 36%, respect to control (Rawat et al., 2018). In the case of Mg, it has been reported that Cu alter cell membranes (Ochoa et al., 2018), forcing an influx of Mg within the roots, in order to maintain permeability (Taiz and Zeiger, 2002). However, data in the literature is not consistent. While Rawat et al. reported no effects of nCuO on Mg accumulation in bell pepper, Zuverza-Mena et al. reported a reduction of Mg in cilantro roots exposed to 20 mg/kg of nCuO (Zuverza-Mena et al., 2015). The fact that the Cu compounds affected the uptake of macroelements only in B-14 suggests an effect of the lignin content. However, more experiments are needed in order to determine why only the highest nCuO increased Mg accumulation in sweetpotato.

Microelements- Besides Cu, only Mn, Ni, and Zn showed significant differences in roots of plants exposed the Cu compounds (Table 3). However, the effects were no clear because in no case the compounds showed a trend. Experiments with higher number of replicates and performed under controlled conditions are needed in order to obtained clear differences.

3.7. Protein analysis

Data on storage roots' protein content are shown in Fig. 1 A. As shown in this figure none of the treatment affected the protein content in both varieties, which suggests the Cu compounds did not affect the production of biopolymers, like proteins, in sweetpotato plants. However, in other of crops, such as bean producers, Cu-based NPs or compounds, have shown significant interaction with protein production. Apodaca et al. found that bCu (50 and 100 mg/kg) increased the protein content by 11% and 12%, respectively, in kidney bean grains

Table 3
Distribution of the macronutrients and micronutrients into the storage root tissues of Beauregard-14 and Covington sweetpotato varieties. Plants were cultivated for 150 days in soil amended nCuO, bCuO, and $CuCl_2$ at 0, 25, 75, and 125 mg/kg ($p \leq 0.05$; n = 3).

Treatments (mg/kg)						
Tissue	Macro nutrient	Variety	Control	nCuO25	nCuO75	nCuO125
Periderm	**P**	B-14 COV	7101.4 ± 314.9a 1398.9 ± 297	5551.1 ± 568.6a 1100.2 ± 664.9	6429.6 ± 325.5a 2853 ± 20.25.8	7063.9 ± 867.6a 1787.5 ± 571.8
	Mg	B-14 COV	54 ± 3.6b 61.3 ± 5	52.2 ± 9.3b 53.6 ± 9.9	69.5 ± 4.4b 57.3 ± 3.7	77.3 ± 12.7a 49.2 ± 4.6
Cortex	**P**	B-14 COV	5732 ± 739.6bc 7220.6 ± 845.8	4373.1 ± 592.1c 9390.4 ± 2069.3	11006.2 ± 2070.4 ab 9794 ± 935.6	11810.2 ± 641.8a 10707.6 ± 744.5
	Mg	B-14 COV	15.2 ± 1.3bc 36.5 ± 4.4	9.2 ± 0.8c 28.6 ± 2.5	43 ± 6.2a 36.4 ± 2.3	46.8 ± 8a 39.7 ± 1.1
Medulla	**P**	B-14 COV	7328 ± 2180.7 7001.2 ± 516.6b	7567.1 ± 1570.7 8351.1 ± 1020.8 ab	8218.9 ± 2884 11050.6 ± 759.7 ab	12455.4 ± 1809.2 8067.1 ± 4216 ab
	Mg	B-14 COV	45 ± 4.4 34.8 ± 2.6	47.1 ± 5.2 44.3 ± 3.5	44.1 ± 10 54.3 ± 12	44.4 ± 8 48.1 ± 6.6
Tissue	**Micro nutrient**	**Variety**	**Control**	**nCuO25**	**nCuO75**	**nCuO125**
Periderm	**Zn**	B-14 COV	0.18 ± 0.01b 0.2 ± 0.02b	0.22 ± 0.04 ab 0.26 ± 0.01 ab	0.3 ± 0.05 ab 0.22 ± 0.03 ab	0.25 ± 0.04 ab 0.22 ± 0.03 ab
Cortex	**Zn**	B-14 COV	0.2 ± 0.01 ab 0.24 ± 0.02	0.14 ± 0.01b 0.25 ± 0.02	0.25 ± 0.05 ab 0.35 ± 0.02	0.35 ± 0.08a 0.28 ± 0.04
Medulla	Mn	B-14 COV	0.18 ± 0.04 0.29 ± 0.08b	0.06 ± 0.02 1.24 ± 0.7b	0.75 ± 0.04 2.5 ± 0.7 ab	0.41 ± 0.05 2.5 ± 0.4 ab
	Zn	B-14 COV	4.56 ± 0.27 0.2 ± 0.02b	0.26 ± 0.05 0.27 ± 0.03 ab	0.24 ± 0.09 0.29 ± 0.04 ab	0.32 ± 0.06 0.2 ± 0.01 ab
	Ni	B-14 COV	28.4 ± 8.2abc 89.8 ± 8	11 ± 5.8c 30.1 ± 7.7	29.7 ± 8.7abc 15.2 ± 1.9	36.3 ± 7.4abc 36.1 ± 8.8

Treatments (mg/kg)						
Tissue	bCuO25	bCuO75	bCuO125	$CuCl_2$ 25	$CuCl_2$ 75	$CuCl_2$ 125
Periderm	4443.7 ± 391.3 ab 1713.3 ± 426.2	5471.8 ± 293a 344.7 ± 165.4	6812.2 ± 147.3a 3268.5 ± 1252.7	6077.1 ± 791.3a 907.5 ± 214.5	5552.6 ± 982a 1230.7 ± 689.8	1393.15 ± 201b 2240.5 ± 806.5
	56.1 ± 2.6 68.9 ± 6.4	61.9 ± 3.3b 57.2 ± 1.2	61.5 ± 0.4b 46.1 ± 7	68.9 ± 8.8b 50 ± 3.5	60.1 ± 7.3b 65.3 ± 0.3	73.9 ± 5.6b 52.4 ± 3.2
Cortex	9329.7 ± 1365.4abc 10298.9 ± 1741.2	10586.4 ± 1092.4 ab 9695.8 ± 707.2	11041.8 ± 1120.4 ab 12284.2 ± 1953.8	12395.3 ± 732.3a 8098.7 ± 639.4	11583 ± 1162.2 ab 10690.9 ± 225.1	9649 ± 1508.1abc 11475 ± 983.7
	37.9 ± 4.3 ab 32.2 ± 3.2	50.7 ± 4.1a 34.9 ± 2.2	53.6 ± 2.5a 57.1 ± 3.3	48.5 ± 2.8a 35.2 ± 1.5	41.7 ± 3.2a 36 ± 3.6	47.8 ± 6.7a 36.9 ± 1.9

(*continued on next page*)

Table 3 (*continued*)

Treatments (mg/kg)						
Tissue	bCuO25	bCuO75	bCuO125	$CuCl_2$ 25	$CuCl_2$ 75	$CuCl_2$ 125
Medulla	8659.9 ± 1165.8	6269.2 ± 499	5226.7 ± 849.4	6316.3 ± 1772.3	6579.6 ± 183	9870.7 ± 1217.5
	6747.5 ± 1694b	7852 ± 630.1 ab	8376 ± 468.4 ab	6750.3 ± 509.4b	13283.4 ± 1440.5a	10837.6 ± 2632.7 ab
	39.1 ± 5.4	37.6 ± 1.3	31.2 ± 2.4	34.9 ± 8.5	45 ± 42	63.7 ± 14.7
	36.7 ± 10	47.2 ± 9.5	43.8 ± 9	31 ± 3.3	63 ± 12.16	57.8 ± 18.3
Tissue	**bCuO25**	**bCuO75**	**bCuO125**	**$CuCl_2$ 25**	**$CuCl_2$ 75**	**$CuCl_2$ 125**
Periderm	0.19 ± 0.02 ab	0.23 ± 0.02 ab	0.16 ± 0.01b	0.27 ± 0.06 ab	0.24 ± 0.03 ab	0.31 ± 0.03a
	0.25 ± 0.01 ab	0.23 ± 0.02 ab	0.26 ± 0.02 ab	0.19 ± 0.01b	0.337 ± 0.04a	0.22 ± 0.05 ab
Cortex	0.19 ± 0.03 ab	0.28 ± 0.03 ab	0.26 ± 0.03 ab	0.23 ± 0.02 ab	0.26 ± 0.04 ab	0.33 ± 0.07 ab
	0.28 ± 0.03	0.23 ± 0.01	0.4 ± 0.03	0.29 ± 0.01	0.3 ± 0.02	0.22 ± 0.06
Medulla	0.36 ± 0.03	0.3 ± 0.04	0.33 ± 0.04	0.55 ± 0.1	0.31 ± 0.02	0.38 ± 0.07
	1.7 ± 0.4b	1.7 ± 0.5b	3.5 ± 0.7a	1 ± 0.4b	0.6 ± 0.4b	3.8 ± 0.3a
	0.23 ± 0.03	0.18 ± 0.02	0.17 ± 0.03	3 ± 0.06	0.3 ± 0.03	0.42 ± 0.08
	0.19 ± 0.04b	0.2 ± 0.01 ab	0.22 ± 0.01 ab	0.14 ± 0.01b	0.35 ± 0.04a	0.24 ± 0.09 ab
	28 ± 5.3 ab	24.8 ± 4.5abc	24 ± 9.1bc	16.8 ± 7c	68.3 ± 7.5a	61.6 ± 3.7 ab
	93.7 ± 9.6	29.1 ± 6.9	51.8 ± 18.5	25.8 ± 1.9	52.9 ± 11.6	103.2 ± 7.3

($p \leq 0.05$) (Apodaca et al., 2018). Conversely, Ochoa et al. reported no significant changes in protein content in green pea exposed to 50 and 100 mg/kg of nCuO, bCuO, and $CuCl_2$ (Ochoa et al., 2018).

3.8. Sugar and starch concentrations

Fig. 1-B shows the concentration of sugar in storage roots of B-14 and COV varieties. As seen in this figure, although some treatment concentrations showed statistical differences in sugar content, none of the varieties showed a clear tendency. Similar results were observed in the starch content data (Fig. 1-C). This suggests that, independently of the root lignin content, the Cu-based compounds, at the concentrations tested, had low to no effects in sugar production of sweetpotato roots. However, Fig. S4 shows some changes in starch grains of the COV variety. As seen in Fig. S4 (A-B), control roots had round starch grains, while nCuO at 125 mg/kg showed mainly round grains, but some of them were a little bit curved (Fig. S4 (C-D). On the other hand, at 125 mg/kg, both the bCuO and $CuCl_2$ showed a reduced number of grains per sample, and most of them were misshaped. However, it is important to point out that, due to time limitations in the use of the SEM, only a small number of samples were analyzed. More samples have to be examined in order to have definitive conclusions.

Varied results have been published regarding the effects of nanoparticles in plant macromolecules. Panou-Filotheou et al. showed that Cu avoided the formation of starch in oregano (Panou-Filotheou et al., 2001). Alaoui- Sossé et al. reported no changes in sugar and starch in cucumber exposed to Cu ions (Alaoui-Sossé et al., 2004). Ochoa et al. reported that nCuO, bCuO, and $CuCl_2$ at 50 and 100 mg/kg did not affect sugar content in green pea seeds (Ochoa et al., 2018). Rico et al. mentioned that, in rice, CeO_2 NPs did not affect sugar but reduced starch content (Rico et al., 2015). Du et al. reported that $nCeO_2$ changed the number and size of starch granules in endosperm cells of wheat (Du et al., 2015). Du et al. also reported that nCu and μCu reduced sugar and starch contents in oregano leaves (Du et al., 2015).

3.9. Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR analysis of lignin in storage roots is shown in Fig. 2(a–f). As seen in this figure, lignin showed the characteristics C=C stretching peak from hydrocinnamic acid at 1632 $cm^{-1,}$ as reported in the literature (Dokken et al., 2013; D'Souza et al., 2008). All the samples showed similar result in the peak position for lignin content. However, there were differences in intensities of treated and untreated samples of both varieties, except in B-14, which had less changes in intensities for bCuO treated plants (Fig. 2e). In addition, there was a dramatic modification in lignin content for the nCuO treatment samples in both cases (Fig. 2 a and d). Results on B-14 could be due to the low Cu release from bCuO (Rawat et al., 2018), which could be trapped by lignin at a lower rate. For the carbohydrate spectra of COV variety, no shift was observed in the band region from 900 cm^{-1} to 1200 cm^{-1} (Fig. S5 a-c)) (Rico et al., 2015). Though, FTIR peak intensities were affected for treated and untreated samples in all cases.

4. Conclusion

In summary, this study demonstrated that both varieties showed differences in response to CuO and $CuCl_2$ exposure. Treatment effects on gas exchange parameters were not clear in B-14; however, varied assertive responses were observed in COV. For instance, at 125 mg/kg, bCuO reduced intercellular CO_2 but ionic Cu increased it. None of the treatments reduced root production, and only B-14 roots exposed to 125 mg $CuCl_2$/kg had more Cu in medulla than control. There were no effects on roots production; however, COV plants exposed to 25 mg nCuO/kg had statistically longer roots, compared with control. Only B-14 showed significant changes in macroelement accumulation. At 125 mg/kg, $CuCl_2$/kg reduced P, while the equivalent concentration of

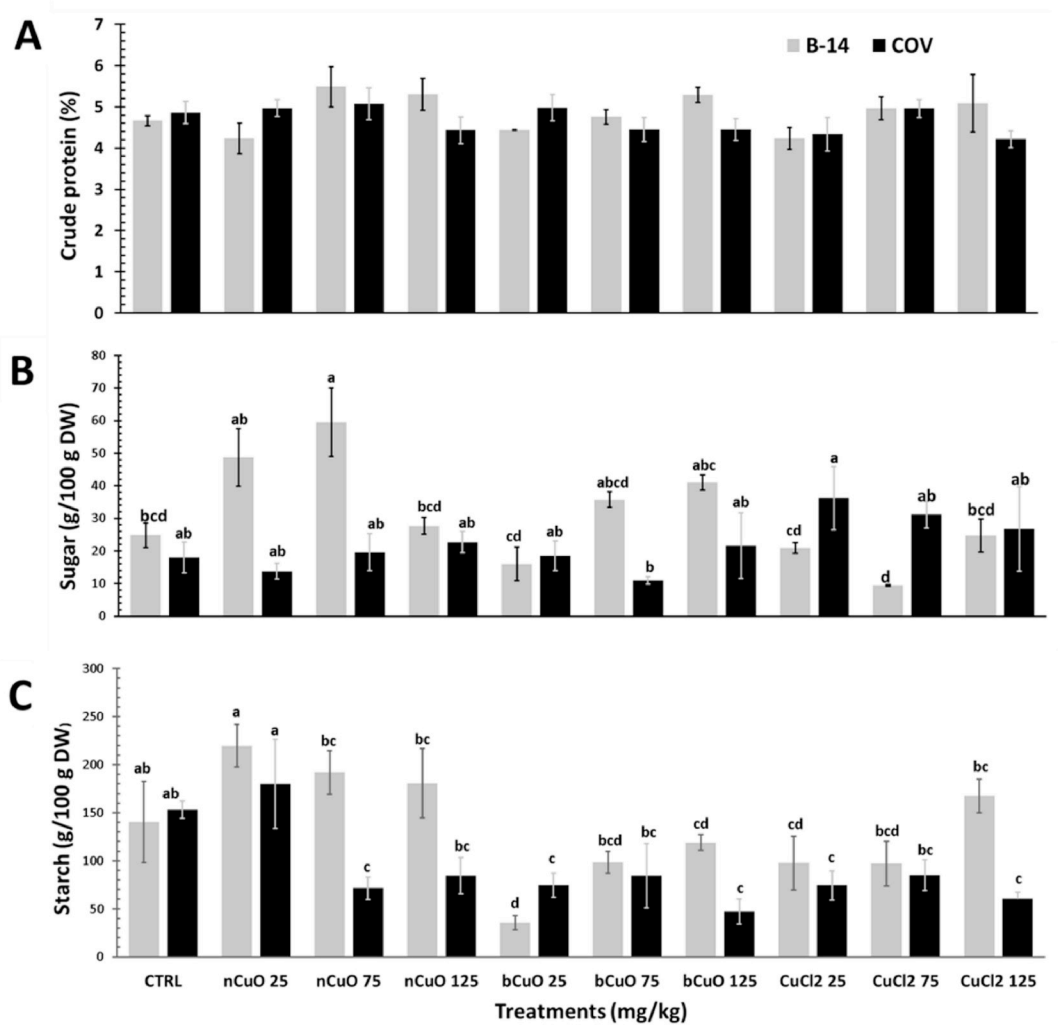

Fig. 1. Crude protein, sugar, and starch in storage roots of Beauregard-14 and Covington sweetpotato varieties developed in soil amended with nCuO, bCuO, and $CuCl_2$ at 0, 25, 75, and 125 mg/kg. Data are average of three replicates ± SE. Different letters stand for statistical differences at $p \leq 0.05$.

nCuO increased Mg in the periderm. None of the treatments affected proteins, sugar, and starch production; however, the FTIR spectra showed changes in lignin of both varieties exposed to nCuO. Electron microscope images showed that at 125 mg/kg, bCuO and $CuCl_2$ produced changes in the shape and morphology of starch grains in storage roots, while minimal changes were produced by nCuO. It is true that, besides lignin, B-14 and COV may differ in other characteristics; however, the obvious difference in root lignin content suggests that this biomarker modulated the response to the Cu treatments. Although, at the concentrations tested, nCuO did not affect protein and sugar content in storage roots, produced longer roots and increased an essential macroelement, which suggest a potential application of these nanoparticles as nanofertilizer for sweetpotato storage root production.

Credit author statement

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Declaration of competing interest

The authors declare no competing or conflict of interest.

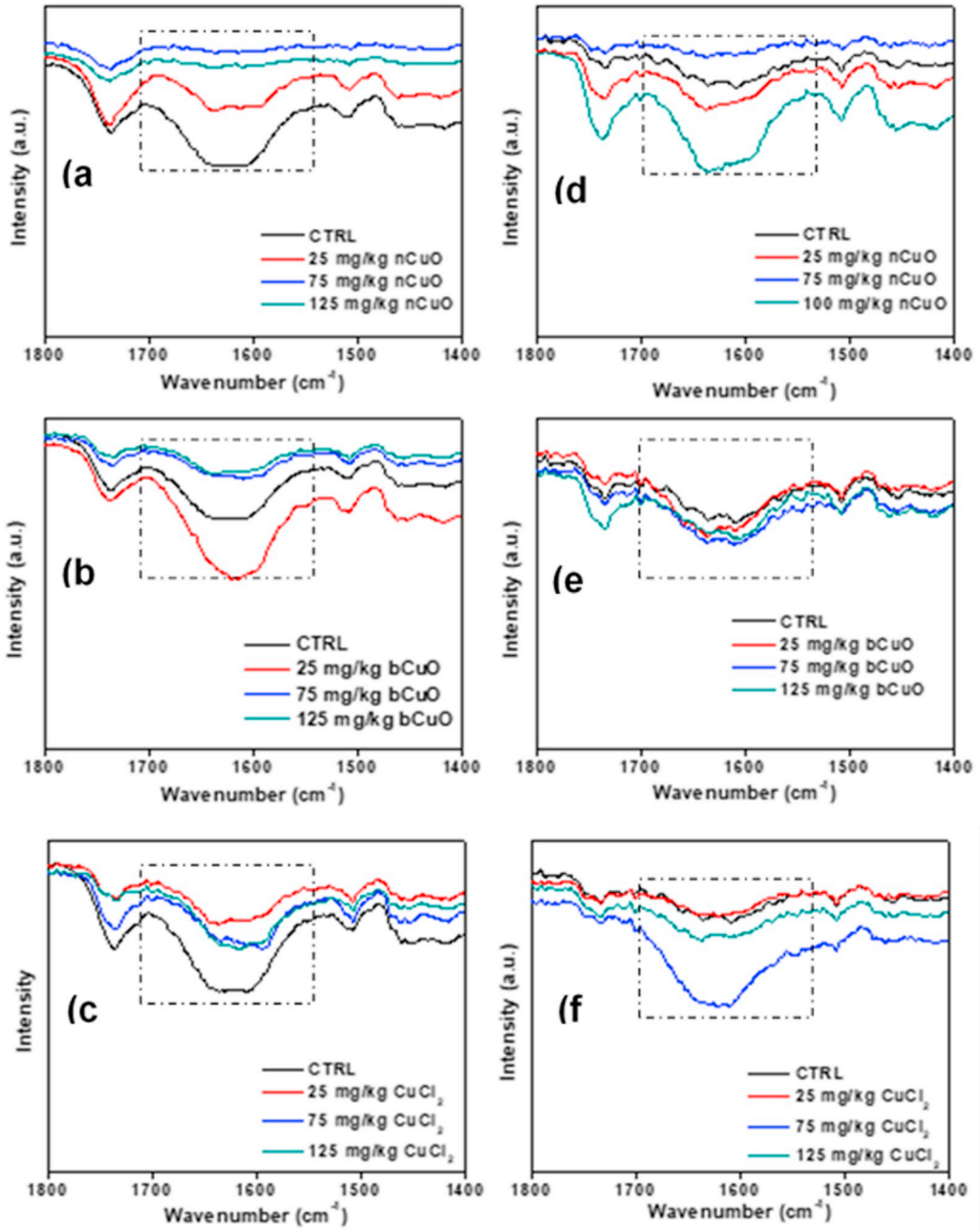

Fig. 2. FTIR spectra for lignin content (shown in rectangular box) in skin of Covintong (a) nCuO; (b) bCuO; (c) $CuCl_2$ and Beauregard-14 varieties (d) nCuO; (e) bCuO; (f) $CuCl_2$.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2020.06.009.

References

Adams, J., Wright, M., Wagner, H., Valiente, J., Britt, D., Anderson, A., 2017. Cu from dissolution of CuO nanoparticles signals changes in root morphology. Plant Physiol. Biochem. 110, 108–117.

Adhikari, T., Kundu, S., Biswas, A.K., Tarafdar, J.C., Rao, A.S., 2012. Effect of copper oxide nano particle on seed germination of selected crops. J. Agric. Sci. Technol. 2 (6A), 815.

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.

Apodaca, S.A., Medina-Velo, I.A., Lazarski, A.C., Flores-Margez, J.P., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2018. Different forms of copper and kinetin impacted element accumulation and macromolecule contents in kidney bean (*Phaseolus vulgaris*) seeds. Sci. Total Environ. 636, 1534–1540.

Barrios, A.C., Rico, C.M., Trujillo-Reyes, J., Medina-Velo, I.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2016. 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.

Bird, N.J.B., 2015. Morphological and Physiological Characterization of Sweetpotato Roots after Skinning. Mississippi State University.

Bonilla-Bird, N.J., Paez, A., Reyes, A., Hernandez-Viezcas, J.A., Li, C., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2018. Two-photon microscopy and spectroscopy studies to determine the mechanism of copper oxide nanoparticle uptake by sweetpotato roots during postharvest treatment. Environ. Sci. Technol. 52 (17), 9954–9963.

Borgatta, J., Ma, C., Hudson-Smith, N., Elmer, W., Plaza Pérez, C.D., De La Torre-Roche, R., et al., 2018. Copper based nanomaterials suppress root fungal disease in watermelon (Citrullus lanatus): role of particle morphology, composition and dissolution behavior. ACS Sustain. Chem. Eng. 6 (11), 14847–14856.

Bradfield, S.J., Kumar, P., White, J.C., Ebbs, S.D., 2017. Zinc, copper, or cerium accumulation from metal oxide nanoparticles or ions in sweet potato: yield effects and projected dietary intake from consumption. Plant Physiol. Biochem. 110, 128–137.

Bremner, J.M., 1996. Nitrogen-total. In: Methods of Soil Analysis. Part 3. Chemical Methods-SSSA. Soil Science Society of America, American Society of Agronomy, pp. 1085–1121.

Burzyński, M., Zurek, A., 2007. Effects of copper and cadmium on photosynthesis in cucumber cotyledons. Photosynthetica 45 (2), 239–244.

Chen, W., He, Z.L., Yang, X.E., Mishra, S., Stoffella, P.J., 2010. Chlorine nutrition of higher plants: progress and perspectives. J. Plant Nutr. 33 (7), 943–952.

Clark, C.A., Smith, T.P., Ferrin, D.M., Villordon, A.Q., 2010. Performance of sweetpotato foundation seed after incorporation into commercial operations in Louisiana. HortTechnology 20 (6), 977–982.

Conway, J.R., Beaulieu, A.L., Beaulieu, N.L., Mazer, S.J., Keller, A.A., 2015. Environmental stresses increase photosynthetic disruption by metal oxide nanomaterials in a soil-grown plant. ACS Nano 9 (12), 11737–11749.

Demirevska-Kepova, K., Simova-Stoilova, L., Stoyanova, Z., Hölzer, R., Feller, U., 2004. Biochemical changes in barley plants after excessive supply of copper and manganese. Environ. Exp. Bot. 52 (3), 253–266.

Dimkpa, C.O., Bindraban, P.S., Fugice, J., Agyin-Birikorang, S., Singh, U., Hellums, D., 2017. Composite micronutrient nanoparticles and salts decrease drought stress in soybean. Agron. Sustain. Dev. 37 (1), 5.

Dokken, K., Davis, L.C., Erickson, L.E., Castro, S., 2013. Fourier-transform infrared spectroscopy as a tool to monitor changes in plant structure in response to soil contaminants. In: 2002 Proceedings-Water Res. Technol. 2002, pp. 1–7 No. December.

Du, W., Gardea-Torresdey, J.L., Ji, R., Yin, Y., Zhu, J., Peralta-Videa, J.R., Guo, H., 2015. Physiological and biochemical changes imposed by CeO_2 nanoparticles on wheat: a life cycle field study. Environ. Sci. Technol. 49 (19), 11884–11893.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28 (3), 350–356.

Duhan, J.S., Kumar, R., Kumar, N., Kaur, P., Nehra, K., Duhan, S., 2017. Nanotechnology: the new perspective in precision agriculture. Biotechnol. Rep, 15, 11–23.

D'Souza, L., Devi, P., Divya Shridhar, M.P., Naik, C.G., 2008. Use of fourier transform infrared (FTIR) spectroscopy to study cadmium-induced changes in padina tetrastromatica (Hauck). Anal. Chem. Insights 2008 (3), 135–143.

Ekanayake, I.J., Collins, W., 2004. Effect of irrigation on sweet potato root carbohydrates and nitrogenous compounds. Food Agric. Environ. 2 (1), 243–248.

EPA (US Environmental Protection Agency), 1994. Land Application of Sewage Sludge and Biosolids.

FAO, 2016. Faostat crops. http://www.fao.org/faostat/en/#data/QC.

Giannousi, K., Avramidis, I., Dendrinou-Samara, Synthesis, C., 2013. Characterization and evaluation of copper based nanoparticles as agrochemicals against phytophthora infestans. RSC Adv. 3 (44), 21743–21752.

Hong, Jie, R, C., 2015. Toxic Effects of Copper-Based Nanoparticles or Compounds to Lettuce (*Lactuca sativa*) and Alfalfa (*Medicago Sativa*), vol. 85. pp. 1–27 0 1.

Kah, M., Tufenkji, N., White, J.C., 2019. Nano-enabled strategies to enhance crop nutrition and protection. Nat. Nanotechnol. 14 (6), 532–540.

Kopek, B.G., Paez-Segala, M.G., Shtengel, G., Sochacki, K.A., Sun, M.G., Wang, Y., Xu, C.S., Van Engelenburg, S.B., Taraska, J.W., Looger, L.L., et al., 2017. Diverse protocols for correlative super-resolution fluorescence imaging and electron microscopy of chemically fixed samples. Nat. Protoc. 12 (5), 916–946.

Lamichhane, J.R., Osdaghi, E., Behlau, F., Köhl, J., Jones, J.B., Aubertot, J.N., 2018. Thirteen decades of antimicrobial copper compounds applied in agriculture. A review. Agron. Sustain. Dev. 38 (3), 28.

Lin, C.C., Chen, L.M., Liu, Z.H., 2005. Rapid effect of copper on lignin biosynthesis in soybean roots. Plant Sci. 168 (3), 855–861.

Ling, Q., Huang, W., Jarvis, P., 2011. Use of a SPAD-502 meter to measure leaf chlorophyll concentration in *Arabidopsis thaliana*. Photosynth. Res. 107 (2), 209–214.

Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S.I., Lee, Y.C., 2005. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. Anal. Biochem. 339 (1), 69–72.

Ochoa, L., Zuverza-Mena, N., Medina-Velo, I.A., Flores-Margez, J.P., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2018. Copper oxide nanoparticles and bulk copper oxide, combined with indole-3-acetic acid, alter aluminum, boron, and iron in Pisum sativum seeds. Sci. Total Environ. 634, 1238–1245.

Panou-Filotheou, H., Bosabalidis, A.M., Karataglis, S., 2001. Effects of copper toxicity on leaves of oregano (*Origanum vulgare subsp. Hirtum*). Ann. Bot. 88 (2), 207–214.

Pimentel, David, Burgess, Michael, 2014. Environmental and economic costs of the application of pesticides primarily in the United States. In: Integrated Pest Management. Springer, Dordrecht, pp. 47–71.

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, 601 2016.

Priyanka, N., Geetha, N., Ghorbanpour, M., Venkatachalam, P., 2019. Role of engineered zinc and copper oxide nanoparticles in promoting plant growth and yield: present status and future prospects. In: Advances in Phytonanotechnology. Academic Press, pp. 183–201.

Rawat, S., Pullagurala, V.L., Hernandez-Molina, M., Sun, Y., Niu, G., Hernandez-Viezcas, J.A., et al., 2018. Impacts of copper oxide nanoparticles on bell pepper (Capsicum annum L.) plants: a full life cycle study. Environ. Sci.: Nano 5 (1), 83–95.

Rico, C.M., Peralta-Videa, J.R., Gardea-Torresdeya, J.L., 2015. Differential effects of cerium oxide nanoparticles on rice, wheat, and barley roots: a fourier transform infrared (FT-IR) microspectroscopy study. Appl. Spectrosc. 69 (2), 287–295.

Rocateli, AlexZhang, Hailin Forage quality interpretations. Oklahoma cooperative extension service PSS-2117. http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Document-2557/PSS-2117web.pdf.

Servin, A., Elmer, W., Mukherjee, A., De la Torre-Roche, R., Hamdi, H., White, J.C., et al., 2015. A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. J. Nanoparticle Res. 17 (2), 92.

Shacklette, H.T., Boerngen, J.G., 1984. Element Concentrations in Soils and Other Surficial Materials of the Conterminous United States. U.S. Geological Survey Professional PAPER No. 1270, *I*.

Spectrum Technologies, I. SPAD 502 Plus Chlorophyll Meter Product Manual.

Sun, H., Mu, T., Xi, L., Zhang, M., Chen, J., 2014. Sweet potato (Ipomoea batatas L.) leaves as nutritional and functional foods. Food Chem. 156, 380–389.

Taiz, L., Zeiger, E., 2002. Plant Physiology. 3. third ed. Sinauer Assoc. Sunderland, , MA, USA, pp. 386.

Tamez, C., Molina-Hernandez, M., Medina-Velo, I.A., Cota-Ruiz, K., Hernandez-Viezcas, J.A., Gardea-Torresdey, J., 2020. Long-term assessment of nano and bulk copper compound exposure in sugarcane (Saccharum officinarum). Sci. Total Environ. 718, 137318.

Villavicencio, L.E., Blankenship, S.M., Yencho, G.C., Thomas, J.F., Raper, C.D., 2007. Temperature effect on skin adhesion, cell wall enzyme activity, lignin content, anthocyanins, growth parameters, and periderm histochemistry of sweetpotato. J. Am. Soc. Hortic. Sci. 132 (5), 729–738.

Von Caemmerer, S., Lawson, T., Oxborough, K., Baker, N.R., Andrews, T.J., Raines, C.A., 2004. Stomatal conductance does not correlate with photosynthetic capacity in transgenic tobacco with reduced amounts of Rubisco. J. Exp. Bot. 55 (400), 1157–1166.

Zhang, Z., Ke, M., Qu, Q., Peijnenburg, W.J.G.M., Lu, T., Zhang, Q., Ye, Y., Xu, P., Du, B., Sun, L., et al., 2018. Impact of copper nanoparticles and ionic copper exposure on wheat (*Triticum aestivum L.*) root morphology and antioxidant response. Environ. Pollut. 239, 689–697.

Zhao, L., Huang, Y., Zhou, H., Adeleye, A.S., Wang, H., Ortiz, C., Mazer, S.J., Keller, A.A., 2016. GC-TOF-MS based metabolomics and ICP-MS based metallomics of cucumber (: *Cucumis sativus*) fruits reveal alteration of metabolites profile and biological pathway disruption induced by nano copper. Environ. Sci. Nano 3 (5), 1114–1123.

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. Process. Impacts 17 (10), 1783–1793.